



# ABERDEEN SCOTLAND

3-5 May 2023



## PROGRAMME

P&J LIVE



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**Food Protection**®

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IAFP'S EUROPEAN  
SYMPOSIUM ON FOOD SAFETY



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## CELL PHONE POLICY

As a courtesy to the presenters, we request that you silence your cell phone while attending sessions.

## RECORDING POLICY

Unauthorized video or still photography or audio recording will not be allowed without prior approval. Thank you for your cooperation.

## CONNECT WITH IAFP



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# ORGANISING COMMITTEE

**Chairperson**, Luca Cocolin, University of Torino

**Vice Chairperson**, Mariem Ellouze, Nestlé Research Center

## **Committee Members**

Ana Allende Prieto, CEBAS-CSIC

Peter Ben Embarek, World Health Organization

Francois Bourdichon, Food Safety, Microbiology and Hygiene

Sara Bover-Cid, IRTA

Christophe Cordevant, ANSES

Noemie Desriac, University of Brest

Elissavet Gkogka, Arla Innovation Centre

Isabelle Guelinckx, ILSI Europe

Liesbeth Jacxsens, Ghent University

Jeffrey LeJuene, Food and Agriculture Organization of the United Nations

Peter McClure, Consultant

Lisa O'Connor, Food Safety Authority of Ireland

David Tomas Fornes, MERCK Life Science

Vasilis Valdramidis, University of Malta

Carol Wallace, University of Central Lancashire

Marjon Wells-Bennik, NIZO

## **IAFP Executive Board Liasons**

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International Association  
for Food Protection

# IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY PROGRAMME AT-A-GLANCE

Room	Suite 2B	Suite 3	Suite 4	Exhibit Area
<b>Wednesday, 3 May 2023</b>				
Wednesday 8.30 - 10.00	Opening Session			
Wednesday 10.00 - 10.30	Coffee/Networking Break Exhibit Hall			<b>Poster Session 1 –</b> Animal and Pet Food Safety, Communication Outreach and Education, Dairy Data Management and Analytics, Epidemiology, Food Fraud, Food Law and Regulation, Food Safety Systems, Low-Water Activity Foods, Meat, Poultry and Eggs, Microbial Food Spoilage, Plant-Based Alternative Products, Pre-Harvest Food Safety, Produce, Seafood, Viruses and Parasites, Water  <i>authors present at coffee breaks and lunch</i>
Wednesday 10.30 - 12.00	S1 – Improving Food Traceability through Global Partnerships, Harmonized Data Elements, and Accessible, Interoperable Software Tools	S2 – <i>Clostridium botulinum</i> – Opportunities and Challenges for New Testing Methods to Maintain Food Safety in Ready-to-Eat Foods	Technical Session 1 – Meat, Poultry and Eggs, Seafood, Microbial Food Spoilage	
Wednesday 12.00 - 13.30	Lunch Exhibit Hall			
Wednesday 13.30 - 15.00	RT1 – When Opposites and Peers Come Together in a Roundtable: Zero-Tolerance Versus Microbial Risk Assessment within a Poultry Case Study	S3 – Latest Developments in International Organisations Making Food Safety Improvements and Successes Measurable	Technical Session 2 – Produce, Beverages and Acid/Acidified Foods, Laboratory and Detection Methods	
Wednesday 15.00 - 15.30	Coffee/Networking Break Exhibit Hall			
Wednesday 15.30 - 17.00	S4 – The New Codex Alimentarius Framework for Safe Water-Reuse in Food Production and Processing Developed for Dairy and Fishery Products and Put to the Test in Practice for Fruit and Vegetable Food Products	S5 – What Should the Food Industry and Risk Communicators Know about People's Kitchen Practices and Beliefs?	Technical Session 3 – Packaging, Sanitation and Hygiene and Food Chemical Hazards	
Wednesday 17.00 - 18.00	Exhibit Hall Reception			
<b>Thursday, 4 May 2023</b>				
Thursday 8.30 - 10.00	S6 – Paving the Avenue for the Application of Natural Antimicrobials	RT2 – Responding to Food Safety Crises: Evolving Role of Food Scientists	Technical Session 4 – Molecular Analytics, Genomics and Microbiome	
Thursday 10.00 - 10.30	Coffee/Networking Break Exhibit Hall			<b>Poster Session 2 –</b> Antimicrobials, Food Processing Technologies, Food Toxicology, General Microbiology, Laboratory and Detection Methods, Modeling and Risk Assessment, Molecular Analytics, Genomics and Microbiome, Packaging, Retail and Food Service Safety, Sanitation and Hygiene  <i>authors present at coffee breaks and lunch</i>
Thursday 10.30 - 12.00	S7 – Microbiological Contaminants in Plant Protein Ingredients – Assessing Potential Risks	RT3 - Creating Capacity of the Next-Generation Food Safety Researchers and Implementers through International Collaboration: Experience of Low-and Middle-Income Countries (LMICs)	Technical Session 5 – Microbial Food Spoilage, Dairy and Antimicrobials	
Thursday 12.00 - 13.30	Lunch Exhibit Hall			
Thursday 13.30 - 15.00	S8 – <i>Bacillus cereus</i> and Related Organisms: Differentiating Friend from Foe	S9 – Food Safety of Infant Foods: Care for Our Most Precious	Technical Session 6 – Food Safety Systems	
Thursday 15.00 - 15.30	Coffee/Networking Break Exhibit Hall			
Thursday 15.30 - 17.00	S10 – Testing and Improving HACCP Team Proficiency to Strengthen Food Safety Culture	S11 – Raw Milk Safety and Climate Dynamics: Integrating Geographical and Seasonal Variation from Farms of Southern Europe and the Middle East	Technical Session 7 – Modeling and Risk Assessment and Viruses and Parasites	
<b>Friday, 5 May 2023</b>				
Friday 8.30 - 10.00	S12 – Pathogens of Concern in Food Processing Environment	RT4 – The Current and Future Landscape of Scientific Publications in the Food Safety Domain	Technical Session 8 – Food Processing Technologies and Viruses and Parasites	
Friday 10.00 - 10.30	Coffee/Networking Break			
Friday 10.30 - 12.00	S13 – Towards the Development of Quantitative Microbiological Risk Assessment for Non-Thermal Technologies	S14 – How Has Wastewater Surveillance Been Useful to Boost Public Health Preparedness and Make Our Food Systems More Resilient? What Does the Future Hold?	Technical Session 9 – Epidemiology, Food Chemical Hazards, Communication and Outreach and Retail and Food Service Safety	
Friday 12.15 - 13.30	Closing Session			
Friday 13.30 - 14.30	Farewell Refreshments			





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**ABERDEEN**

**SCOTLAND**

**3–5 MAY 2023**

**PROGRAMME**

Wednesday, 3 May

IAFP'S EUROPEAN  
SYMPOSIUM ON FOOD SAFETY



ABERDEEN

SCOTLAND

3–5 MAY 2023

WELCOME TO THE  
IAFP EUROPEAN  
SYMPOSIUM

*All times listed in Central European Time (CET).*

**WEDNESDAY, 3 MAY**

**7.30 – 17.00 Registration Open**

**7.30 – 8.30 Morning Coffee**

**10.00 – 18.00 Exhibit Hours**

**OS Opening Session**

Suite 2B

**Chairs: Luca Cocolin, Mariem Ellouze**

8.30 Introduction to IAFP  
MICHELLE DANYLUK, University of Florida  
CREC, Lake Alfred, FL, USA

8.45 Introduction to IAFP's European Symposium,  
Programme Notes and Recognition of the  
Organising Committee  
LUCA COCOLIN, Department of Agricultural,  
Forest and Food Sciences, University of Turin,  
Grugliasco (TO), Italy

9.00 Current Challenges and Opportunities for Food  
Safety  
DAVID GALLY, Food Standards Scotland, Aber-  
deen, Scotland, United Kingdom

9.30 Food System Transformation: Policy Coherence  
through Standard Setting. The Role of Codex  
STEVE WEARNE, Chairperson, Codex Alimenta-  
rius Commission, on secondment from the Food  
Standards Agency, UK, London, United Kingdom

10.00 – 10.30 Networking Coffee in the Exhibit Hall

■ – Symposia

■ – Roundtables

■ – Technicals

\*Student Award Competitor



## S1 Improving Food Traceability through Global Partnerships, Harmonized Data Elements, and Accessible, Interoperable Software Tools

Suite 2B

**Organizers:** Marion Gottschald, Xin Liu, Eric Stevens

**Convenors:** Marion Gottschald, Xin Li, Eric Stevens

- 10.30 Global Partnerships and Interoperable Software Tools: Towards a Universal Data Format to Enable Data Exchange and Interoperability between National, European, and International Traceability Stakeholders  
MARION GOTTSCHALD, German Federal Institute for Risk Assessment, Berlin, Germany; Alexander Falenski, German Federal Institute for Risk Assessment, Berlin, Germany
- 11.00 Global Partnerships and Harmonized Data Elements: Speaking the Same Traceability Language through the Use and Standardization of Critical Tracking Events and Key Data Elements  
ADAM FRIEDLANDER, FDA, College Park, MD, USA
- 11.30 Food Industry Traceability Initiatives – Interoperability in Real Life  
ERIC STEVENS, FDA, College Park, MD, USA
- 12.00 Lunch Available in the Exhibit Hall

## S2 *Clostridium botulinum* – Opportunities and Challenges for New Testing Methods to Maintain Food Safety in Ready-to-Eat Foods

Suite 3

**Organizers:** Matthew McCusker, Janneke Wijman

**Convenors:** Gijs Lommerse, Janneke Wijman

- 10.30 *Clostridium botulinum* – An Age Old Problem and Its Impact on Food Safety in RTE Foods  
KRISTIN SCHILL, Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA
- 11.00 *Clostridium botulinum* Growth Boundary Models for Development of Stabilized New Food Products  
PAW DALGAARD, Research Group for Food Microbiology and Hygiene, National Food Institute (DTU Food), Technical University of Denmark, Kgs. Lyngby, Denmark
- 11.30 Nontoxic Surrogates of Proteolytic and Nonproteolytic *Clostridium botulinum* for Food Challenge Studies  
CHRIS MICHIELS, KU Leuven, Leuven, Belgium
- 12.00 Lunch Available in the Exhibit Hall

## T1 Technical Session 1 – Meat, Poultry and Eggs, Seafood, Microbial Food Spoilage

Suite 4

**Convenors:** Cangliang Shen, Koen De Reu

- T1-01** Nanosystems to Successfully Delivery Aptamers: a Disruptive Strategy to Prevent Salmonellosis in the Poultry Industry  
10.30 Carina F. Almeida, Márcia Faria, Josué Carvalho, EVA PINHO, AliCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, Porto, Portugal
- T1-02** Biofilm-Associated Traits and Tolerance to Didecyltrimethylammonium Chloride are Involved in Enhanced Ability of *Listeria monocytogenes* to Persist in Seafood Processing Environments  
10.45 Benjamin Duqué, Alice Michel, François Gravey, Graziella Midelet, Thomas Brauge, Guylaine Leleu, Sabine Debuiche, Anthony Colas, Christophe Soumet, Béatrice Anger, Emeline Bourgault, Simon Le Hello, AURELIE HANIN, ACTALIA, Food Safety Department, Saint-Lô, France
- T1-03** The Effect of Cysteine on the Motility Behaviour and Biofilm Formation of *Listeria monocytogenes*; Cysteine Intake through CTAP Plays a Role  
11.00 MAHIDE MUGE YILMAZ TOPCAM, Kimon-Andreas Karatzas, University of Reading, Reading, United Kingdom
- T1-04** Growth Potential of *Clostridium botulinum* and *Clostridium perfringens* in Nitrite-Free Ham Model during Cooling in Thermal Abuse Conditions  
11.15 ELENA DALZINI, Elena Cosciani-Cunico, Paola Monastero, Daniela Merigo, Stefania Ducoli, Alessandro Norton, Marina-Nadia Losio, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", Brescia, Italy
- T1-05** Exploring the Diversity of Biofilm Formation by the Food Spoiler *Brochothrix thermosphacta* and its Ability to Form Biofilms on Food Industrial Surfaces  
11.30 ANTOINE GAILLAC, Oniris, Nantes, France
- T1-06** The Effect of Meat Processing Techniques on Hepatitis E Virus Infectivity in Real-Life Pork Meat Matrices  
11.45 TATJANA LOCUS, Ellen Lambrecht, Sjarlotte Willems, Michael Peeters, Thomas Vanwollegem, Steven Van Gucht, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium
- 12.00 Lunch Available in the Exhibit Hall

## RT1 When Opposites and Peers Come Together in a Roundtable: Zero-Tolerance Versus Microbial Risk Assessment within a Poultry Case Study

Suite 2B

**Organizers: Daniele Sohier, Purnendu Vasavada**  
**Convenor: Daniele Sohier**

GARY ACUFF, Acuff Consulting LLC, College Station, TX, USA

ALVIN LEE, Institute for Food Safety and Health, Bedford Park, IL, USA

PANAGIOTIS SKANDAMIS, Agricultural University of Athens, Athens, Greece

ANETT WINKLER, Cargill, Inc., Unterschleißheim, Germany

MARCEL ZWIETERING, Wageningen University, Wageningen, The Netherlands

15.00 – 15.30 Networking Coffee in the Exhibit Hall

## S3 Latest Developments in International Organisations Making Food Safety Improvements and Successes Measurable

Suite 3

**Organizers: Caroline Smith DeWaal, Leon Gorris**

**Convenor: Leon Gorris**

13.30 A Conceptual Framework and Indices Linking Food Safety and Nutrition to Support the Investment in and Management of Food Systems Programs

CAROLINE SMITH DEWAAL, Global Alliance for Improved Nutrition (GAIN), Washington, D.C., USA

14.00 Experience in the Use of Food Safety Indicators in Different Contexts (Regional; Internal)

KANG ZHOU, Food and Agriculture Organization (FAO), Rome, Italy

14.30 WHO Global Strategy for Food Safety: Indicators to Monitor Progress towards Reducing the Burden of Foodborne Diseases

PETER SOUSA HOEJSKOV, World Health Organization, Geneva, Switzerland

15.00 – 15.30 Networking Coffee in the Exhibit Hall

## T2 Technical Session 2 – Produce, Beverages and Acid/Acidified Foods, Laboratory and Detection Methods

Suite 4

**Convenor: Christina Serra**

**T2-01** Acidity of Fruit Puree Determines the Kinetics of the High Pressure Inactivation in *Escherichia coli* Strains

13.30 BERTA TORRENTS-MASOLIVER, Anna Jofre, Albert Ribas-Agustí, Sara Bover-Cid, IRTA (Institute of Agrifood Research and Technology). Food Safety and Functionality Program, Monells, Girona, Spain

**T2-02** Development and Validation of a Digital PCR Assay for Detection and Quantification of Norovirus and Hepatitis A Virus

13.45 Inês Santos, Joana Diogo, João Melo, Maria Fonseca, JOANA CRUZ, Competence Centre for Molecular Biology, SGS Portugal, Lisbon, Portugal

**T2-03** Metagenomics Detection of *Salmonella*-Contaminated Lettuce

14.00 DELE OGUNREMI, Tianbi Tan, Sohail Naushad, Marc-Olivier Duceppe, Hongsheng Huang, Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency, Ottawa, ON, Canada

**T2-04** Development of an OmpG Nanopore Sensor for Norovirus Detection

14.25 MINJI KIM, Joshua C. Foster, Min Chen, Matthew Moore, University of Massachusetts Amherst, Amherst, MA, USA

**T2-05** Efficacy of a Triple-Wash with a Combination of Peroxyacetic Acid and Hydrogen Peroxide to Reduce Populations and Mitigate Cross-Contamination of *Salmonella* Typhimurium and the Surrogate *Enterococcus faecium* on Tomatoes

14.30 CANGLIANG SHEN, Rebecca Stearns, Corey Coe, Kristen Matak, Annette Freshour, West Virginia University, Morgantown, WV, USA

**T2-06** Whole Genome Sequencing of Historical Scottish *Salmonella*

14.45 SVETLOZARA CHOBANOVA, Derek Brown, Food Standards Scotland, Aberdeen, United Kingdom

15.00 – 15.30 Networking Coffee in the Exhibit Hall

## **S4 The New Codex Alimentarius Framework for Safe Water Reuse in Food Production and Processing Developed for Dairy and Fishery Products and Put to the Test in Practice for Fruit and Vegetable Food Products**

Suite 2B

**Organizers and Convenors: Leon Gorris, Kang Zhou**

- 15.30 Guidance for Implementing the Codex Framework for Safe Water Reuse in the Dairy Sector  
CLAUS HEGGUM, Danish Agriculture & Food Council F.M.B.A., Aarhus, Denmark
- 16.00 Guidance for Implementing the Codex Framework for Safe Water Reuse in the Fisheries Sector  
KANG ZHOU, Food and Agriculture Organization of the United Nations, Rome, Italy
- 16.30 Assessing the Feasibility and Practicality of the Decision-Making Processes and Microbiological Criteria for Fresh Fruits and Vegetables Developed within the Codex Framework  
ANA ALLENDE, CEBAS-CSIC, Murcia, Murcia, Spain

### **5.00 – 6.00 p.m. – Exhibit Hall Reception**

## **S5 What Should the Food Industry and Risk Communicators Know about Peoples' Kitchen Practices and Beliefs?**

Suite 3

**Organizer and Convenor: Trond Moretro**

- 15.30 Which are the Riskiest Home Cooking Practices, and How Can We Change Them?  
SOLVEIG LANGSRUD, Nofima, Ås, Norway
- 16.00 Food Safety Challenges: Relevant Myths to Debunk  
PAULA TEIXEIRA, Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
- 16.30 Understanding Older Adult Food Safety Habits through the “Dimensions of Wellness”  
ELLEN EVANS, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

### **5.00 – 6.00 p.m. – Exhibit Hall Reception**

## **T3 Technical Session 3 – Packaging, Sanitation and Hygiene and Food Chemical Hazards**

Suite 4

**Convenor: Anett Winkler**

- T3-01** Results of a Retrospective Study on the Application of Restrictive Attention Limits and Corrective Measures Applied for Aflatoxin M1 Contamination in Commercial Milk Supply Chains  
15.30 FEDERICA SAVINI, Federica Giacometti, Federico Tomasello, Valentina Indio, Anelka Bacak, Alessandra Canever, Paolo Bonilauri, Alessandra De Cesare, Andrea Serraino, Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy
- T3-02** Plasma Treatment Application for Improving Liquid Retention in Plastic Food Packaging  
15.45 ALAA ALAIZOKI, Davide Deganello, Christopher Phillips, Chris Griffiths, Craig Hardwick, David Parker, Exponent International and Swansea University, Swansea, United Kingdom
- T3-03** Sustainable Solutions for Smart and Active Packaging for Shelf-Life Extension and Spoilage Monitoring of Processed Meats  
16.00 ROWAIDA KHALIL, Alexandria University, Alexandria, Egypt
- T3-04** Effect of Temperature and Isolation Source on the Susceptibility of *Salmonella* to Biocides  
16.15 MARIA HOFFMANN, Jae Hee Jang, Yu Cao, Jie Zheng, Victor Jayeola, U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA
- T3-05** Induction of VBNC *L. monocytogenes* by Chlorine-Based Disinfectants: Presence in Process Water and Transfer from the Process Water to the Product during Washing  
16.30 PILAR TRUCHADO, Maria I. Gil, Marisa Gómez-Galindo, Ana Allende, CEBAS-CSIC, Murcia, Spain
- T3-06** Development, Implementation, and Evaluation of Targeted Cleaning Optimisation Interventions in a UK-Based Small and Medium Sized (SME) Ready-to-Eat Food Manufacturer  
16.45 ALIN TURILA, Ellen Evans, James Blaxland, John Holah, Elizabeth C. Redmond, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom

### **5.00 – 6.00 p.m. – Exhibit Hall Reception**

## S6 Paving the Avenue for the Application of Natural Antimicrobials

Suite 2B

**Organizer and Convenor: Heidy den Besten**

- 8.30 Prenylated Isoflavonoids as Natural Preservatives Against *Listeria monocytogenes*: Application and Mode of Action  
ALBERTO BOMBELLI, Carla Araya-Cloutier, Jean-Paul Vincken, Tjakko Abee, Heidy den Besten, Wageningen University and Research, Wageningen, The Netherlands
- 9.00 Going Viral – Bacteriophages as Biocontrol Agents for Foodborne Pathogens  
OLIVIA MCAULIFFE, Teagasc Food Research Centre, Fermoy, Cork, Ireland
- 9.30 Selection and Evaluation of Natural Antimicrobials – The Industry Perspective  
JAN WILLEM SANDERS, Unilever Foods Innovation Centre Wageningen, Wageningen, The Netherlands

10.00 – 10.30 Networking Coffee in the Exhibit Hall

## RT2 Responding to Food Safety Crises: Evolving Role of Food Scientists

Suite 3

**Organizers: John O'Brien, Purnendu Vasavada**  
**Convenor: Purnendu Vasavada**

FRANCOIS BOURDICHON, Università Cattolica Del Sacro Cuore, Cremona, Italy

MICHELLE PATEL, UK Food Standards Agency, London, United Kingdom

DONALD PRATER, U.S. Food and Drug Administration, Silver Spring, MD, USA

HELEN TAYLOR, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom

PURNENDU VASAVADA, University of Wisconsin-River Falls, River Falls, WI, USA

10.00 – 10.30 Networking Coffee in the Exhibit Hall

## T4 Technical Session 4 – Molecular Analytics, Genomics and Microbiome

Suite 4

**Convenors: Kalmia Kniel, Celina To**

- T4-01** Metataxonomic Surveillance of Contamination Pathways in Food Processing Environments: From Observational Studies to Practical Applications  
8.30  
CRISTIAN BOTTA, Dimitra Tsourekaki, Elisabetta Chiarini, Davide Buzzanca, Ilario Ferrocino, Valentina Alessandria, Selene Rubiola, Francesco Chiesa, Kurt Houf, Luca Cocolin, Kalliopi Rantsiou, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy
- T4-02** First Enterotoxigenic Confirmation of *Staphylococcus argenteus* as a Foodborne Pathogen  
8.45  
MARINA CAVAIUOLO, Donatien Lefebvre, Isabelle Mutel, Noémie Vingadassalon, Déborah Merda, Jacques-Antoine Hennekinne, Yacine Nia, Laboratory for Food Safety, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France, Paris, France
- T4-03** Colonisation Dynamics of *Listeria monocytogenes* in Food Processing Environments, and Genetic Features of Strains Showing Persistent Contamination  
9.00  
EDWARD FOX, Jessica Gray, Lydia Fox, Séamus Fanning, Northumbria University, Newcastle Upon Tyne, United Kingdom
- T4-04** Design of a Biofilm Model Based on Metagenomic Characterization of Drains in Seafood and Dairy Processing Facilities  
9.15  
MARTIN LAAGE KRAGH, Nanna Hulbaek Scheel, Pimlapas Leekitcharoenphon, Paw Dalgaard, Lisbeth Truelstrup Hansen, Research Group for Food Microbiology and Hygiene, National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark
- T4-05** Understanding *Listeria monocytogenes* Behavior That Triggers Survival Under Severe Acidity  
9.30  
DIMITRA TSOUREKI, Cristian Botta, Sara Bover-Cid, Heidy den Besten, Luca Cocolin, Kalliopi Rantsiou, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy
- T4-06** Bettercalls: Analysis Tool for Precise Detection of Multiple *Salmonella* Serovars from Culture Enrichments Using Shotgun Metagenomic Profiling and Its Application in an Outbreak Setting  
9.45  
PADMINI RAMACHANDRAN, Elizabeth Reed, Mark Mammel, Rebecca Bell, Karen Jarvis, Christina M. Ferreira, Rachel Binet, Andrea Ottesen, Amanda Windsor, Christopher Grim, Kranti Konganti, U.S. Food and Drug Administration – CFSAN, College Park, MD, USA

10.00 – 10.30 Networking Coffee in the Exhibit Hall



## S7 Microbiological Contaminants in Plant Protein Ingredients – Assessing Potential Risks

Suite 2B

**Organizer: Marjon Wells-Bennik**

**Convenor: Karin Beekmann**

- 10.30 Predominance of Bacterial Spore Formers in Plant Protein-Based Ingredients  
MARJON WELLS-BENNIK, NIZO Food Research, Ede, The Netherlands
- 11.00 What Do We Know about *Bacillus licheniformis* in Plant-Based Dairy Alternatives?  
MARIEM ELLOUZE, Nestle, Lausanne, Switzerland
- 11.30 Microbiological Risk Assessment of *Bacillus cereus* Considering Diverse Phenotypic Traits  
YVAN LE MARC, ADRIA Food Technology Institute – UMT ACTIA 19.03 ALTER'IX, France, Quimper, France
- 12.00 Lunch Available in the Exhibit Hall

## RT3 Creating Capacity of the Next-Generation Food Safety Researchers and Implementers through International Collaboration: Experience of Low- and Middle-Income Countries (LMICs)

Suite 3

**Organizers: Kebede Amenu, Delia Grace**

**Convenor: Kebede Amenu**

DELIA GRACE, NATURAL Resource Institute, University of Greenwich, Kent, United Kingdom

MESERET BEKELE, Uppsala University in Sweden, Uppsala, Uppsala, Sweden

WIGDAN OMER, University of Khartoum, Khartoum, Sudan

HIMADRI PAL, Natural Resources Institute, Chatham, United Kingdom

SHWE PHUE SAN, University of Greenwich, Yangon, Yangon, Myanmar

STACEY DUVENAGE, Natural Resource Institute, University of Greenwich, Kent, United Kingdom

15.00 – 15.30 Networking Coffee in the Exhibit Hall

## T5 Technical Session 5 – Antimicrobials, Dairy and Microbial Food Spoilage

Suite 4

**Convenor: Francois Bourdichon**

- T5-01** Heat Inactivation of *Bacillus licheniformis* Spores in Plant-Based, Bovine Milk and Broth  
10.30 CHRYSANTHI CHAMPIDOU, Mariem Ellouze, Nabila Haddad, Jeanne-Marie Membré, Oniris INRAE Secalim & Nestlé Research, Lausanne, Switzerland
- T5-02** Cold Shock Proteins Promote Nisin Tolerance in *Listeria monocytogenes* through Modulation of Cell Envelope Modification Responses  
10.45 FRANCIS MUCHAAMBA, Joseph Wambui, Roger Stephan, Taurai Tasara, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- T5-03** Genome Mining within the Psychrophilic *Clostridium estertheticum* Complex Uncovers Estericin A, A Novel and Potent Bacteriocin with Bio-Preservative Potential Against Major Foodborne Pathogens  
11.00 JOSEPH WAMBUI, Marc J.A. Stevens, Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- T5-04** Comparison between MIC and WGS-Predicted Antimicrobial Resistance of *Staphylococcus aureus* from Bovine Mastitis Milk from Italy  
11.15 GIULIA MAGAGNA, Lorenzo Gambi, Paolo Daminelli, Michela Tilola, Virginia Filipello, Franco Paterlini, Food Safety Department, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy
- T5-05** Dairy Powder Industry: Risk Area Associated with Thermophilic Sporeforming Bacteria Using Their Growth Limits  
11.30 Louis Delaunay, Florence Postollec, Anne-Gabrielle Mathot, IVAN LEGUERINEL, LUBEM UBO University – UMT ACTIA 19.03 ALTER'IX, Quimper, France
- T5-06** Monitoring of Antimicrobial Resistance Indicator Genes in a Benthic Food Web in the English Channel and the North Sea  
11.45 ERWAN BOURDONNAIS, Cédric Le Bris, Thomas Brauge, Graziella Midelet, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Laboratory for Food Safety, Boulogne-sur-Mer, France
- 12.00 Lunch Available in the Exhibit Hall



## S8 *Bacillus cereus* and Related Organisms: Differentiating Friend from Foe

Suite 2B

**Organizers:** Mariem Ellouze, Laura Carroll, Maria Teresa Da Silva Felicio  
**Convenor:** Claudia Guldemann

- 13.30 Keeping up with the *Bacillus cereus* Group in the Whole-Genome Sequencing Era  
LAURA CARROLL, Umeå University, Umeå, Sweden
- 14.00 Same but Different: Modeling *Bacillus cereus* Behavior in Plant-Based Milk Alternatives and Bovine Milk  
MARIEM ELLOUZE, Nestle, Lausanne, Switzerland
- 14.30 Risk Assessment of *Bacillus cereus* Group in Food  
MARIA TERESA DA SILVA FELICIO, European Food Safety Authority (EFSA), Parma, Italy
- 15.00 – 15.30 Networking Coffee in the Exhibit Hall

## S9 Food Safety of Infant Foods: Care for Our Most Precious

Suite 3

**Organizer and Convenor:** Marcel Zwietering

- 10.30 Hazard Identification and Risk Ranking for Microbial Risks in Infant Foods  
KAH YEN CLAIRE YEAH, Wageningen University, Wageningen, Gelderland, The Netherlands
- 11.00 Hazard Control in Infant Foods Using Emerging Processes Technologies  
SARA BOVER-CID, IRTA (Institute of Agrifood Research and Technology), Food Safety and Functionality Program, Monells, Girona, Spain
- 11.30 Traditional and DNA-Based Analytics for Microbial Hazard Detection and Behaviour in Infant Foods  
KALLIOPI RANTSIOU, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy
- 12.00 Lunch Available in the Exhibit Hall

## T6 Technical Session 6 – Food Safety Systems

Suite 4

**Convenors:** Neela Badrie, Lisa O'Connor

- T6-01** The Effect of Dry Salting on the Survival of *Escherichia coli*, *Vibrio* spp., *Listeria monocytogenes*, and *Salmonella* on Inoculated Sugar Kelp during Storage  
13.30 JENNIFER PERRY, Richa Arya, Denise Skonberg, University of Maine, Orono, ME, USA
- T6-02** Practical Experiences in Setting up *Listeria monocytogenes* Environmental Sampling in the Food Industry  
13.45 KOEN DE REU, Geertrui Rasschaert, Liesbeth Jacxsens, Ellen Lambrecht, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium
- T6-03** Development and Evaluation of Low-Cost, Easily Deployable Molecularly Imprinted Polymer for Norovirus Detection  
14.00 Sarbjeet Kaur, Jake McClements, Pankaj Singla, Amy Dann, Mark V. Sullivan, Nicholas W. Turner, Minji Kim, SLOANE STOUFER, Matthew D. Moore, Inderpreet Kaur, Marloes Peeters, University of Massachusetts Amherst, Amherst, MA, USA
- T6-04** Whole-Genome Sequence Analysis of *Listeria monocytogenes* CC7 Associated with Clinical Infections and Persistence in the Food Industry  
14.15 TROND MØRETRØ, Eva Wagner, Even Heir, Solveig Langsrud, Annette Fagerlund, Nofima, Ås, Norway
- T6-05** Maturing Food Safety Culture with Nudging in Food Manufacturing Environments in the UK  
14.30 CAROL WALLACE, Lone Jespersen, Sophie Tongyu Wu, University of Central Lancashire, Preston, United Kingdom
- T6-06** Impact of Mlst Genetic Diversity on *Listeria monocytogenes* Growth  
14.45 NATHALIE GNANOU BESSE, Carolina Rosa Rodrigues de Souza, Patricia NG, Laurent Guillier, Benjamin Félix, Alexandre Leclercq, Helene Bergis, ANSES Laboratory for Food Safety, Maisons Alfort, France
- 15.00 – 15.30 Networking Coffee in the Exhibit Hall

## S10 Testing and Improving HACCP Team Proficiency to Strengthen Food Safety Culture

Suite 2B

**Organizers:** Lone Jespersen, Shingai Nyarugwe  
**Convenor:** Veronika Bulochova

- 15.30 Using HACCP Proficiency Testing to Upskill HACCP Teams and Build the Foundations for Culture Improvement  
CAROL WALLACE, University of Central Lancashire, Preston, Lancashire, United Kingdom
- 16.00 Connecting HACCP and Food Safety Data to Mindset and Cultures, How Data Is Gathered and Utilized to Generate Behavioural Insights and Drive Change  
LONE JESPERSEN, Cultivate Food Safety, Hauterive, Switzerland
- 16.30 Hazard and Risk Awareness: Foundational Food Safety Knowledge, Risk Awareness and Culture  
SHINGAI NYARUGWE, University of Central Lancashire, Preston, United Kingdom

## S11 Raw Milk Safety and Climate Dynamics: Integrating Geographical and Seasonal Variation from Farms of Southern Europe and the Middle East

Suite 3

**Organizers and Convenors:** Jan F. M. Van Impe, Jeanne-Marie Membré, Vasilis Valdramidis

- 15.30 Predictive Modelling of Maltese Raw Milk Production Traits Under Climate Dynamics  
LYDIA KATSINI, KU Leuven, Ghent, East Flanders, Belgium
- 16.00 Dairy Farming in the Middle East: Insights from Raw Milk Microbiology and Quality  
RODNEY FELICIANO, INRAE, Nantes, France
- 16.30 Raw Milk Safety Under the Climatic Influence in North Spain  
STYLIANI ROUFOU, University of Malta, Msida, Malta

## T7 Technical Session 7 – Modeling and Risk Assessment and Viruses and Parasites

Suite 4

**Convenors:** Ákos Józwiak, Aricia Possas

- T7-01** Trends and Early Signals of Emerging Risks Identified in the Food Chain  
15.30 Zsuzsa Farkas, Erika Ország, Szilveszter Csorba, Tekla Engelhardt, Andrea Zentai, ÁKOS JÓZWIAK, University of Veterinary Medicine, Digital Food Institute, Budapest, Hungary
- T7-02** Sym'Previus MAP: A Web Application for the Design of Food Packaging to Improve the Preservation of Food Products  
15.45 Jonathan Thévenot, YVAN LE MARC, Catherine Denis, Janushan Christy, Valérie Michel, Didier Majou, Valérie Stahl, Emilie Gauvry, Emmanuel Jamet, Fanny Tenenhaus, Jean-Christophe Augustin, Narjes Mtimet, Sabin Jeuge, Jeanne-Marie Membré, Anna Jofre, Alizée Guérin, Aline Rault, Stella Planchon, Véronique Huchet, Olivier Couvert, Louis Coroller, ADRIA Food Technology Institute - UMT ACTIA 19.03 ALTER'IX, France, Quimper, France
- T7-03** Implementation and Application of Quality and Predictive Microbiology Models of Strawberries and Tomatoes in Microhibro for a Holistic Approach for Shelf-Life Assessment  
16.00 ARICIA POSSAS, Francisco Jiménez-Jiménez, Laura Rabasco-Vilchez, Cristina Díaz-Martínez, Zeynep Turgay, Matthias Brunner, Fernando Perez-Rodríguez, University of Córdoba, Córdoba, Spain
- T7-04** Comparing the Performance of Two Predictive Models When Fitting Noisy Data  
16.15 MAHA ROCKAYA, Mariem Ellouze, Jozsef Baranyi, University of Debrecen, Debrecen, Hungary
- T7-05** Method for Tick-Borne Encephalitis Virus Detection in Raw Milk Products  
16.30 CATHERINE HENNECHART-COLLETTE, Gaëlle Gonzalez, Lisa Fourniol, Audrey Fraisse, Cécile Beck, Sara Moutailler, Laure Bournez, Nolwenn Dheilly, Sandrine Lacour, Sylvie Lecollinet, Sandra Martin-Latil, Sylvie Perelle, ANSES, Laboratory for Food Safety, University of Paris-Est, Maisons-Alfort, France
- T7-06** Zebrafish Embryo: A Simple and Robust Tool for the Cultivation of Human Noroviruses  
16.45 MALCOLM TAN, National University of Singapore, Singapore

## S12 Pathogens of Concern in Food Processing Environment

Suite 2B

**Organizers: Preetha Biswas, Frederic Martinez**

**Convenor: Preetha Biswas**

- 8.30 Persistent Versus Transient States: Strain Adaptations to the Environment  
FRANCOIS BOURDICHON, Università Cattolica Del Sacro Cuore, Cremona, Italy
- 9.00 Recent Advances in Rapid Detection, Data Interpretation and Trend Analysis of Environmental Pathogens  
PREETHA BISWAS, Neogen Corporation, Lansing, MI, USA
- 9.30 Emerging Environmental Pathogens and Unusual Outbreaks  
SUZANNE JORDAN, Campden BRI, Chipping Campden, United Kingdom

10.00 – 10.30 Networking Coffee

## RT4 The Current and Future Landscape of Scientific Publications in the Food Safety Domain

Suite 3

**Organizer and Convenor: Marcel Zwietering**

KOSTAS KOUTSOUMANIS, Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, Aristotle University of Thessaloniki, Thessaloniki, Greece

PANAGIOTIS SKANDAMIS, Agricultural University of Athens, Athens, Greece

LUCA COCOLIN, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy

JOHN DONAGHY, Nestec Ltd., Vevey, Switzerland

JENNIFER WOOD, Elsevier, London, UK, United Kingdom

10.00 – 10.30 Networking Coffee

## T8 Technical Session 8 – Food Processing Technologies and Viruses and Parasites

Suite 4

**Convenors: Edward Sliwinski, Rosa Heydenreich**

- T8-01** Potential of High Pressure Processing (HPP) to Inactivate Pathogens and Extend Shelf Life of Cold Brew Coffee  
8.30 MARIO GONZÁLEZ-ANGULO, Rodrigo García, Berta Polanco, Iris Valla, Rui Queiros, Dolores Rivero, Isabel Jaime, Carole Tonello, Hiperbaric S.A., Burgos, Spain
- T8-02** Better Together? Effect of Isostatic High Pressure and Nutrients in Bacterial Spore Control  
8.45 ROSA HEYDENREICH, Alessia Delbrück, Viviane Baeriswyl, Alexander Mathys, ETH Zurich, Institute of Food, Nutrition and Health, Sustainable Food Processing Laboratory, Zurich, Switzerland
- T8-03** Inactivation Variability of Food-Associated Microorganisms Following Ultrasound Treatment  
9.00 ESTHER OKAFOR, Foteini Pavli, Joerg Hummerjohann, Vasilis Valdramidis, Department of Food Science and Nutrition, University of Malta, Msida, Malta
- T8-04** Looking for the Most Appropriate Inoculum Pre-Culture Conditions for High-Pressure Processing Validation Studies  
9.15 CRISTINA SERRA-CASTELLÓ, Anna Jofre, Sara Bover-Cid, WUR, Wageningen University & Research, Wageningen, The Netherlands
- T8-05** Microbial Species and Strain Heterogeneity Affect Resistance to High Pressure Processing  
9.30 THEOCHARIA TSAGKAROPOULOU, Kimon-Andreas Karatzas, University of Reading, Reading, United Kingdom
- T8-06** Efficiency of Non-Thermal Plasma Treatment Against Food Pathogens and Spoilage Microorganisms  
9.45 DOMIZIANA BATTAGGIA, Hannah Sperlich, Masja Nierop Groot, Tjakko Abee, Heidi den Besten, Wageningen University, Wageningen, The Netherlands

10.00 – 10.30 Networking Coffee

## S13 Towards the Development of Quantitative Microbiological Risk Assessment for Non-Thermal Technologies

Suite 2B

**Organizers:** Heidy den Besten,  
Vasilis Valdramidis

**Convenor:** Elissavet Gkogka

- 10.30 Risk Assessment Needs Regarding New and Emerging Non-Thermal Technologies – The Perspective of the Regulatory Science  
SARA BOVER-CID, Institute of Agrifood Research and Technology (IRTA), Monells, Catalunya, Spain
- 11.00 Generic Model Development for Non-Thermal Processing Technologies  
GEORGE PAMPOUKIS, Wageningen University, Wageningen, The Netherlands
- 11.30 A Quantitative Exposure Assessment for *Listeria monocytogenes* in HHP-Treated Meat Products – A Case Study  
ARICIA POSSAS, University of Córdoba, Córdoba, Spain

## S14 How Has Wastewater Surveillance Been Useful to Boost Public Health Preparedness and Make Our Food Systems More Resilient? What Does the Future Hold?

Suite 3

**Organizers:** Christophe Cordevant,  
Maria Hoffmann

**Convenors:** Christophe Cordevant,  
Padmini Ramachandran

- 10.30 Wastewater-Based Epidemiology: A Useful Tool in Public Health Preparedness across One Health  
PADMINI RAMACHANDRAN, U.S. Food and Drug Administration – CFSAN, College Park, MD, USA; MARIA HOFFMANN, U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA
- 11:00 Flows of AMR from Environment to People - A Context for Combining Surveillance of Wastewater and Environment Towards Addressing the AMR Crisis  
LISA AVERY, James Hutton Institute, Aberdeen, Scotland, United Kingdom
- 11.30 Inter-comparison Strategy to Evaluate SARS-CoV-2 Methodologies Used for the Detection and Quantification in Wastewater  
ALI ATOUI, Anses Nancy Laboratory for Hydrology, Maisons-Alfort, France

## T9 Technical Session 9 – Epidemiology, Food Chemical Hazards, Communication and Outreach and Retail and Food Service Safety

Suite 4

**Convenor:** Ellen Shumaker

- T9-01** Are Meal Kits Promoting Food Safety? A Transatlantic Comparison Study  
10.30 Alicyn Dickman, NAOMI MELVILLE, Joseph Baldwin, Elizabeth C. Redmond, Sanja Ilic, Ellen Evans, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom
- T9-02** Development of a Food Safety Culture Course for U.S. Regulators  
10.45 ELLEN SHUMAKER, Lone Jespersen, Mary Yavelak, Mitzi Baum, Stephanie Cotter, Clint Stevenson, Lynette Johnston, Ellen Buchanan, Michael Rogers, Benjamin Chapman, Department of Agricultural and Human Sciences, North Carolina State University, Raleigh, NC, USA
- T9-03** “Pearls of Wisdom”: Canada’s Experience Investigating Outbreaks of Norovirus in Oysters from 2017 to 2022  
11.00 LEANN DENICH, Courtney Smith, Heather Bond, Bijay Adhikari, Hannah Caird, Tara Hluchy, Gabrielle Kosmider, Victor Mah, Lorraine McIntyre, Tim Wenman, Public Health Agency of Canada, Guelph, ON, Canada
- T9-04** Do Polysorbate 80 and Sodium Nitrite Affect Differently the Gut Microbiota of Healthy Individuals and IBD Patients?  
11.15 IRMA ELIZABETH GONZA QUITO, Elizabeth Goya-Jorge, Caroline Douny, Marie Louise Scippo, Edouard Louis, Véronique Delcenserie, Food Quality Management, Food Science Department, FARAH, University of Liège, Liège, Belgium
- T9-05** Characterization of Proteolytic *Clostridium botulinum* Isolated from Residuals Food Involved in Hospitalized Cases with Botulism Diagnosis  
11.30 ELENA COSCIANI-CUNICO, Elena Dalzini, Paola Monastero, Sara Arnaboldi, Daniela Merigo, Stefania Ducoli, Alessandro Norton, Marina-Nadia Losio, Istituto Zooprofilattico Sperimentale Della Lombardia e dell’Emilia Romagna “B. Ubertini”, Brescia, Italy
- T9-06** Management Attitudes and Perceptions Towards Factors That Influence Food Safety Culture in UK-Based Small and Medium-Sized Food-Service Establishments  
11.45 OMOTAYO IRAWO, Arthur Tatham, Elizabeth C. Redmond, Cardiff Metropolitan University, Cardiff, United Kingdom

## Closing Session

Suite 2B

**Chairs: Luca Cocolin, Mariem Ellouze**

- 12.15 Food Safety and Sustainable Food Systems  
WAYNE ANDERSON, Food Safety Authority  
of Ireland, Dublin, Ireland
- 12.45 Managing Microbiological Risks in Agricultural  
Waters  
MICHELLE DANYLUK, University of Florida  
CREC, Lake Alfred, FL, USA
- 1.15 Awards Presentation and Concluding Remarks  
MICHELLE DANYLUK, University of Florida  
CREC, Lake Alfred, FL, USA

## 13.30 – 14.30 Farewell Refreshments

■ – Symposia

■ – Roundtables

■ – Technicals

\*Student Award Competitor



IAFP'S EUROPEAN  
SYMPOSIUM ON FOOD SAFETY

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**ABERDEEN**

**SCOTLAND**

**3–5 MAY 2023**

**SYMPOSIUM  
ABSTRACTS**

## SYMPOSIUM ABSTRACTS

### S1 **Improving Food Traceability through Global Partnerships, Harmonized Data Elements, and Accessible, Interoperable Software Tools**

**Marion Gottschald**, German Federal Institute for Risk Assessment, Berlin, Germany

**Adam Friedlander**, FDA, College Park, MD, USA

**Eric Stevens**, FDA, College Park, MD, USA

The German Federal Institute for Risk Assessment (BfR) and the U.S. Food and Drug Administration (FDA) are collaborating on a project to build technology-enabled systems that improves digital food traceability. Through the development of accessible, interoperable software systems and by capturing harmonized traceability data elements within the supply chain, government agencies around the world can better identify and remove potentially contaminated foods from the market and bolster consumer transparency and trust.

In 2016, BfR built the open-sourced, web-based software platform FoodChain-Lab Web (FCL Web) in an EFSA-funded project to collect, visualize and analyze large amounts of food traceability data. This tool creates reports and simulations for outbreak investigators to hypothesize and validate where potentially contaminated product(s) were distributed within the supply chain. Recently, FDA launched the New Era of Smarter Food Safety to help create a more digital, efficient and safe food supply, and is currently developing an open-sourced, web-based software platform (Product Tracing System (PTS) Prototype) to modernize outbreak response and promote data interoperability. The data ingestion and interoperability feature of the PTS Prototype aims to promote data harmonization by converting any data format or file into an event-driven industry standard. These data are then leveraged within FCL Web for further analysis and visualization.

As food supply chains become more globalized and complex, the need for smarter tools and approaches to enhance rapid data exchange and outbreak response is critically important to create a safer and more digital food system. In this symposium FDA, BfR, and Industry will highlight how global partnerships, harmonized data elements, and building accessible interoperable software systems can spark technological innovation while reducing the burdens of foodborne disease and increasing trust within the food system for all consumers. Additionally, an interactive demonstration FCL and PTS and Industry perspective will be highlighted.

### S2 ***Clostridium botulinum* – Opportunities and Challenges for New Testing Methods to Maintain Food Safety in Ready-to-Eat Foods**

**Kristin Schill**, Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA

**Paw Dalgaard**, Research Group for Food Microbiology and Hygiene, National Food Institute (DTU Food), Technical University of Denmark, Kgs. Lyngby, Denmark

**Chris Michiels**, Laboratory of Food Microbiology, Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium

Consumer demand for ready-to-eat (RTE) foods with long shelf life has grown rapidly worldwide. The requirement for more convenient fresher foods with reduced thermal processing, lower salt and sugar, removal of chemical preservatives, has significant implications for the safety of these types of products. Furthermore, these products are packaged in modified atmosphere packaging (MAP) or under vacuum, increasing the likelihood of growth of anaerobic pathogens such as the spore former *Clostridium botulinum*.

*C. botulinum* is the causative agent of botulism, a rare but severe disease resulting from consumption of food containing the preformed botulinum neurotoxin (BoNT). *C. botulinum* groups I and II are primarily associated with human food-borne botulism. Control of group II *C. botulinum* spore germination and BoNT production in chilled (3-8°C) RTE foods is limited to <10 days unless specific parameters are met.

Where a combination of factors is required to inhibit group II *C. botulinum* growth and toxin production, validation is necessary to ensure product safety. Predictive models are useful tools to help design safe food products with a number of models currently available. However, these models are indicative and therefore may under- or overestimate the safety risk. Consequently, expensive challenge tests are required to validate the safety of refrigerated RTE foods regarding group II *C. botulinum*.

The aim of this symposium is to understand the food safety risks associated with *C. botulinum* contamination in chilled RTE foods along with the 'state-of-art' in predictive microbiology modelling and challenge testing. We will discuss the future developments required for predictive models to be more robust and alternative approaches to reduce the costs associated with challenge testing models.

Together these talks will provide information about the risks of *C. botulinum* in RTE foods, and how predictive modelling combined with cost effective challenge studies can promote further innovation in the RTE market.

### S3 **Latest Developments in International Organisations Making Food Safety Improvements and Successes Measurable**

**Caroline Smith DeWaal**, Global Alliance for Improved Nutrition (GAIN), Washington, D.C., USA

**Markus Lipp**, Food and Agriculture Organization (FAO), Rome, Italy

**Peter Sousa Hoejskov**, World Health Organization, Geneva, Switzerland

Food safety and quality are increasingly at the hearts and minds of society and regulators around the globe. There are many organisations and initiatives that drive further improvements to food safety in the complex context of food security and sustainability.

Such initiatives typically aim at infrastructural improvements as well as capability and capacity building of nations and key stakeholders such as governments, consumers, industry and academia. Many models and strategies have been developed and implemented, but an overarching challenge that remains is how one can confidently measure real progress and improvement in food safety and quality or nutrition for consumers.

Several Governmental, Intergovernmental and Non-governmental organizations have recently been establishing frameworks and metrics such as food safety indicators for quantitatively or qualitatively assessing the outcomes of their interventions in term of food safety improvements.

Such frameworks and metrics are typically designed and selected with respect to the scope and responsibility of individual international organisations, but there may be common principles and indices that are important to identify and that may help harmonize approaches to measuring food safety advancements at a more international or multi-sectorial level.

This short symposium will provide the audience examples of the frameworks and food safety indicators developed or being developed by three different international organizations and an opportunity to discuss ambitions and learnings with the presenters.

The session is designed for food safety professionals from academia, industry, trade associations, consumer organizations and government interested in learning about the approaches and metrics that are being used or considered to monitor the outcome of food safety strategies and interventions.

## S4 **The New Codex Alimentarius Framework for Safe Water Reuse in Food Production and Processing Developed for Dairy and Fishery Products and Put to the Test in Practice for Fruit and Vegetable Food Products**

**Claus Heggum**, Danish Agriculture & Food Council F.M.B.A., Aarhus N, Denmark

**Kang Zhou**, Food and Agriculture Organization of the United Nations, Rome, Italy

**Ana Allende**, CEBAS-CSIC, Murcia, Spain

Water is an increasingly precious commodity in many geographies. Codex Alimentarius is for the first-time developing guidance on the safe reuse of water for food production and processing that can help address issues of water scarcity where relevant. Guidance has been elaborated for fresh fruits and vegetables, fishery and dairy products. Competent authorities around the world may adopt the new guidance but will be scrutinizing the feasibility and practicality of the new guidance for their sectors.

In the past several years, the Food and Agricultural Organization (FAO) and World Health Organization (WHO) have established a new framework for science- and risk-based decision-making on fit-for-purpose water use and reuse in primary production and food operations. This framework has been developed through the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA). The principles of the framework and elaboration for fruits and vegetable products has featured at past IAFP EU meetings. Now the guidance for fishery and to dairy products has been elaborated. Moreover, the feasibility and practicality of the guidelines for fruits and vegetables are scrutinized by experts and stakeholders, amongst others concerning the management of safe water use and reuse in situations where human, expertise and financial resources are scarce.

This short symposium will bring together key JEMRA experts involved in the elaboration of guidance concerning use of the Codex framework for various sectors, allowing for direct discussion on the feasibility and practicality of the science and principles underlying the advice of FAO/WHO to Codex Alimentarius.

## S5 **What Should the Food Industry and Risk Communicators Know about Peoples' Kitchen Practices and Beliefs?**

**Solveig Langsrud**, Nofima, Ås, Norway

**Paula Teixeira**, Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

**Ellen Evans**, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

The main responsibility for providing safe food lies on food producers, processors, and retailers, but as the last line of defense against foodborne illness, households are a part of the equation. About 40% of foodborne illnesses are acquired from homemade food and both observational studies and self-reporting shows that common practices among consumers contribute to higher risk.

In contrast to earlier actors in the food system, household food practices cannot be controlled by regulation and people sometimes lack basic requirements for safe food preparation, for example, awareness about food risk and how to reduce it, access to safe food and tools and skills necessary to control and measure the food preparation process. To help consumers mitigate risk, it is crucial to know common food preparation practices, the consumers' knowledge and skills as well as an understanding of barriers for changing towards more safe behaviour. With this insight, risk communicators can develop food safety advice that has a real uptake in the population. Similarly, the insight can enable producers of food and kitchen appliances to improve their products to reduce the risk of foodborne infection.

This session will provide information about what is the most important consumers' food choices, preparation, and storage practices that may affect risk. Furthermore, it will give insight in the factors that can explain risky practices, such as common myths, and physical, emotional, social, spiritual, financial, and environmental factors.

## S6 **Paving the Avenue for the Application of Natural Antimicrobials**

**Alberto Bombelli**, Wageningen University and Research, Wageningen, The Netherlands

**Olivia McAuliffe**, Teagasc Food Research Centre, Fermoy, Cork, Ireland

**Jan Willem Sanders**, Unilever Foods Innovation Centre, Wageningen, The Netherlands

The trends toward mild processing of foods and the consumer preference for natural food ingredients advocate the search for natural antimicrobials that control the growth of foodborne pathogens and/or spoilage agents. Plants synthesize *de novo* a plethora of structurally diverse compounds, and this intrinsic defense capacity of the plant that harbors potential novel antimicrobials opens avenues to discover new antimicrobials for food safety. Moreover, control of the growth of foodborne pathogens may be achieved with biocontrol by using bacteriophages. This symposium will discuss the challenges and opportunities of natural-derived antimicrobials and biocontrol strategies to ensure food safety and extend shelf life. The presenters will focus on the characterisation of natural antimicrobials and examples of their use for food preservation. Moreover, research advances to unravel the mode of action of these compounds to inhibit

microorganisms will be highlighted. The industry plays an important role in the successful application of novel antimicrobials, and the symposium will also discuss the industrial perspective on the selection and evaluation of promising antimicrobial candidate compounds for food protection.

## S7 Microbiological Contaminants in Plant Protein Ingredients – Assessing Potential Risks

**Marjon Wells-Bennik**, NIZO Food Research, Ede, The Netherlands

**Mariem Ellouze**, Nestlé, Lausanne, Switzerland

**Yvan Le Marc**, ADRIA Food Technology Institute - UMT ACTIA 19.03 ALTERIX, Quimper, France

A wide variety of innovative foods made from alternative protein sources are now available on the market to replace animal proteins (meat, dairy and egg protein), and this trend is expected to continue. Alternative proteins may be derived from plants, including legumes, cereals, nuts, stone fruits, or seeds, or can be produced using yeast, fungi, or insects. Clearly, the composition of food products made with these ingredients is significantly different from the traditional animal products. Often, there is little information available on the loads and types of microbes that are present in such ingredients – however, this is needed to assess microbiological risks associated with ingredients used to produce new types of foods.

This symposium will focus on microbiological risks associated with plant protein-based foods. While many plant protein sources are not new (e.g., soy, oat, pea), their use and application in new food products may bring new challenges. A survey carried out on plant-based ingredients from different sources and in different forms (e.g., isolates, concentrates, kernels, flours) showed that bacterial spore formers are often predominant. Commonly encountered species include *Bacillus subtilis* and *Bacillus licheniformis*, and the pathogen *Bacillus cereus* is isolated regularly as well. Insight into the behaviour of these organisms is needed for the design of effective processing conditions and stable product formulations of plant-based foods, and to support trouble shooting activities in case microbial contaminants are encountered in finished products.

Microbial challenges and approaches taken to ensure the safety and quality of innovative plant-based products throughout the chain will be discussed. First, an overview of microbes in plant protein-based ingredients will be given. Subsequently, findings on inactivation and growth of *B. licheniformis* in plant-based food products and risk assessments of *B. cereus* will be presented taking diversity in phenotypic characteristics into consideration.

## S8 *Bacillus cereus* and Related Organisms, Differentiating Friend from Foe

**Laura Carroll**, Umeå University, Umeå, Sweden

**Mariem Ellouze**, Nestlé, Lausanne, Switzerland

**Maria Teresa Da Silva Felicio**, European Food Safety Authority (EFSA), Parma, Italy

The *Bacillus cereus* group, also known as *B. cereus sensu lato*, is a complex of closely related species, which are widespread throughout the environment and frequently isolated from a wide variety of foodstuffs. Numerous illnesses have been attributed to *B. cereus* group members, including foodborne gastrointestinal diseases (emesis and diarrhea), severe non-gastrointestinal infections, and anthrax/anthrax-like illnesses. Simultaneously, some *B. cereus* group members play important roles in industrial and agricultural settings, including as biocontrol agents and food spoilage organisms.

In order to minimize both human illness cases and economic losses, it is critically important that food producers differentiate high-risk *B. cereus* group strains from their lower-risk counterparts. However, evaluating risks posed by the presence of some *B. cereus* group members in foods remains extremely

challenging, particularly when *B. cereus* group members are present in novel or under-studied food matrices. The proposed symposium will discuss the current status of the *B. cereus* group and the public health risks its members pose in food. Specifically, the symposium will cover, (i) state-of-the-art genomic approaches, which are being used for *B. cereus* group surveillance, source tracking, outbreak detection, and risk assessment efforts, but require care in their interpretation; (ii) modeling approaches for predicting growth and inactivation of *B. cereus* group members in under-studied food matrices, with an emphasis on increasingly popular plant-based dairy alternative products; (iii) public health risks related to the presence of *B. cereus* group members in food assessed by the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA). Overall, the symposium proposed here will provide academic, industry, and EFSA perspectives and offer the most up-to-date portrayal of the *B. cereus* group, including current challenges and data gaps relevant for microbiological risk assessment.

## S9 Food Safety of Infant Foods, Care for Our Most Precious

**Kah Yen Claire Yeak**, Wageningen University, Wageningen, Gelderland, The Netherlands

**Sara Bover-Cid**, IRTA (Institute of Agrifood Research and Technology), Food Safety and Functionality Program, Monells, Girona, Spain

**Kalliopi Rantsiou**, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy

Infants are more vulnerable to foodborne diseases. To ensure food safety, in general but even more so for infant foods, it is relevant to identify and rank hazards, to control the risk by properly validated interventions and to test for the hazards in the food and food processing environment for verification. In this symposium, all three aspects will be addressed by presenting the work carried out in the framework of the SAFFI project (Safe Food for Infants in the EU and China). An approach using a variety of databases on hazards, foods, outbreaks, and epidemiological data is used to develop a decision support system for hazard identification and risk assessment. The impact of emerging processing and preservation technologies on the behaviour of prioritized pathogens in baby food is assessed. A decision support system prototype will be presented for setting the conditions of non-thermal processes to control hazards in fruit purees as case-study. For verification of control, studies on traditional and molecular techniques relating ingredients, environmental and end products are analysed and correlated. The work performed supplies crucial information for use in setting efficient monitoring and sampling strategies at operational (infant food companies) and governmental (food safety agencies) level, designing or evaluating HACCP programs, performing quantitative risk assessments, and for example auditing activities and are thus relevant for food industry, governments and academia.

## S10 Testing and Improving HACCP Team Proficiency to Strengthen Food Safety Culture

**Carol Wallace**, University of Central Lancashire, Preston, Lancashire, United Kingdom

**Lone Jespersen**, Cultivate Food Safety, Hauterive, Switzerland

**Shingai Nyarugwe**, University of Central Lancashire, Preston, United Kingdom

For years, food companies have invested in collecting food safety data to demonstrate how compliant HACCP programs are and more recently to measure where their food safety cultures are at on a culture maturity continuum. These measures are often based on data collected through surveys and number of non-conformances. Decisions are then made



for how to be compliant in the next inspection or audit but, the data are often forgotten, hidden away in filing systems, providing no further value to the company. This information wastage goes against the philosophy of HACCP as a preventative continuous improvement approach to food safety, reducing HACCP to a paperwork exercise. HACCP data and review activities provide learning opportunities for companies to modernise risk management and overcome the complacency often associated with ageing HACCP plans. This requires understanding of HACCP Team dynamics and HACCP proficiency to maximise HACCP program upgrades as a basis for strengthening food safety culture.

Participants will hear about factors influencing HACCP effectiveness, including the influence of human factors. Using a case-study of a North American pet food company, data were collected at three manufacturing locations for food safety culture and HACCP proficiency. Results were tested using ANOVA and found to be significantly different before and after intervention for all three plants. The proficiency levels improved on average 49% post-intervention and the significant factors causing this improvement were identified through factor analysis. Speakers will share results from this case study and other global studies of HACCP teams, HACCP team interventions, and actions required from a practitioner perspective to make lasting and significant changes. This will include correlation of HACCP team and individual team member proficiency to food safety culture and discussion of practice methods and tools leaders have used to make decisions and change behaviours and improve food safety management and culture.

## **S11 Raw Milk Safety and Climate Dynamics, Integrating Geographical and Seasonal Variation from Farms of Southern Europe and the Middle East**

**Lydia Katsini**, KU Leuven, Ghent, East Flanders, Belgium

**Rodney Feliciano**, INRAE, Nantes, France

**Styliani Roufou**, University of Malta, Msida, Malta

The issue of climate change has become a significant concern worldwide with multiple affected scientific areas, including food safety. The first step in planning adaptation and mitigation strategies is to evaluate the food safety risks due to climate change. Assessing the potential of these risks requires a deep understanding of the effect of climate on food safety. However, performing controlled experiments on such complex systems as the climate is impractical. To this end, large-scale, mainly observational, data sets can be utilised.

This symposium aims to highlight the effect of climate factors on the quality and microbial food safety aspects of raw milk by identifying seasonal patterns and correlations and building predictive models. Raw milk data at the farm level for several years, along with weather observation data, are assessed with multivariate analysis. Three case studies with data sets corresponding to European and Western Asia countries, i.e., Malta, North Spain, and Saudi Arabia, will be presented. These regions are characterised by different climates as well as various farm settings. Moreover, the hot weather conditions in some of these countries may provide a projection of the possible impact of climate change on microbial safety and milk quality. The challenges and opportunities for the dairy sector under climate change conditions will be presented. Finally, this symposium will discuss the application and scalability potential of analysing observational raw milk data in the context of food safety.

This work is part of the PROTECT project, funded under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 813329.

## **S12 Pathogens of Concern in Food Processing Environment**

**Francois Bourdichon**, Università Cattolica Del Sacro Cuore, Cremona, Italy

**Preetha Biswas**, Neogen Corporation, Lansing, MI, USA

**Suzanne Jordan**, Campden BRI, Chipping Campden, United Kingdom

The food industry usually controls the potential cross-contamination of the finished product through an environmental monitoring program (EMP), a critical component for verifying that pathogens or spoilage organisms are under check and preventive measures are in place for safe food production. However, even with well-established EMP standards for controlling microbial contamination, the production and distribution chains' complexities make the microbial contamination more challenging to control. Previous risk analyses have focused on persistent pathogens traditionally associated with food processing environments, recalls and food related outbreaks. More recently new pathogen-food combination outbreaks are on the rise. There is growing consensus on the importance of effective EMP as an essential part of food safety and quality systems, additionally supported through the regulatory approaches that are in place for food safety compliance. In-depth understanding of overall survival and adaptation of these pathogens to diverse types of environmental conditions will help apply rapid detection and mitigation strategies. Complimenting EMP with data tracking systems allows for application of modern practices to prevent future outbreaks.

The expert speakers in this symposium will provide updates and review key aspects of design and implementation of environmental monitoring program and analytical methods with examples. Moreover, cover aspects about unexpected/emerging environmental pathogens of concern in food processing environments. How routine effective environmental monitoring practices need to be implemented for checks at critical points along the production chain. Discussions will also include recent advances in rapid detection of environmental pathogens, as well as data interpretation, source tracking, and strategic use of environmental monitoring for food safety assurance and regulatory compliance.

## **S13 Towards the Development of Quantitative Microbiological Risk Assessment for Non-Thermal Technologies**

**Sara Bover Cid**, Institute for Agrifood Research and Technology (IRTA), Monells, Spain

**George Pampoukis**, Wageningen University, Wageningen, The Netherlands

**Aricia Possas**, University of Córdoba, Córdoba, Spain

Over the last few decades, major efforts have been made to develop innovative food technologies that deliver safe, nutritious foods with high organoleptic qualities that meet consumer demands. Sustainable (environmentally friendly) non-thermal technologies such as High Hydrostatic Pressure and Non-Thermal Plasma offer consumer benefits like improved microbiological safety, clean-label food products with a better freshness appearance, and an extended shelf life. Nevertheless, for some technologies the application is currently limited or not yet available in the food industry and quantitative microbiological risk assessments (QMRA) assessing food safety issues are lacking. This symposium will discuss the assessment questions and the steps to be taken to develop QMRAs for non-thermal technologies.



Among the examples we will mention a recent scientific opinion of the EFSA BIOHAZ panel that focused on the assessment of the efficacy and safety of non-thermal high-pressure processing of food, identifying the minimum HPP requirements to reduce the relevant hazards in raw milk and other ready-to-eat foods. Also we will address the challenges in model development for upcoming technologies, and we will illustrate the potential of sustainable technologies to control food safety in a case study and discuss their viability and efficacy and highlighting their impacts on food quality.

**S14 How Wastewater Surveillance Been Useful to Boost Public Health Preparedness and Make Our Food Systems More Resilient? What Does the Future Hold?**

**Elisabetta Suffredini**, Istituto Superiore di Sanità, Rome, Italy

**Lisa Avery**, James Hutton Institute, Aberdeen, Scotland, United Kingdom

**Ali Atoui**, Anses Nancy Laboratory for Hydrology, Maisons-Alfort, France, France

Wastewater based epidemiology (WBE) is a non-invasive surveillance method and has become a routine public health monitoring tool for detecting and tracking several emerging pathogens and its variants at a community level. Theoretically, any infectious agent that passes through or infects the

gastrointestinal tract of humans or animals could be detected from wastewater. As we have seen from SARS-CoV-2 and now Monkeypox or Poliovirus, wastewater-based surveillance can provide a broader picture of disease burden at low cost and as well as pathogen subtyping for a discrete population in a non-invasive, culture-independent manner. Hospital and community wastewaters are potential reservoirs of antimicrobial resistance and the data collected through these studies will complement surveys of rivers and streams to give a more complete picture of AMR in freshwater ecosystems. Additionally, by focusing the surveillance on watersheds that serve food-production areas or food-production workers, this system can serve as part of a warning system for food security and vulnerabilities.

WBE in combination with sequencing approaches has the potential for making culture-independent pathogen and AMR surveillance a reality. To achieve this, methods are being developed and baseline data is being gathered, reviewed, and disseminated.

In this symposium, we propose to explore how WBE will help us to build better public health assessment tools and consequently, make our food systems more resilient. Speakers will cover the whole breadth of these issues; from rapidly and unambiguously identifying emerging pathogens to understanding the ecology of community through wastewater surveillance. The Symposium will also examine and discuss the unbiased community based One Health approach to addressing microbial resistance and discuss some of the public facing dashboard that supports data-driven public health efforts in near real time.

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**ROUNDTABLE  
ABSTRACTS**

# ROUNDTABLE ABSTRACTS

## RT1 When Opposites and Peers Come Together in a Roundtable, Zero-Tolerance Versus Microbial Risk Assessment within a Poultry Case Study

**Gary Acuff**, Acuff Consulting LLC, College Station, TX, USA

**Alvin Lee**, Institute for Food Safety and Health, Bedford Park, IL, USA

**Panagiotis Skandamis**, Agricultural University of Athens, Athens, Greece

**Anett Winkler**, Cargill, Inc., Unterschleißheim, Germany

**Marcel Zwietering**, Wageningen University, Wageningen, The Netherlands

There is no such thing as zero risk when it comes to food safety!

Sampling is one of the bottle necks to overcome. Due to its limitation, pathogens can get into the food chain without being detected.

Microbial Risk Assessment evaluates risks and can identify control strategies that reduce risks to an acceptable level, while zero-tolerance approaches set the lowest limit for specific pathogens. Authorities balance between the two concepts. In Rhode Island, a roundtable was paving the way of this debate. Where are we 10 years later?

The topic could be illustrated with the U.S. and EU policies to manage *Listeria* in ready-to-eat foods. But let's surf on hot news from 2022 in the poultry segment. USDA is conducting risk assessment for *Salmonella* in poultry and has announced action to declare *Salmonella* an adulterant in breaded stuffed raw chicken products, with a proposed limit of 1 CFU/g. Additionally, annual targets will be used to track progress toward reducing a TOP-3 serotypes. However, the focus is currently restricted to *S. Enteritidis* in the U.S. primary production.

The food business operators must comply with criteria defined in the EU Regulation EC 2073/2005. Absence of *Salmonella* is expected in the tested portions for poultry meat products intended to be eaten cooked. Regulation EC 2160/2003 and related amendments aim at controlling *Salmonella* prevalence together with a TOP-2 serovars that are relevant for public health. But an increased human salmonellosis triggered EFSA investigation, and the recommendations published recently are to track a TOP-5-serovars.

U.S. and EU are not fully harmonized ... yet!

Key opinion leaders will animate this roundtable. The debate will be data-driven with key figures and supported with a live-poll with the audience. Sparks can fly when opposites come together; however, it is very well known that opposites do attract.

## RT2 Responding to Food Safety Crises, Evolving Role of Food Scientists

**Francois Bourdichon**, Università Cattolica Del Sacro Cuore, Cremona, Italy

**Michelle Patel**, UK Food Standards Agency, London, United Kingdom

**Donald Prater**, U.S. Food and Drug Administration, Silver Spring, MD, USA

**Helen Taylor**, Zero2five Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom

**Purnendu Vasavada**, University of Wisconsin-River Falls, River Falls, WI, USA

Food safety professionals joke that careers are remembered according to the crises lived through. The experience of many such crises teaches us the value of the scientific quality of available information together with public trust in the information. Credible, scientific information about food safety, food science and nutrition is available through scientific publications, subject matter experts in food science, nutrition and public health and consumer advocates. Unfortunately, in recent years, consumers and society leaders are also faced with the spread of myths, misinformation and conspiracies dealing with food safety, food and nutrition, especially through social media. Misinformation can spread quickly online leading to confusion, damage to public health and distrust in science, public agencies and businesses. The term "infodemic" is appropriate. False or distorted information is more than of academic interest, it can lead to a small food safety issue becoming a major media crisis (e.g., fipronil) or common food safety concerns not receiving enough attention (e.g., *Salmonella*, norovirus, *Cronobacter*).

Responding to food safety crisis involves elements of risk communication and risk management. Food scientists and food safety professionals can play an important role in communicating science-based information and help stop the spread of myths and misinformation. That, the spread of misinformation can be highly destructive and a leading cause of confusion, and distrust in science was illustrated recently by the worldwide "Infodemic" regarding SARS CoV-2 and COVID-19 and food. This interactive roundtable featuring food scientists from academia and industry will highlight the lessons learned from dealing with misinformation and popular myths in food science and food safety. Using examples drawn from risk communication, crisis management and prevention in the academic, public and private sector, the panel will discuss the industry and regulatory developments designed to assure food safety and the evolving role of the food scientists.

## RT3 Creating Capacity of the Next-Generation Food Safety Researchers and Implementers through International Collaboration, Experience of Low- and Middle-Income Countries (LMICs)

**Meseret Bekele**, Uppsala University, Uppsala, Sweden

**Delia Grace**, Natural Resource Institute, University of Greenwich, Kent, United Kingdom

**Florence Mutua**, International Livestock Research Institute, Nairobi, Kenya

**Wigdan Omer**, University of Khartoum, Khartoum, Sudan

**Himadri Pal**, Natural Resources Institute, Chatham, United Kingdom

**Shwe Phue San**, University of Greenwich, Yangon, Yangon, Myanmar

**Stacey Duvenage**, Natural Resource Institute, University of Greenwich, Kent, United Kingdom

Foodborne diseases cause 600 million illnesses and 420,000 deaths annually, with most of the burden falling on people in low- and middle-income countries (LMICs), especially children and the immune compromised. Rapidly changing global food systems bring other health challenges including spillover of novel diseases from animals to people. To address the complexity of food systems and associated challenges, LMICs need food safety professionals who can apply risk assessment, management, and communication to the mass informal and traditional domestic markets where most of the population buy and sell food. However, historically food safety education and research has focused on formal markets and export, drawing largely on the approaches that have been relatively successful in high income countries (HICs) but have so far failed to be applied widely or achieve food safety impact in informal and traditional markets of LMICs.

This roundtable discussion will bring two levels of food safety professionals from LMICs, senior researchers and educators with decades of experience in food safety and early-career professionals which we can consider as 'the next-generation food safety researchers and implementers. Participants are drawn from Ethiopia, Kenya, Sudan, India, Myanmar and Ireland. The issues to be discussed include, what core competencies are needed for food safety professionals in LMICs? What are the most pressing evidence gaps and what methods can be used to fill them? What successful and unsuccessful experiences of training and educating food safety professionals especially in the context of LMICs? How do young food safety professionals envisage the future of food safety in LMICs? How are food safety researchers incorporate and integrate the technical and socio-cultural aspects of food production and consumption in their research activities towards generating evidence for actions? The discussion will be interactive and engage the audience identifying training needs and opportunities.

#### **RT4 The Current and Future Landscape of Scientific Publications in the Food Safety Domain**

**Luca Cocolin**, Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (To), Italy

**John Donaghy**, Nestec Ltd., Vevey, Switzerland, Switzerland

**Kostas Koutsoumanis**, Aristotle University of Thessaloniki, Thessaloniki, Greece, Greece

**Panagiotis Skandamis**, Agricultural University of Athens, Athens, Greece, Greece

**Jennifer Wood**, Elsevier, London, United Kingdom

One of the foundations of science is the reporting of scientific papers, since this is a crucial communication channel, but also the foundation for further science. Just like for food, quality control of publication is crucial. In the last decade we see large changes. One positive is that food journals get higher and higher impact factors, meaning that the domain gets more attention. But of course impact factor is not a generic quality indicator. We also see very negative aspects like more

and more predatory journals, or semi-predatory. Results in these journals are not *per se* incorrect, but they do not always receive a decent quality check. This does not imply that reputed journals must be fully trusted, but information in predatory journals should a priori not be trusted by academics either, nor by the industry, the governments and the journalists. To note that also with reputed publishers there are trends for more and more reviews, opinion papers and new journals. Furthermore, there seems to be more pressure towards quantity than quality. For example, the number of special issues is largely increasing. Nothing wrong with good and timely reviews, as well as good and timely special issues, but are we not floating really to too many? Scientific recognition and reputation should not only be measured on number of publications and citations. In this symposium we will share the vision of a publisher and of two editors of food safety journals.

#### **RT5 Food Safety and Microbial Dark Matter – Decrypting the Genomes of Microbiomes**

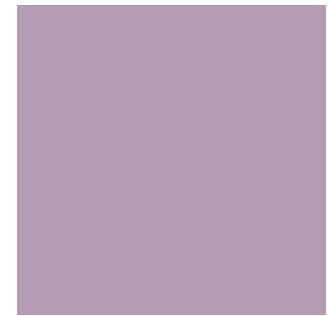
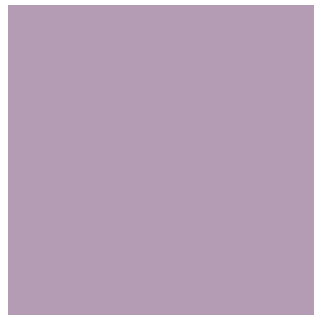
**Francesco Chiesa**, Department of Veterinary Sciences, University of Turin, Grugliasco (To), Torino, Italy

**Maria Diaz**, Quadram Institute, Norwich, United Kingdom, United Kingdom

**Ilario Ferrocino**, University of Turin, Grugliasco (To), Italy

**Alison Mather**, Quadram Institute, Norwich, United Kingdom

Microbial Dark Matter has become a popular term to describe the vast uncultured majority of microorganisms that populate most of the environments, foods included, and that are currently only known through the use of culture-independent techniques such as sequencing and metagenomics-approaches. Even though molecular methods may reveal the presence of a pathogen in a sample through the detection of specific virulence gene(s) associated with pathogenic microorganisms, often, the culture isolation of the strain is not possible. This significant discrepancy may be related to low concentration of the cells, presence of dead cells, presence of competitive microflora, injured cells and cells in a viable but non-culturable state, free DNA but also due to the presence of free bacteriophages which can carry the searched virulence factor, causing the PCR-positive/culture-negative phenomena. Studying the food microbiota allows the detection of non-culturable microorganisms and nowadays, intensive computational efforts have allowed to reconstruct their genomes and study backwards the metabolomics promoting the knowledge required for their consequent cultivation. The novel technologies have given the possibility to study more in-depth the microbial-spoilage dark matter of food and the enormous potential of discovering novel foodborne agents. The roundtable will provide a forum for discussion of the impact of metagenome assembled genome, the contributions technologies are making toward more readily achieving complete microbial genome sequence assemblies and how they should be incorporated into public databases. This roundtable will discuss the current state of the research in this field, bringing together researchers interested in exploring the vast uncultured majority of microorganisms in the hope of stimulating a lively culture-dependent vs. culture-independent discussion.



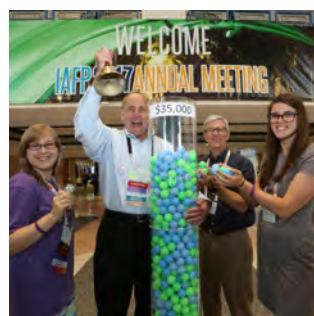
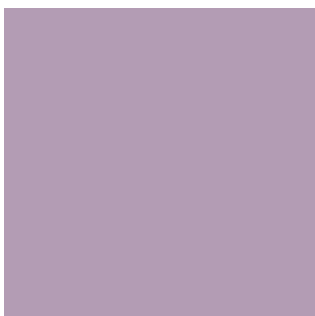
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**TECHNICAL  
ABSTRACTS**

# TECHNICAL ABSTRACTS

\*Student Award Competitor

## T1-01 Nanosystems to Successfully Delivery Aptamers: A Disruptive Strategy to Prevent Salmonellosis in the Poultry Industry

Carina F. Almeida<sup>1</sup>, Márcia Faria<sup>2</sup>, Josué Carvalho<sup>2</sup> and Eva Pinho<sup>3</sup>

<sup>1</sup>INIAV – National Institute for Agrarian and Veterinarian Research, Vairão, Portugal, <sup>2</sup>ALS Life Sciences Portugal, Tondela, Portugal, <sup>3</sup>AliCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, Porto, Portugal

**Introduction:** Eggs and egg-derived products are the main cause of human salmonellosis which is the second most reported foodborne disease, leading to economic losses of over €3 billion/year. Besides human and animal health risks, egg production decreases with the presence of *Salmonella* spp. on hens' reproductive organs. The prophylactic application of antibiotics was one of the strategies to control *Salmonella* spp. infections in poultry, however, the spread of antimicrobial resistance led to the banning of such strategies. Aptamers, single-stranded oligonucleotides, have been applied as antimicrobial agents with promising neutralizing properties to combat pathogenic microorganisms.

**Purpose:** Herein, a nanosystem based on cyclodextrins able to transport aptamers to the hen's intestine was developed, promoting a controlled release in the small intestine, colon and cecum to prevent colonization by *Salmonella* spp.

**Methods:** Chitosan-cyclodextrins nanocapsules were synthesized via the anionic tripolyphosphate-based gelation mechanism. The encapsulation of the aptamers was performed simultaneously with the gelation reaction. The controlled release profile of the system was evaluated under conditions that mimic the chickens' gastrointestinal system (low pH). The size of the nanoparticles was analyzed by DLS, SEM, FTIR and DSC. These techniques were also used to evaluate the integrity of the nanoparticles in buffers that mimic the gastrointestinal environmental conditions of chickens.

**Results:** The composition of the encapsulation system was defined ensuring aptamer protection during the digestive process of chickens and their release in the gut of these animals. The effectiveness of aptamers after encapsulation and release was also confirmed. The nanocapsules remained in the epithelium sufficient amount of time for total release of the aptamers.

**Significance:** The present work is part of the project NAMShield that aims to develop novel, species-specific antimicrobials, that are able to prevent or fight *Salmonella* spp. infections in poultry while keeping the natural microbiome intact and consequently hindering the spread of AMR and decreasing the burden of salmonellosis.

## T1-02 Biofilm-Associated Traits and Tolerance to Didecyldimethylammonium Chloride are Involved in Enhanced Ability of *Listeria monocytogenes* to Persist in Seafood Processing Environments

Benjamin Duqué<sup>1</sup>, Alice Michel<sup>2</sup>, François Gravey<sup>2</sup>, Graziella Midelet<sup>3</sup>, Thomas Brauge<sup>3</sup>, Guylaine Leleu<sup>3</sup>, Sabine Debuiche<sup>3</sup>, Anthony Colas<sup>3</sup>, Christophe Soumet<sup>4</sup>, Béatrice Anger<sup>4</sup>, Emeline Bourgault<sup>4</sup>, Simon Le Hello<sup>2</sup> and Aurelie Hanin<sup>1</sup>

<sup>1</sup>ACTALIA, Food Safety Department, Saint-Lô, France, <sup>2</sup>Normandie University, UNICAEN, UNIROUEN, DYNAMICURE U1311, Caen University Hospital, Caen, France, <sup>3</sup>French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Laboratory for Food Safety, Boulogne-sur-Mer, France, <sup>4</sup>French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Fougères Laboratory, Antibiotics, Biocides, Residues and Resistance Department, Fougères, France

**Introduction:** *Listeria monocytogenes* is a foodborne pathogen that can persist over a period of several years in seafood processing environments despite frequent implementation of cleaning and disinfection protocols. Further understanding of bacterial traits enabling such a long-term survival in industries would allow food operators to optimize their procedures for an increased control of this pathogen.

**Purpose:** This study aimed to identify genetic and phenotypic traits differentiating strains that colonized and persisted in food processing environments and strains that failed in colonizing factories.

**Methods:** Ninety isolates from eight different industries were characterized by Whole Genome Sequencing. A SNP analysis was implemented and persisting strains were identified. Resistome and virulome were characterized using the Bionumerics software. The ability of the 90 isolates to adhere to surfaces and to form biofilms were also measured as well as MICs for 10 biocide molecules. Statistical analyses were finally implemented to associate the ability of strains to persist and their genetic and phenotypic attributes.

**Results:** *L. monocytogenes* strains were distributed in 15 clonal complexes (CC). CC121, CC321 and CC9 were the most represented CC ( $n=34$ , 17 and 10, respectively). CC121 isolates were further subtyped and divided into four cgMLST types, each of which being specific to a production site. SNPs analyses showed that several clones colonized processing environment, one of which having persisted in a factory for at least four and a half years. These persistent strains showed enhanced biofilm abilities and an increased MIC for didecyldimethylammonium chloride compared to presumed sporadic contaminants. Genes involved in virulence, stress response, tolerance or resistance to antimicrobials are differentially distributed between and within CC and can be correlated to phenotypic traits.

**Significance:** WGS-based analyses combined to phenotypic characterization provided new information about the genetic and phenotypic traits affecting the ability of *Listeria monocytogenes* to colonize production environments.

## T1-03\* The Effect of Cysteine on the Motility Behaviour and Biofilm Formation of *Listeria monocytogenes*; Cysteine Intake through *ctaP* Plays a Role

Mahide Muge Yilmaz Topcam<sup>1</sup> and Kimon-Andreas Karatzas<sup>2</sup>

<sup>1</sup>University of Reading, Reading, United Kingdom, <sup>2</sup>Department of Food & Nutritional Sciences, University of Reading, Reading RG6 6AD, United Kingdom

**Introduction:** Various bacteria can swim or swarm in liquid and solid environments while they can form immobile multicellular clumps called biofilms. It is generally assumed that there is a motility-biofilm transition wherein the limited motility of bacteria triggers biofilm formation. Biofilm formation and motility are important factors in the ability of pathogenic bacteria to persist and cause disease. The persistence of *Listeria monocytogenes* in the environment is associated with its biofilm formation ability. It colonizes surfaces under different conditions depending on nutrient availability and surface conditions.

**Purpose:** This study aimed to indicate the effect of the important for virulence of *L. monocytogenes* cysteine (cys), on swimming and swarming motility, and biofilm formation of *L. monocytogenes*. We also investigated the role of the *ctaP* gene, a cys transporter protein, in the above properties.

**Methods:** Defined Medium (DM) (containing 0.82mM cys) and cys supplemented DM with the final concentrations of 1.57mM, 3.17mM, 6.34mM, 9.5mM and 12.69mM were used for both motility and biofilm formation analysis. 10403S WT, 10403S $\Delta$ sigB, 10403S $\Delta$ ctaP, EGD-eWT, EGD-e $\Delta$ sigB, EGD-e $\Delta$ Imo0799, LO28WT were used in this study. Visible light was tested to investigate the effect of light on motility and biofilm formation. 0.3% agar for swimming motility and 0.4% agar for swarming motility was added into DM for motility analysis. Crystal violet colouring methods (under 595nm reading) were conducted for biofilm formation analysis.

**Results:** We showed that increasing levels of cys increased biofilm formation. On the other hand, different concentrations of cysteine affected motility behaviour differently. In the same concentration increase, we found different phenomena than literature. Results for 10403S  $\Delta$ ctaP showed that cysteine plays an important role in both biofilm formation and motility.

**Significance:** We showed that cys has an effect on virulence of *L. monocytogenes*, playing a role in biofilm formation and motility. Different cys concentrations affected swimming and swarming motility in different trends.

## T1-04 Growth Potential of *Clostridium botulinum* and *Clostridium perfringens* in Nitrite-Free Ham Model during Cooling in Thermal Abuse Conditions

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**Introduction:** Proteolytic *Clostridium botulinum* (Cb) and *Clostridium perfringens* (Cp) are spore-forming bacteria, which can contaminate meat products and their growth is usually inhibited by additives such as nitrites.

**Purpose:** The objective of this study was to evaluate the growth of Cb and Cp in nitrite-free cooked ham during the cooling in abuse conditions.

**Methods:** For each pathogen, two batches of 15 kg of minced meat were divided in a) Meat (M), b) with Salt (2.5%) (MS), c) with Salt and Sodium Ascorbate (0.03%) (MSA), d) with Salt and Sodium Nitrite (0.015%) (MSN) and e) with Salt and Sodium Ascorbate (0.03%) and Sodium Nitrite (0.015%) (MSNA). Meat was inoculated with 1% v/w of spore suspension (mix of three strains) to obtain a final concentration of 4 log CFU/g in meat. Samples (100 g of vacuum-packed meat or broth) were cooked (75°C for 20 min) and quickly cooled until 54.4°C, and then they were cooled in a programmable water bath to 10°C in 6 hrs (Cooling A) or in 21 hrs (Cooling B) (linear cooling profiles). The clostridia enumeration was performed according to ISO 15213 (2003). The growth potential ( $\Delta_{max}$ ) was calculated according to ISO 20976-1 (2019).

**Results:** During the cooling A, the  $\Delta_{max}$  of Cb was <0.2 log CFU/g in broth and negative for the other tested groups, while the  $\Delta_{max}$  of Cp was 2.08, 4, 0.82, 0.61, -0.17 and -0.15 log CFU/g in Broth, M, MS, MSA, MSN and MSNA, respectively. Extending the abuse condition during the cooling for 21 hrs, the  $\Delta_{max}$  of Cb was 2.85 and 2.5 log CFU/g in broth and in M, respectively, while the growth of Cp was inhibited only MSNA samples.

**Significance:** The reduction or the removal of nitrites in foods can increase the risk of illness caused by foodborne pathogens if additional hurdles other than the control of fast cooling or low storage temperature are not included.

## T1-05 Exploring the Diversity of Biofilm Formation by the Food Spoiler *Brochothrix thermosphacta* and Its Ability to Form Biofilms on Food Industrial Surfaces

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**Introduction:** Biofilms play an important role in residence and persistence of microorganisms in the food industry. *Brochothrix thermosphacta* is considered as a major food spoiler (Illikoud et al., 2019). This bacterium has been identified in biofilms on multiple surfaces of the food processing environment (Wagner et al., 2020, 2021).

**Purpose:** The biofilm formation ability and the biofilm structural diversity of 30 multi-origin *B. thermosphacta* strains has been explored. Also, to simulate the food industrial environment conditions, the high biofilm producer strain CD337(2) was cultivated in a CDC biofilm reactor on polycarbonate (PC), polystyrene (PS) and stainless steel (SS) surface coupons.

**Methods:** The biofilms were analysed using a set of complementary biofilm assays (biofilm ring test, crystal violet staining, confocal laser scanning microscopy and BiofilmQ images analysis software). The biofilms on coupons were analysed with plate counting, specific rpoC-qPCR, confocal laser scanning microscopy and BiofilmQ.

**Results:** Two major groups corresponding to low and high biofilm producers were identified. High biofilm producers presented flat architectures characterized by high surface coverage, high biofilm volume, and high surface area (Gaillac et al 2022). Biofilm growth on those three surfaces show similar kinetics. However, highly structured biofilm was observed on PC and PS instead of flat biofilm observed on SS. The biofilm on the SS coupons show a higher biofilm outer surface area per volume unity, contact area of the biofilm to the substrate and percentage of substratum covered by microbial cell compared to biofilms on PC and PS coupons. Conversely, a lower biofilm density and a lower biofilm volume per substrate area unity was highlighted from biofilm on the SS coupons.

**Significance:** Ability to form biofilm on food industrial surfaces, with various structures, suggests a strong ability of *B. thermosphacta* to persist in the food manufacturing environment.

## T1-06 The Effect of Meat Processing Techniques on Hepatitis E Virus Infectivity in Real-Life Pork Meat Matrices

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**Introduction:** Foodborne Hepatitis E virus (HEV) infection through the consumption of pork and wild boar meat is the most frequent cause of acute viral hepatitis in Europe.

Theoretical prevalence analyses performed in our research group indicated that RTE pork products pose the highest risk for HEV contamination, but the effect of meat processing techniques on virus infectivity remain unclear. Hence, there is a need to investigate the effect of these technique in relevant matrices.

**Purpose:** We examine here the effect of common meat processing techniques (heating, drying, fermentation, etc.) on inactivation of HEV.

**Methods:** Pork liver and fat were bought and screened for (natural) presence of HEV by RT-PCR. HEV-negative pork liver and fat was used to prepare pâté according to a standard recipe.

A batch of raw pâtés ( $n = 3$ ) was spiked with HEV (6.4 log PFU/g), heated to a core temperature of 68°C, removed from the oven and cooled at room temperature. The next day a soft extraction, capsid-integrity assay (PtCl<sub>4</sub> pretreatment), RNA extraction and RT-qPCR was performed. In parallel, soft extracts were put on cell culture to assess remaining infectivity.

**Results:** An average reduction in HEV genome copy numbers of 0.8 and 1.3 log was observed in untreated and PtCl<sub>4</sub> pretreated samples, respectively (average residual concentration = 5.5 log PFU/g and 5.0 PFU/g, respectively) ( $P < 0.001$ ).

**Significance:** Heating pâté to an internal core temperature of 68°C is insufficient to destroy all viral genomes. It is thus needed to study the effect of other meat processing practices (i.e., other time-temperature profiles) on viral infectivity to define preparatory conditions that will render food free of infectious HEV. Thus, we are currently doing by testing higher core temperatures for pâté and preparing pH-adjusted dried sausages.

## T2-01\* Acidity of Fruit Puree Determines the Kinetics of the High Pressure Inactivation in *Escherichia coli* Strains

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**Introduction:** High-pressure processing (HPP) is a non-thermal technology used to inactivate foodborne pathogens while preserving freshness. There are several predictive models for HPP-inactivation of pathogens for animal-based food products but none of them has been developed for fruit puree intended for infants.

**Purpose:** The aim of this study was to quantitatively characterize the effect of HPP on the inactivation of *Escherichia coli* on two types of fruit puree, with differing acidity (apple and banana).

**Methods:** Apple (pH 3.6) and banana puree (pH 4.2) were independently inoculated with *E. coli* CECT5947 (non-toxicogenic serotype O157:H7) and CTC1029 strains at 7 log CFU/g and treated at different pressures (300 to 600 MPa) and holding times (up to 10 min). The effect of 24 h-acid exposure before HPP was also evaluated. The best fitting primary model to the log reduction data was determined by GInaFit Tool. The one-step global fitting procedure was applied to estimate the parameters of the secondary Bigelow-type model.

**Results:** HPP-inactivation was the product (acidity) and strain dependent. After 2 min at 400 MPa, CECT5947 was reduced by 3.21 and 0.55 log in apple and banana puree, respectively; while CTC1029 showed 5.91 (apple) and 0.65 (banana) log reductions. Inactivation ranges in banana puree treated at 500 MPa (0 to 10 min) were 0.69–4.48 log (CECT5947) and 0.06 to >7 log (total inactivation after 7 min) (CTC1029), while in apple total inactivation was achieved after 2 min of treatment. Interestingly, acid exposure before HPP provided certain cross-protection in CTC1029, showing less inactivation than samples pressurised immediately after inoculation. A log-linear



inactivation trend was preceded by a shoulder shape in banana puree between 400 to 600 MPa, while a tail was observed in apple puree between 300 to 500 MPa.

**Significance:** The mathematical models can be used by baby food producers as a decision support tool to define HPP process criteria for puree meeting *E. coli* food safety standards.

## T2-02 Development and Validation of a Digital PCR Assay for Detection and Quantification of Norovirus and Hepatitis A Virus

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**Introduction:** In 2021, viruses were the third most frequently reported foodborne outbreak (FBO) causative agents in Europe. Of these, the enteric norovirus (NGI and NGII) is responsible for more widely reported and significant outbreaks, although hepatitis A virus (HAV), while less frequently transmitted, carries a higher hospitalization rate.

**Purpose:** Testing for these viruses is critical to minimize the risk of FBOs, guaranteeing the safe consumption of commonly infected goods – raw or barely cooked food, such as fruits, salads and vegetables, bivalve molluscan shellfish (BMS), and water.

**Methods:** The gold-standard RT-qPCR protocol for detection and quantification (D&Q) of these pathogens is described in ISO15216. Sample processing is time-consuming and yields small amounts of viral RNA that are usually loaded with PCR inhibitors. Therefore, an adaptation of this procedure to a more sensitive and less prone to inhibition technology is paramount.

**Results:** A tetraplex digital PCR (dPCR) method was developed for the D&Q of NGI, NGII and HAV, according to ISO15216, using the same target sequences and controls, and following the recommendations for validation stated in ISO20395:2019.

Applicability in real samples was tested by spiking commonly affected matrices – BMS, soft fruits, food surfaces and water – with certified reference material for each target. RNA extraction followed a matrix-appropriate protocol, adapted from ISO15216. Extraction efficiency and inhibition were assessed as quality controls. All dPCR tests were performed in parallel using the standard RT-qPCR method and in duplicate.

**Significance:** The dPCR method's performance was evaluated following the established criteria, presenting 100% specificity and an LOQ > 6 viral genome copies/μL. In real samples, target quantification was significantly higher for dPCR than with RT-qPCR, highlighting the more sensitive approach and the resistance to inhibitors.

The dPCR approach presented can be used as a sensitive and robust alternative to the RT-qPCR recommended by ISO15216.

## T2-03 Metagenomics Detection of *Salmonella*-Contaminated Lettuce

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**Introduction:** Advancement in genomics is promising to deliver gains in food safety by addressing obstacles that have long hindered prevention.

**Purpose:** A single, deep coverage, metagenomics testing of a contaminated food sample could provide reliable data in support of food safety, including the detection, identification, and characterization of the contaminant.

**Methods:** We spiked lettuce (25 g) with low (1 to 5 CFU) and higher numbers (10 to 50 CFU) of *Salmonella*, and carried out a culture-independent, metagenomics sequencing assay developed using the Oxford Nanopore Technologies platform (Mk1C; Food Metagenomics Oxford Nanopore Test, FOMONT). Duplicate samples were tested by a standard culture method. We also applied FOMONT to a blind panel of lettuce spiked with a bacterium, multiple bacteria, or phosphate-buffered saline (PBS,  $n = 10$ ), and to another set of non-spiked samples ( $n = 5$ ).

**Results:** We used FOMONT to detect all lettuce samples contaminated with either low ( $n = 20$ ; 100% sensitivity) or higher number of *Salmonella* ( $n = 20$ , 100% sensitivity). The culture procedure detected all samples contaminated with the higher number of *Salmonella* (100% sensitivity), but only 15 out of 20 samples with a low number (75% sensitivity;  $P < 0.05$ ). Neither test detected *Salmonella* among lettuce samples spiked with PBS only ( $n = 5$ ). All the blindly

tested samples ( $n = 10$ ) were accurately identified (100% accuracy). Serovar designation was accurately determined in 34 of 40 samples (85%), six samples were indeterminate, and no sample was wrongfully called. The FOMONT assay took four days to complete in contrast with the culture method which took 7–10 days for *Salmonella* confirmation.

**Significance:** Metagenomics-based culture-independent testing holds a considerable advantage over culture method based on speed and test accuracy, and thereby provides an enabling tool for preventive and precision food safety. The cost of FOMONT is high (CAN\$200 per sample) but is commensurate with the amount of information obtained, in addition to the labour costs saved from its rapid turnaround time.

## T2-04\* Development of an OmpG Nanopore Sensor for Norovirus Detection

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**Introduction:** Human noroviruses are the leading cause of foodborne illness globally. Numerous properties of norovirus make them difficult to control, including low infectious dose, stability, and diversity, making rapid detection and subtyping crucial. Nanopore outer membrane protein G (OmpG)-based sensing has numerous advantages, such as high sensitivity, tolerance of inhibitors, rapidity, portability, and the ability to subtype; however, it has not been utilized for microbial targets.

**Purpose:** In this study, we developed an OmpG nanopore technology for norovirus detection and subtyping.

**Methods:** Norovirus capsid protein was cloned and expressed in *Escherichia coli*. Norovirus affinity peptides were presented on OmpG in two different ways; tethering or sequence replacement of peptide in an OmpG loop. Initial work resulted in a detectable signal of norovirus target; however, signal needed to be improved for more repeatable and sensitive signal. Thus, optimization of bait peptide location in OmpG was performed using a FLAG tag and 3 anti-FLAG antibodies.

**Results:** The electrical current of peptide-tethered OmpG after adding target norovirus protein exhibited a 20pA drop in signal, showing the potential of OmpG to detect norovirus, however aforementioned optimization was needed to increase sensitivity and subtyping ability. We thus optimized the binding motif presentation in OmpG with a FLAG tag. As a result, sequence replacement in OmpG<sup>G222FLAG</sup> not only detected multiple target antibodies, but also was capable of discriminating different isotypes of monoclonal antibodies, and different isotypes of antibodies in a polyclonal antibody, each with characteristic signal at the tested antibody concentration of 17nM. We further tested the antibody detection in 10% FBS, and it generated target unique signals.

**Significance:** This work reports the first successful detection of norovirus by OmpG nanopores, and further optimization demonstrates the potential of the OmpG nanopore sensing to rapidly, sensitively, and portably detect target protein, as well as sensitively discriminate even closely related proteins and mixture thereof.

## T2-05 Efficacy of a Triple-Wash with a Combination of Peroxyacetic Acid and Hydrogen Peroxide to Reduce Populations and Mitigate Cross-Contamination of *Salmonella* Typhimurium and the Surrogate *Enterococcus faecium* on Tomatoes

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**Introduction:** West Virginia Small Farm Center suggested applying triple-wash to improve microbial safety of locally grown produce. Applying antimicrobials in produce wash water is a critical step in preventing cross-contamination instead of reducing pathogen cells.

**Purpose:** To determine the efficacy of a triple-wash system to reduce and mitigate cross-contamination of *Salmonella* Typhimurium and the surrogate *Enterococcus faecium* on tomatoes

**Methods:** Tomatoes were dip-inoculated with *S. Typhimurium* or *E. faecium* followed by triple-washing for 45 s at each wash step: water+antimicrobial+water (WAW) or water+water+antimicrobial (WWA). A mixture [23% of hydrogen peroxide + 5.3% of peroxyacetic acid (SaniDate-5.0)] was tested at 0, 0.0064, 0.1, and 0.25%. Microbial population was estimated using a modified most probable number (MPN) method. Multiple comparisons of reduction rates of inoculated tomatoes were analyzed using Mixed Model Analysis using JMP ( $P = 0.05$ ). The Mixed Model Procedure of SAS was used to analyze cross-contamination rates of tomatoes ( $P = 0.05$ ).

**Results:** Reductions of *S. Typhimurium* were similar ( $P > 0.05$ ) to *E. faecium* (1.83-3.53 vs 1.72-3.65  $\log_{10}$  MPN/g) with the greatest ( $P < 0.05$ ) reductions at 0.25% of the mixer. No differences ( $P > 0.05$ ) were seen in reductions of *S. Typhimurium* or *E. faecium* regardless of wash strategy. Application of 0.25% of the mixer in WAW or WWA wash strategy resulted in the lowest ( $P < 0.05$ ) cell counts than the lower concentration treatments for *S. Typhimurium* (0.16-0.69  $\log_{10}$  MPN/g) and *E. faecium* (-0.41  $\log_{10}$  MPN/g). There were no differences ( $P > 0.05$ ) in survival between *S. Typhimurium* and *E. faecium* after treatment with the triple-wash strategies.

**Significance:** Results suggest that *E. faecium* could be an acceptable surrogate for *S. Typhimurium* when validating antimicrobial washing systems on tomatoes.

two AL levels. Moreover, a comparison between the AFM1 values of plants with different ALs was performed. After the test for normality and equality of variance, the data were analysed using the Chi-squared test, Mann-Whitney U test, Kruskal-Wallis test and Dunn's multiple comparison test considering significant a  $P \leq 0.01$ .

**Results:** An overall decreasing trend during the years were recorded for samples overcoming the EU compliance limit, and the ALs of 40 and 30 ng  $\text{kg}^{-1}$ . Furthermore, a statistically significant ( $P \leq 0.01$ ) reduction in the proportion of samples above the AL was observed in plants with a 30 ng  $\text{kg}^{-1}$  compared to plants with 40 ng  $\text{kg}^{-1}$  AL as well as an overall reduction of AFM1 levels for the 2013–2019 period.

**Significance:** The data demonstrate how the application of restrictive AL and implementation of management actions can significantly decrease AFM1 presence in milk, and consequently human health risks.

## T2-06 Whole Genome Sequencing of Historical Scottish *Salmonella*

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**Introduction:** *Salmonella* is an established foodborne pathogen. Of over 2500 described serotypes, fewer than 100 are associated with human disease, with *Salmonella* Enteritidis and Typhimurium being most commonly isolated. This project used whole genome sequencing (WGS) to genotypically characterise *Salmonella* isolates from human, food, and animal sources and identify possible relationships.

**Purpose:** The objective was to characterise >500 *Salmonella* strains isolated between 1988 and 2017 from Scottish human clinical cases, isolates from domestic food animals, plus food and environmental sources.

**Methods:** DNA sequences from Illumina paired-end sequencing were analysed to derive serotype, markers of pathogenicity and antimicrobial resistance (AMR) genes, and to determine relatedness by core genome MLST and SNP typing (certain serotypes only). Results were compared with those from routine sequencing of Scottish isolates carried out since 2017, and international isolates in the Enterobase dataset (<https://enterobase.warwick.ac.uk>) to allow direct comparison of historic isolates with those identified through current routine sequencing-based surveillance.

**Results:** The collection of sequenced isolates comprised 60 different serotypes, belonging to 85 different MLST (7 locus) sequence types. At a cgMLST cut-off of 10 allelic differences, 57 clusters of two or more isolates were identified (total 302, range 2 to 57). Changes in cgMLST and SNP profiles were observed over time, but it is possible to demonstrate the persistence of particular clones of *Salmonella* in animals, food and humans throughout the entire study period from the 1994 to the present day.

**Significance:** This study has demonstrated the persistence of specific *Salmonella* clones in Scotland for up to 27 years. The obtained results improve our understanding of and ability to investigate the sources of foodborne salmonellosis in Scotland, and provide important baseline data to assist the implementation of a "One Health" approach to reduce the burden of disease.

## T3-01 Results of a Retrospective Study on the Application of Restrictive Attention Limits and Corrective Measures Applied for Aflatoxin M1 Contamination in Commercial Milk Supply Chains

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**Introduction:** European Regulation 1831/2006 sets the limit for AFM1 in raw milk to 50 ng  $\text{kg}^{-1}$ . Nonetheless, the Italian Ministry of Health applied an attention limit (AL) of 40 ng  $\text{kg}^{-1}$  since 2013 and single dairy plants voluntarily set the AL to 30 ng  $\text{kg}^{-1}$  since 2017.

**Purpose:** To perform a retrospective study for assessing the effectiveness of the application of a more restrictive AL and consequent management actions on the AFM1 concentration in milk on time.

**Methods:** We analysed the data obtained from the self-control plan of six commercial dairy plants (67,944 samples) for AFM1 contamination of milk during the years 2004–2008 and 2013–2019. Descriptive statistical parameters were calculated for all the years, as well as the percentages of samples above the EU compliance limit and the

## T3-02 Plasma Treatment Application for Improving Liquid Retention in Plastic Food Packaging

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**Introduction:** Exudate released from fresh meat products remains a challenge in meat packaging and presentation. Meat exudate accumulated in plastic packaging trays is unsightly and facilitates the proliferation of pathogenic and spoilage microorganisms, compromising the safety and quality of packaged fresh meat. Absorbent meat pads are primarily used to soak away the excessive meat exudate. Soaked pads are non-recyclable components and restrict recycling of the plastic trays themselves. An innovative and fully recyclable plastic package was developed to act not only as primary packaging but also to scavenge the meat exudate, improving the meat shelf life. This packaging solution improved liquid retention through localized plasma treatment of liquid-holding recesses integrated into the plastic tray.

**Purpose:** Innovation – developing sustainable packaging solution for the management of exudate in plastic meat trays.

**Methods:** Design and thermoforming of a PET substrate with capillary recesses, oxygen plasma treatment of the recesses, surface characterization (wettability) of PET surface, liquid retention test of recess samples, evaluation of the longevity of plasma treatment effects.

**Results:** Localised oxygen plasma treatment improved surface wettability of recesses and led to higher liquid retention by ~2.24 times. The new packaging solution provides a comparable capacity of liquid retention to the conventional absorbent pads while ensuring trays are fully recyclable.

**Significance:** The developed technology provides a novel, fully recyclable meat tray. This design manages exudate in meat packaging, helping limit the growth of microorganisms on meat surface, thus improving the safety and quality of fresh meat. It also reduces the environmental footprint of plastic packaging, avoiding the difficult-to-recycle plastic waste.

## T3-03 Sustainable Solutions for Smart and Active Packaging for Shelf-Life Extension and Spoilage Monitoring of Processed Meats

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**Introduction:** Polymer molecules extraction from agro-industrial wastes for production of food packaging materials is an effective valorization approach aligning with circular economy concept and contributes positively to accumulation problems of synthetic packaging. However, there is scarce information on exploring "underutilized" natural and sustainable resources for their implementation in innovative food packaging materials production.

**Purpose:** For the first time, a sustainable, active/smart edible packaging film was fabricated only from unutilized, inexpensive discarded crop residues, and reliably used for preservation and spoilage monitoring purposes of processed meat under refrigerated storage.

**Methods:** Cellulose and carboxymethyl cellulose (CMC) production from cotton linter, rice husk, and melon rind (MR) was first optimized. MR-CMC (selected as the best matrix for active/smart film fabrication) was loaded with onion scale extract (OSE) as an antimicrobial ingredient and a visual indicator of quality. The well-characterized, glycerol-plasticized film was applied on chill-stored pastrami for ten days, where coated/uncoated samples were evaluated for their aerobic mesophilic population counts (AMC), weight loss, pH, and total volatile basic nitrogen (TVBN).



**Results:** The novel active/smart film demonstrated strong DPPH scavenging, potent antimicrobial activity against foodborne pathogens and unique color responsiveness at pH values 1-14. The film exhibited remarkable light transmittance, solubility, soil biodegradability, reduced water vapor permeability, excellent tensile strength and high thermal stability compared to commercial CMC prepared counterparts. AMC in active/smart film wrapped samples were significantly ( $P < 0.05$ ) reduced by 1.3 log units after 1 day of storage, with further reductions for up to 7 days at 4°C, where the film's color changed from red to green denoting spoilage under alkaline condition as verified by TVBN.

**Significance:** A novel, eco-friendly, low-cost, active/smart film based entirely on agro-industrial wastes was developed with real-time sensing capability facilitating quick consumers' judgement to freshness and quality of processed meats. Promising results may qualify the novel film for future production upscaling and commercialization.

**Methods:** Four types of PW were contaminated with *L. monocytogenes* and then treated at established operational limits for chlorine (20 to 25 mg/L) and chlorine dioxide ( $\text{ClO}_2$ ) (2 to 3 mg/L) for 1 min. Once VBNC cells were induced in the PW, shredded lettuce was washed for 1 min. Then, the washed product was stored for 15 days at 7°C. The presence of VBNC was determined by viability quantitative PCR (v-qPCR) complemented with two dyes, ethidium and propidium monoazide, and plate count.

**Results:** The results obtained showed that 20 mg/L of chlorine inactivated the inoculated *L. monocytogenes* cells in all types of PW. However,  $\text{ClO}_2$  showed differences in its efficacy depending on the type of PW. Shredded lettuce was cross-contaminated with the VBNC cells, indicating that VBNC cells were able to attach to the product during washing. After storage, VBNC *L. monocytogenes* cells present in the product were able to resuscitate, although the levels of culturable bacteria remained very low.

**Significance:** Recommended operational limits by the fresh-cut processing industry for chlorine (20 mg/L) are effective in inactivating pathogenic bacteria present in the PW, but the efficacy of other disinfectants on the induction of VBNC stage such as chlorine dioxide should be revised. The presence of VBNC cells in PW can cross-contaminate the product during washing and they can be able to resuscitate during shelf-life, representing a potential safety issue.

### T3-04 Effect of Temperature and Isolation Source on the Susceptibility of *Salmonella* to Biocides

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**Introduction:** Sanitation is an important mitigation step to control *Salmonella* in the food industry. However, the occurrence of isolates with less susceptibility to biocides raises public health concerns. To date, there are no standard methods for biocide susceptibility testing and factors that affect susceptibility to biocides are not fully characterized.

**Purpose:** Determine baseline susceptibility of *Salmonella* to biocides and evaluate the impact of isolation source and incubation temperature on biocide tolerance.

**Methods:** Broth dilution was used to determine minimum inhibitory concentrations (MICs) and minimum biocidal concentrations (MBCs) of 55 isolates (26 serotypes) from low-moisture foods (LMFs), 62 isolates (19 serotypes) from meat sources, and 17 environmental Newport isolates. An additional spot plate assay was used to determine the MBCs of all the isolates. Testing was done in three replicates at 25°C or 37°C. Both methods had a 96-well layout with a 2-fold increase in concentrations of the biocides acetic acid (AA), citric acid (CA), lactic acid (LA), dodecyl trimethyl ammonium chloride (DC), benzalkonium chloride (BC), hexadecyltrimethylammonium chloride (HC), chlorhexidine (CH), trisodium phosphate (TP), acidified sodium chlorite (ASC), sodium hypochlorite (SH), sodium arsenate (ARA), sodium arsenite (ARI), Copper (Cu) and Silver (Ag). Statistical analysis was done using SAS 9.4. All isolates used were sequenced and the data was analyzed for antimicrobial resistance genes using AMRFinder plus.

**Results:** Temperature played a significant role in determining both MIC and MBC values for each biocide regardless of the method used ( $P < 0.001$ ). Serovar didn't affect the MICs and MBCs for most biocides. Furthermore, isolates from a different source (LMF vs. meat vs. environment) exhibited significant differences in MBCs ( $P < 0.001$ ) using spot plate assay, while no difference was detected in MICs. Outliers (isolates that had a significantly higher MBC) were seen for ARA, ARI, CA, TP, CU and SH. Interestingly these isolates carried a different genetic profile.

**Significance:** Findings could greatly improve the sanitation plan against *Salmonella*.

### T3-05 Induction of VBNC *L. monocytogenes* by Chlorine-Based Disinfectants: Presence in Process Water and Transfer from the Process Water to the Product during Washing

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**Introduction:** Water disinfectants are used during washing of fresh and fresh-cut produce to maintain the microbial quality of the process water (PW) and prevent water-mediated cross-contamination. However, water disinfectants are not always able to inactivate all microorganisms present in the PW, causing a sub-lethal damaged and may cause the bacteria to enter a viable but non-culturable (VBNC) state.

**Purpose:** To determine the significance of commonly applied water disinfectant treatments in the induction of VBNC *Listeria monocytogenes* and the potential cross-contamination during washing of leafy greens as well as resuscitation during shelf life.

### T3-06 Development, Implementation, and Evaluation of Targeted Cleaning Optimisation Interventions in a UK-Based Small- and Medium-Sized (SME) Ready-to-Eat Food Manufacturer

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**Introduction:** Cleaning practices are an important component in assuring food safety. There is a lack of studies that focus on improving cleaning operations through interventions focused on employees involved with cleaning.

**Purpose:** Develop, implement, and evaluate interventions to promote adherence to specific cleaning procedures in a UK-based RTE SME food manufacturer.

**Methods:** A mixed-methods research approach was developed to capture baseline and post-intervention data. Approaches included qualitative interviews (n=13), quantitative surveys (n=37), direct observations (n≥35 hours), company documentation review, focus groups (n=2) and environmental cleaning performance indicators aerobic colony counts (n=676), adenosine triphosphate bioluminescence assay (n=708), casein assay (n=40), cleaning chemicals concentration (n=305), associated with the cleaning practices in the food-manufacturer. Developed interventions focused on eight targeted behaviours and consisted of posters (n=7) placed in the production area, hands-on procedure demonstrations (n=3), cleaning instructions amendments (n=3), and environmental restructuring.

**Results:** Baseline observations, interviews, and survey data indicated that barriers towards adherence of specific cleaning procedures improvement were 'time', 'lack of refresher training', and 'lack of risk awareness'. Further observational data indicated the need for improvement of detergent rinsing, detergent, and disinfectant preparation, and cleaning of hard-to-reach areas of the equipment. Surveys and focus groups evidenced the need for providing prompts/cues-to-action and messages to increase risk awareness, demonstrations of the correct practice, verbal communication of health consequences and the need for additional equipment to facilitate detergent rinsing. Overall, the implementation of the intervention had a positive impact upon company cleaning practices. There was a significant reduction ( $P < 0.05$ ) of surface microbiological, allergen, and organic matter resulting from improved cleaning processes and observational data evidenced implementation of required cleaning practices including the rinse step.

**Significance:** The use of the mixed-methods approach enabled robust company-specific, targeted intervention development, as well as evaluation of the practicability and acceptability of developing interventions that resulted in improvement of cleaning within the company.

## T4-01 Metataxonomic Surveillance of Contamination Pathways in Food Processing Environments: From Observational Studies to Practical Applications

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**Introduction:** Metataxonomics represents a user-friendly omics technique for culture-independent characterisation of microbiota composition. Although it is a well-established approach applied for a decade in medical and environmental microbiology, most of the studies in food science are only observational and its practical implementation in the food industry seems still far away.

**Purpose:** Therefore, we aimed to assay the pros and cons of using metataxonomic surveillance (MS) to characterise contamination pathways in food processing establishments and discover MS-based biomarkers of pathogen and spoilage bacteria presence.

**Methods:** Over 700 environmental and food samples were collected from cattle slaughterhouses, a poultry abattoir, and a baby food facility. The 16S rRNA amplicon-based sequencing outputs were compared to the targeted detection of microbes by counts, enrichment, and quantitative PCR (RT-qPCR).

**Results:** Cattle slaughterhouses' microbial biogeography showed that resident bacteria varied between premises as a function of temperatures and longitudinally along temporal phases of cleaning/sanitising. Moreover, selective bacterial inactivation by the cleaning/sanitising was only detected through MS. Contamination patterns plotted in the poultry abattoir showed that resident microbiota mainly originated from broilers' skin, in which *Arcobacter butzleri* was largely present. Targeted detection confirmed the high environmental persistence of this pathogen and flocks' cross-contaminations conveyed by carcass transport lines. In the baby food processing plant, the fluctuation of biodiversity and the succession of resident communities along a one year period were strongly influenced by the type and the microbiota composition of incoming raw materials. The parallel detection of alive spore-forming pathogens by RT-qPCR significantly correlated with low biodiversity and the presence of specific taxa.

**Significance:** Our studies have shown that MS is a valid approach to defining contamination patterns in the processing plant, from the environment to the food products and back. As demonstrated by the benchmark with targeted cultural methods, the time for MS integration in microbiology quality control of food industries has now come.

## T4-02 First Enterotoxigenic Confirmation of *Staphylococcus argenteus* as a Foodborne Pathogen

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**Introduction:** When present in food, some coagulase-positive staphylococci can produce staphylococcal enterotoxins, whose ingestion has been reported as the cause of staphylococcal food poisoning outbreaks (SFPOs). SFPO investigations revealed that *S. aureus* is the most prevalent species responsible for foodborne diseases. For this reason, current detection and typing methods have been developed for *S. aureus* and its enterotoxins. In the last ten years, however, few studies have highlighted the potential involvement of *S. argenteus*, another coagulase-positive *Staphylococcus*, in foodborne outbreaks. Nonetheless, its ability to produce enterotoxins remains questionable.

**Purpose:** The objective of this work was to determine if *S. argenteus* is a causative agent of food poisoning. We also addressed the limitations of the various methods available to study SFPOs and for monitoring staphylococci in food.

**Methods:** In this study, we characterized in-depth two staphylococci strains isolated from two independent outbreaks that occurred in France. We used a combination of methods currently used for SFPO investigations including PCR, whole genome sequencing (WGS), liquid chromatography-mass spectrometry (LC-MS), and ELISA.

**Results:** While both PCR and phenotypic analyses did not allow identification of staphylococci isolates to the species level, WGS allowed classification of them as *S. argenteus* and to determine the full content of virulence genes. Some enterotoxins were produced in artificially *S. argenteus*-contaminated milk and detected with LC-MS and ELISA methods. The toxin concentration measured in milk was comparable to concentrations reported from SFPO. From a collection of 250 publicly available genomes, the complete enterotoxin gene set of *S. argenteus*, including variants and pseudogenes was determined. The most prevalent genes were *sex*, followed by *sel26*, *sel27* and *sey*. The *egc* cluster was less frequent and most of the time carried a dysfunctional *seg* gene.

**Significance:** Our results highlighted the enterotoxigenic properties of *S. argenteus* and will help to improve the characterisation of SFPO and to monitor *S. argenteus* as an emerging foodborne pathogen.

## T4-03 Colonisation Dynamics of *Listeria monocytogenes* in Food Processing Environments, and Genetic Features of Strains Showing Persistent Contamination

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**Introduction:** The foodborne pathogen *Listeria monocytogenes* presents a significant contamination challenge for food processing environments (FPEs). Once strains of the bacterium enter these environments, they can colonise niches present, and persist for months or even years in the environment. This presents an increased risk for ongoing contamination of food products produced. Strains possess a range of genetic features influencing biofilm production, stress tolerance, and cell-to-cell communication, which can impact these colonisation dynamics.

**Purpose:** This study examined the colonisation mechanisms of *L. monocytogenes* in simulated FPE conditions, as well as the genetic markers associated with persistent contamination.

**Methods:** In this study, 52 food system-associated isolates of *Listeria monocytogenes* were characterised for their ability to colonise stainless steel surfaces, using phenotypic and genotypic approaches. In addition, persistent strains from a single FPE were subjected to whole genome sequencing to characterise their genetic landscape and compare with that of presumed non-persistent strains also isolated from the same FPE.

**Results:** Results suggested differences in colonisation dynamics were not due to *agr*-dependant cell signaling, and that strain-specific transcriptional responses were observed, largely characterised by upregulation of diverse metabolic pathways associated with nutrient acquisition/scavenging. Interestingly, persistent strains showed greater heterogeneity in their virulence gene markers, with mutations suggesting a loss of virulence relative to other strains.

**Significance:** Taken together, these results provide new insights into how *L. monocytogenes* adapts to, and colonises, the FPE, and describes the genetic features of persistent strains.

## T4-04 Design of a Biofilm Model Based on Metagenomic Characterization of Drains in Seafood and Dairy Processing Facilities

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**Introduction:** Mock biofilm drain models that reflect the microbiota in food drains in industrial food processing environments are needed to understand and optimize sanitation schemes.

**Purpose:** The purpose of this study was to develop a mock drain biofilm model suitable for studies investigating the efficacy of biocides.

**Methods:** Eleven stainless steel floor drains in different food industries (shrimp, n=4; fish, n=4; dairy, n=3) were sampled. Dairy drains were sampled twice, nine months apart. For culture-dependent



analysis, 20 aerobic culturable bacteria were isolated from each drain (n=220) and identified using MALDI-TOF and 16s rRNA sequencing. DNA extracted from each drain were whole genome amplified before shotgun metagenomic sequencing (Illumina). Taxonomic classification, alpha- and beta diversity and statistical analyses were assessed using CLC Genomics Workbench.

**Results:** Forty-two unique genera were found among the aerobic cultivable drain isolates with *Pseudomonas* spp., *Chryseobacterium* spp., *Microbacterium* spp., and *Acinetobacter* spp. dominating (37%). Fourteen genera were found in both seafood and dairy drains. No differences in alpha diversity were observed among the drain types. The microbiota of cheese and shrimp drains were significantly ( $P_{adj} < 0.05$ ) different, however, there were no significant ( $P_{adj} > 0.05$ ) differences among other drain types (cheese vs. fish, shrimp vs. fish, cheese<sub>2021</sub> vs. chesse<sub>2022</sub>). Across all samples (n=14) Pseudomonadaceae constituted 22% of the drain microbiome. Individual taxonomic profiling showed considerable microbiome variance between drains within the same production environment. Highly abundant genera in the metagenomic analysis and frequently isolated drain bacteria were selected for the final multispecies biofilm model (n=31, 24 genera) to be tested for tolerance to different biocides at industrial concentrations.

**Significance:** The present study developed a biofilm model that reflects the microbiota in industrial floor drains and can be used to test the efficacy of biocides under simulated industrial conditions. Results from this research will contribute to the design and optimization of current and new sanitation schemes.

#### T4-05\* **Understanding *Listeria monocytogenes* Behaviour That Triggers Survival Under Severe Acidity**

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**Introduction:** Foodborne diseases are a constant threat to public health and an important onus for the socioeconomic status worldwide. *Listeria monocytogenes* is the causative agent of listeriosis, the fifth most commonly reported zoonosis in humans in European Union nowadays, with a progressive rise of outbreaks and an elevated number of deaths. This foodborne pathogen is ubiquitous and survives in a wide range of strong stresses, like refrigeration temperatures and harsh food processing conditions, with the ability to create reservoirs and persist for years.

**Purpose:** This study aimed to investigate the mechanisms that allow *Listeria monocytogenes* to persist in acidic conditions, obtain acid resistance, and thus sustain viability upon severe acidic treatment.

**Methods:** The growth of one *L. monocytogenes* strain, isolated from a human skin lesion, was monitored through conventional culturing methods, measuring of optical density, and pH values. Three were the *in vitro* cultures, two submitted to citric acid to reach pH values of 5.5 and 5.2, as well as a control culture without any pH adjustment. The differences in robustness at various phases of growth were quantified by exposure the cultures in different growth phases to an acidic shock at pH 2 for 30 min.

**Results:** Low pH-adapted cultures showed an increasing resistance in comparison with the control culture and the robustness was physiologically expressed at the stationary phase. The strain showed during the adaptation at pH 5.2 a biphasic growth curve, where more resistance was reported at the two stationary phases, contrarily to the two exponential phases. The same behaviour was exhibited in cultures grown at both 10°C and 37°C under anaerobic conditions.

**Significance:** Coupled to the physiological observations described above, RNAseq was performed to delineate the transcriptomic response of the strain. This information should shed light to molecular mechanisms involved.

#### T4-06 **Bettercallsal: Analysis Tool for Precise Detection of Multiple *Salmonella* Serovars from Culture Enrichments Using Shotgun Metagenomic Profiling and its Application in an Outbreak Setting**

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**Introduction:** Current surveillance for *Salmonella* is limited to only detecting the most abundant serovars from culture-based approaches. Here we introduce a tailored, publicly available workflow, "bettercallsal," for *Salmonella* serovar identification using shotgun metagenomics or quasi-metagenomics datasets.

**Purpose:** To develop a comprehensive analysis tool for precise identification of multiple serovars of *Salmonella* in metagenomic datasets and test its utility in a complex outbreak scenario.

**Methods:** An *in-silico* benchmark dataset, comprising 29 unique *Salmonella*, 46 non-*Salmonella* bacterial and 10 viral genomes, was generated using InSilicoSeq with read depths from 0.5 million to 5 million read pairs. Metagenomic data of *Salmonella* culture positive nonselective 24 h (H24), and selective 48 h (H48) papaya outbreak sample enrichments were analyzed. Analyses were performed using a custom-built k-mer tool, SeqSero2 and bettercallsal. A detailed method and workflow is publicly available at <https://github.com/CFSAN-Biostatistics/bettercallsal>.

**Results:** The *in-silico* dataset analyzed with bettercallsal revealed that precision and recall increased as read depth increased for single-end and concatenated reads, to 96% and 90% respectively. In the papaya outbreak, proportional abundance of *Salmonella* ranged from undetectable to 2.5% in H24 enrichments. On average, 10-25% proportional abundances of *Salmonella* were identified through k-mer analysis in H48 enrichments (n=9), with multiple serovars, Newport and Infantis being detected in some samples (n=3). SeqSero2 identified partial antigen profiles in (n=2) H48 selective enrichments and 3 enrichments harbored Kiambu, Senftenberg and Gaminara. In contrast, bettercallsal identified multiple serovars in concordance with previous Bioplex assay results from papaya enrichments (n=9), and the genome hits assigned to the samples clustered with *Salmonella* isolates from the papaya outbreak clearly evident by the SNP cluster information seen through this pipeline.

**Significance:** Precise identification of multiple *Salmonella* serovars from complex food and environmental matrices is essential for successful outbreak investigations pertaining to public health.

#### T5-01\* **Heat Inactivation of *Bacillus licheniformis* Spores in Plant-Based, Bovine Milk and Broth**

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**Introduction:** The consumer demand for plant-based milk alternatives has been increasing in the last years. The potential food spoilage induced by spore-forming bacteria from plant-based ingredients is an emerging research topic.

**Purpose:** The objective of the present study was to characterize the thermal inactivation of spores of spoilage organisms in plant-based and bovine milk and assess the potential protective matrix effect.

**Methods:** *Bacillus licheniformis* was chosen as a model micro-organism due to its common involvement in spoilage incidents in milk and vegetables. To investigate the impact of food matrix on the inactivation profile of spores, experiments were carried out in several plant-based milk alternatives, half-skimmed bovine milk and BHI broth, used as a reference. *B. licheniformis* CTCPA 3107001 spores were inoculated to the selected matrices with an initial concentration of 9 log CFU/mL. Samples were subjected to heating at five different temperature levels, 97.5, 100, 102.5, 105 and 110°C, following the methodology of thermal treatment with capillary tubes in an oil bath. All matrix/temperature combinations were analyzed in biological triplicates.

**Results:** In BHI broth, inactivation followed a linear trend, however, the kinetics obtained in food products included shoulders and tails. Thus, the linear regression model fitted to the data showed non-satisfactory goodness of fit; therefore, non-linear models were fitted. In addition, the inactivation parameter estimates revealed differences between the various plant-based milk alternatives, indicating the need for more precise inactivation assessments to fully characterize spore inactivation in these products.

**Significance:** This study is of a great importance since, to the best of our knowledge, it is the first attempt to describe inactivation kinetics

of spore-forming bacteria in plant-based milk alternatives and can contribute to the reduction of spoilage incidents, hence, major financial losses for the food industry globally.

## T5-02 Cold Shock Proteins Promote Nisin Tolerance in *Listeria monocytogenes* through Modulation of Cell Envelope Modification Responses

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**Introduction:** To resist nisin, *Listeria monocytogenes* deploys several defense mechanisms that are not yet fully understood. *Lm* possesses three genes (*cspA*, *cspB*, and *cspD*) encoding cold shock proteins (Csps) with regulatory roles in various stress responses.

**Purpose:** Evaluating the contribution of Csps to nisin tolerance in *L. monocytogenes*.

**Methods:** *L. monocytogenes* EGDe and its *csp* gene deletion mutants ( $n=7$ ) were compared based on survival [7.5 ppm nisin or 10 ppm benzalkonium chloride (BC)], growth in Brain Heart Infusion media under nisin (5 ppm) or BC (1.25 ppm) stress, antibiotic sensitivity, and cell surface charge using the cytochrome c binding assay. Quantitative RT-PCR assays were used to compare gene expression under nisin stress. Data were analysed using *t*-tests and ANOVA.

**Results:** Without *csp* genes, a *L. monocytogenes*  $\Delta cspABD$  mutant displayed significantly ( $P<0.05$ ) compromised growth under nisin stress (growth rate:  $\Delta cspABD$ : 0.0028 vs 0.43  $OD_{600}/hr$ : wild-type). Examination of single ( $\Delta cspA$ ,  $\Delta cspB$ , and  $\Delta cspD$ ) and double ( $\Delta cspBD$ ,  $\Delta cspAD$ , and  $\Delta cspAB$ ) *csp* gene deletion mutants revealed a hierarchy ( $cspD>cspB>cspA$ ) of importance in *csp* gene contributions toward *L. monocytogenes* nisin tolerance. Individual eliminations of either *cspA* or *cspB* increased nisin tolerance (2.4- and 1.5-fold, respectively), suggesting that their expression has inhibitory effects on CspD mediated nisin resistance. Gene expression analysis revealed that nisin induced *csp* expression upregulation (16.8- to 50.4-fold) and Csp deficiency significantly ( $P<0.05$ ) altered expression (>2-fold) of genes encoding proteins involved in cell envelope modification such as DltA, MprF, RmlT, and penicillin-binding proteins. A  $\Delta cspABD$  mutation induced a more electronegative cell surface charge increasing sensitivity to nisin and other cationic cell-envelope-targeting antimicrobials such as daptomycin and BC.

**Significance:** Csps contribute to *L. monocytogenes* stress tolerance of food preservation-related antimicrobial peptides such as nisin through regulatory functions that contribute to modulation of cell envelope protective responses. Such knowledge can be harnessed in the development of better *L. monocytogenes* control strategies.

## T5-03\* Genome Mining within the Psychrophilic *Clostridium estertheticum* Complex Uncovers Esterцин A, A Novel and Potent Bacteriocin with Bio-Preservative Potential Against Major Foodborne Pathogens

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**Introduction:** Antimicrobial resistance in pathogenic foodborne bacteria is considered a major public health issue necessitating an urgent need for the discovery of novel antimicrobial compounds for biopreservation. Targeted genome mining for antimicrobial compounds, including bacteriocins, in under-characterized bacteria that occupy unexplored ecological niches, such as the psychrophilic and anaerobic *Clostridium estertheticum* complex (CEC), has the potential to close this gap by revealing exploitable novel biosynthetic gene clusters.

**Purpose:** Determine and validate the bacteriocin biosynthetic potential of CEC through a combination of genome mining, heterologous expression and phenotypic assays.

**Methods:** The genome mining was carried out in 38 CEC genomes using antiSMASH. The bacteriocin biosynthetic gene clusters (BBGC) were phenotypically validated through agar well and disk diffusion assays. To validate bacteriocin production, one of the encoded novel bacteriocins, esterцин A, was partially purified from its native produce strain and also heterologously expressed in *Escherichia coli*. Its antimicrobial activity, mode of action and physicochemical stability were determined phenotypically.

**Results:** Eight novel BBGC encoding novel lantibiotics ( $n=6$ ) and sactipeptides ( $n=2$ ) were discovered in 17 genomes. Of interest was BBGC2 which was the most prevalent BBGC and encoded in three different CEC species. Partial extracts of one strain encoding BBGC2

showed activity against *B. cereus*. Heterologously produced esterцин A displayed broad activity against clinically relevant pathogens including vancomycin-resistant *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus*. Stability tests showed esterцин A is stable against different pH, temperature and enzyme conditions. The mode of action of esterцин A has been linked to lipid II and lipoteichoic acid binding.

**Significance:** Through a combination of genome mining and phenotypic screening assays, and heterologous expression, we have shown the under-characterized CEC is a potential source for novel and stable, including esterцин A, that have activity against clinically relevant foodborne pathogens.

## T5-04 Comparison between MIC and WGS-Predicted Antimicrobial Resistance of *Staphylococcus aureus* from Bovine Mastitis Milk from Italy

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**Introduction:** Mastitis is a major disease of dairy cattle farming, negatively affecting animal welfare and economic aspects. *Staphylococcus aureus* is one of the main pathogens involved in clinical and subclinical disease manifestations. Antibiotic treatments are a useful tool for mastitis control; however, due to bacterial evolution and antimicrobial abuse, multi-resistant *S. aureus* have become a growing concern for the dairy industry, also for the potential foodborne spread to the human population.

**Purpose:** The aim of this study was to investigate AMR of *S. aureus* isolated from bovine mastitis milk and to compare the results obtained using phenotypic MIC and genotypic WGS data to assess the reliability of *in silico* prediction.

**Methods:** In 2022, 66 *S. aureus* isolates were collected from bovine milk in Italy. All samples were analyzed using the broth microdilution method to determine the MIC of antimicrobials, and WGS to identify *in silico* antimicrobial resistance genes. Finally, ST and spa type were determined *in silico*.

**Results:** According to Cohen's Kappa, the AMR results obtained with MIC and WGS were in substantial agreement ( $=0.73$ ). Both methods identified 14 samples resistant to at least one of the tested antimicrobials and 45 samples susceptible to all the tested antimicrobials. A total of four isolates showed antibiotic resistance with MIC, but no resistance was identified using WGS. On the contrary, 3 isolates were resistant to one antimicrobial using WGS, but not with MIC. Most isolates belonged to the ST398 and spa type t529.

**Significance:** Agreement between the two techniques was found in 90% of the samples, demonstrating that WGS may be a valid alternative to MIC. Unlike MIC, WGS allows the simultaneous analysis of a wide range of antibiotic resistance determinants and the identification of those genes which are not expressed but may nevertheless be horizontally transmitted to other bacteria.

## T5-05 Dairy Powder Industry: Risk Area Associated with Thermophilic Sporeforming Bacteria Using Their Growth Limits

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**Introduction:** *Anoxybacillus flavithermus*, *Geobacillus stearothermophilus* and *Bacillus licheniformis*, are the most commonly found thermophilic spore forming spoilage bacteria in dairy powders.

**Purpose:** The objective of this study is to determine at which steps of the dairy powder manufacturing process these species may develop.

**Methods:** Five strains of each species were selected to determine minimal and maximal values of temperature, pH and  $a_w$  enabling growth as well as their growth-rate in milk. These growth limit values supplemented by bibliographic data were used in cardinal growth models to evaluate the growth capacities of each species according to the industrial process conditions. Indeed, at each step of the manufacture of dairy powders, the pH and  $a_w$  environmental conditions were obtained from bibliographic data. The gamma concept was then used to evaluate the bacterial growth capacities at each step of the dairy powder manufacturing processes.



**Results:** The growth ability ranges appear wider for *B. licheniformis* (pH over 4 units and  $a_{w, \min} > 0.881$ ) than for *A. flavithermus* and *G. stearothermophilus* which show equivalent and narrower growth range (pH over 2 units and  $a_{w, \min} > 0.978$  and  $>0.966$ , respectively). During the early stages of manufacturing, i.e., pre-treatment, standardization, and pre-heating prior to concentration, the encountered physicochemical conditions are suitable for the development and growth of the three species. While during the evaporation step, conditions appear to be most suitable for *G. stearothermophilus* growth. High temperatures limit the growth of *B. licheniformis* and low water activity strongly limits the development of *A. flavithermus*.

**Significance:** These results enable visualization of the critical process steps for targeted microbial hazards during the manufacture of dairy powder. The determination of mathematical equations to calculate  $a_w$  from dry content as well as the approach used constitute a fruitful tool to optimize dry powder manufacture processes to limit/mitigate the development of thermophilic spore-forming contaminants.

**Methods:** Fresh sugar kelp was washed and cut into blades of uniform size (15 cm length). Blades were inoculated with  $\sim 10^6$  log CFU/g of each pathogen (separately) and processed with kosher salt (30% w/w) to achieve a target water activity of  $\leq 0.7$ . Dry salted samples were stored in sealed bags at 4 or 22°C for up to 10 weeks. Survival of pathogens was assessed each week using cultural techniques. One-way ANOVA ( $P < 0.05$ ) was used to assess the effects of storage temperature and time on the survival of inoculated pathogens.

**Results:** Pathogen survival was primarily affected by storage duration ( $P < 0.001$  for all pathogens). Storage at higher temperature resulted in slightly better survival of *Salmonella* only ( $P=0.048$ ). Treatment showed the strongest antimicrobial effect against STEC, which was enumerable (lower limit of 2 log CFU/g) for no more than 2 weeks of storage. STEC, *Vibrio*, *L. monocytogenes* and *Salmonella* were detectable ( $\geq 1$  CFU/10 g) for up to 7, 5, 9 and 8 weeks of storage, respectively.

**Significance:** The results of this study suggest that kelp intended to be processed by dry salting should undergo an additional processing step to reduce levels of foodborne pathogens in order to ensure safety.

## T5-06 Monitoring of Antimicrobial Resistance Indicator Genes in a Benthic Food Web in the English Channel and the North Sea

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**Introduction:** Antimicrobial resistance genes (ARGs) are environmental contaminants that spread to the marine environment, the final recipient of various anthropogenic effluents. These ARGs can be associated with marine species (plants/animals) organized in food webs. There is thus a risk for human health associated to water activities and the consumption of seafood products, subject to the accumulation of ARGs.

**Purpose:** Evaluation of antimicrobial resistance contamination of the English Channel and the North Sea at the scale of a benthic food web.

**Methods:** We analyzed 16 phytoplankton and zooplankton, 35 flatfish (skin, gills and viscera) and four bivalve mollusk (total flesh) samples collected in the English Channel and the North Sea during the IBTS 2020 oceanographic campaign. The prevalence and absolute abundance of the antimicrobial resistance indicator genes *tetA*, *bla*<sub>TEM</sub>, *sul1* and *int1* were determined by qPCR.

**Results:** The overall prevalence of the indicator genes in the benthic food web was 83.7%, with higher detection rate of *int1* (63.8%) and *sul1* (59.6%) genes followed by *tetA* (47.5%) and *bla*<sub>TEM</sub> (46.1%) genes. A higher prevalence of the indicator genes was observed in the flatfish and bivalve mollusk samples compared to the phytoplankton and zooplankton ones, which represent the basis of benthic food webs. The absolute abundance of the indicator genes was higher in some areas of the English Channel and the North Sea, reflecting potential anthropogenic inputs, for example at the Thames mouth (river effluents) but also in areas more distant from the coasts such as in the middle of the North Sea (in the surroundings of offshore platforms).

**Significance:** The monitoring of the indicator genes has provided information on the contamination of marine food webs in the English Channel and the North Sea by antimicrobial resistance. These results demonstrate that the seas are reservoirs of ARGs to which humans may be exposed.

## T6-01 The Effect of Dry Salting on the Survival of *Escherichia coli*, *Vibrio* spp., *Listeria monocytogenes*, and *Salmonella* on Inoculated Sugar Kelp during Storage

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**Introduction:** Sugar kelp (*Saccharina latissima*) is a widely consumed edible seaweed. Since it is highly perishable in fresh form, techniques including drying, freezing, and salting are used to extend its shelf life. Kelp may get contaminated with pathogens during pre- or postharvest processing and handling, resulting in foodborne illness if ingested. Salting is one of the oldest preservation methods used to increase shelf life of sugar kelp, however, its effect on pathogen survival is not well-researched.

**Purpose:** The aim of this study was to investigate the effect of dry salting and storage temperature on survival of pathogens (STEC, *Vibrio*, *Listeria monocytogenes*, *Salmonella*) in inoculated sugar kelp during storage.

## T6-02 Practical Experiences in Setting Up *Listeria monocytogenes* Environmental Sampling in the Food Industry

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**Introduction:** *Listeria monocytogenes* is an ongoing food safety pathogen. Their persistence in the food processing environment is an important source of food contamination and needs monitoring with sampling plans.

**Purpose:** In 2020–2022 15 food business operators (FBO) were guided in drafting and executing those plans.

**Methods:** FBOs were informed and guided in setting up environmental sampling plans. They need to be risk-based considering sector, raw materials, sources and routes, process types and end products. Further, a tailored risk-evaluation on structure, infrastructure, hygienic zones but also on risk factors such as contact type, humidity, ease of cleaning, cross-contamination possibilities, and non-conform results, is needed. This determined the choice of sampling location(s), number of sampling points, sampling frequency, etc. Examples were worked out with the FBOs. The need to sample large areas to increase the probability to detect *L. monocytogenes* using appropriate wipe-sampling techniques was demonstrated. To estimate the contamination pressure and persistence in the production environment, samplings were performed during processing, after cleaning and disinfection and just before start-up of production. In all cases, disassembling production equipment and running the production equipment making carry-over possible from hard-to-reach places was demonstrated.

**Results:** Practical experiences at the 15 FBOs showed that the sampling plans were quite often too limited in both frequency and number of samples, not enough risk-based, not structured, with choice of sampling location insufficiently justified, sampling techniques and size of the surfaces not effective. Based on the results, corrective and preventive measures were advised, and sampling plans were adjusted. The need for molecular characterization of *L. monocytogenes* isolates was advised to distinguish between persistors and passers-by.

**Significance:** Experiences showed that *L. monocytogenes* control is in some cases a long and difficult process which needs sufficient time and resources with the required corrective and preventive measures.

## T6-03\* Development and Evaluation of Low-Cost, Easily Deployable Molecularly Imprinted Polymer for Norovirus Detection

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**Introduction:** Human noroviruses are responsible for the majority of acute gastroenteritis, diarrhoea, and food poisoning cases worldwide. Even tiny quantities (~102 copies/mL) can create outbreaks and cause significant harm. Consequently, there is a critical need to develop sensitive and selective technologies for norovirus detection in food samples.

**Purpose:** Most current norovirus detection methods use natural recognition elements such as antibodies which are high-cost, have limited stability, and exhibit significant batch-to-batch variation. Therefore, this study developed novel molecularly imprinted polymer nanoparticles (nanoMIPs), which act as synthetic antibody mimics, for the accurate, low-cost, and portable detection of norovirus.

**Methods:** An innovative solid phase synthesis technique was used to synthesize nanoMIPs that bind to a specific protein (epitope of P-domain) on the external surface of norovirus. The nanoMIPs were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM), and their binding affinities were determined using surface plasmon resonance (SPR).

**Results:** DLS and SEM showed the nanoMIPs were 100 to 250 nm in size with spherical morphologies. The  $K_D$  values of three batches of nanoMIPs towards the template epitope were 328, 750, and 1920 nM. Furthermore, the binding affinities of the nanoMIPs were determined for whole P-domain and virus-like particles (VLPs) of the GII.4 strain and the values were similar to those for the epitope. Thus, by imprinting a small epitope of the virus, nanoMIPs could effectively bind with the P-domain and VLPs. Based on these findings, the nanoMIPs will be utilized for norovirus detection using electrochemical and thermal methods.

**Significance:** This work successfully developed nanoMIPs for the GII.4 strain of norovirus using an epitope imprinting approach. The SPR results demonstrated that the sensing capabilities of the nanoMIPs were comparable to commercial antibodies, which is extremely promising as unlike antibodies, they are also low-cost, highly versatile, and can be utilized within diagnostically challenging environments.

## T6-04 Whole-Genome Sequence Analysis of *Listeria monocytogenes* CC7 Associated with Clinical Infections and Persistence in the Food Industry

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**Introduction:** Different clonal complexes (CCs) of *L. monocytogenes* may have different potential for causing human listeriosis and for persisting in food-processing environments. In Norway, *L. monocytogenes* CC7, belonging to lineage II, is commonly found in both the food industry and outdoor environments, and was the most common CC found among clinical listeriosis isolates for the period 2010–2015.

**Purpose:** The purpose of this study was to determine if the prevalence of clinical CC7 isolates was higher in Norway compared to other European countries, if the Norwegian CC7 isolates were characterized by phylogenetic differences and if they showed different potentials for virulence and persistence.

**Methods:** The prevalence of different CCs among clinical isolates in different European countries was determined from publicly available data. The whole genome sequences of 115 *L. monocytogenes* CC7 isolates from Norway were analyzed and compared with sequences of 255 CC7 reference strains from other countries to study genetic relationships and genetic determinants linked to stress responses and virulence properties.

**Results:** The relative prevalence of CC7 among clinical isolates varied considerably between European countries and was higher in Norway than in most other countries. The majority of CC7 isolates from Norway formed a separate cgMLST cluster that was widely distributed in different habitats in Norway. Further wgMLST analysis showed that a pervasive CC7 clone was found in two meat processing plants and one salmon processing plant. All analyzed CC7 isolates harbored the same set of 72 stress-related genes involved in both general and specific stress responses. Virulence attributes were highly conserved among the different CC7 isolates.

**Significance:** The study shows that *L. monocytogenes* CC7 is widespread in Norway, with a pervasive clone present in food processing plants. The study highlights the importance of CC7 and lineage II strains in causing listeriosis and shows that more research is needed to understand the reasons for country differences in CC prevalence.

## T6-05 Maturing Food Safety Culture with Nudging in Food Manufacturing Environments in the UK

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**Introduction:** A recent surge in including food safety culture in various public guidelines and private standards, such as GFSI benchmarking standards 2020, Codex HACCP Principles and Guidelines, EU legislation, and FDA Food Safety Blueprint 2020, has prompted global interest and challenged industry to drive evidence-based cultural change. The nudge theory is effective in influencing people's behaviours and shaping culture, embraced worldwide in various initiatives – but no empirical study on its adoption in food safety culture context.

**Purpose:** This research study aims to improve food safety culture via nudging in a weekly change cycle using a validated machine learning tool.

**Methods:** Each person was nudged every day through answering one question on food safety in nine UK food manufacturing companies (13 sites) from June 2021 to September 2022, resulting in over 180,000 answers. Machine learning generated a weekly action report for each company, from which the companies picked one incremental action to execute every week. Culture maturity was calculated as the running average of all responses; ANOVA and logistic regression were conducted to determine how culture of food safety changed through nudging and to identify key drivers of change.

**Results:** Using the GFSI food safety culture position paper, companies have varying strengths on the five dimensions. Heatmap analysis indicates that "Adaptability" is the least mature dimension and "People" the second least mature. "Values and Mission" is the most mature in the participating companies, although how and why food safety was prioritised during business-critical decision-making was not always shared with team members. In the course of 16 months, nudging leads to improvement in food safety culture in several of the companies.

**Significance:** This study contributes to the currently scarce empirical evidence on how culture of food safety is improved. It is also the first study to use nudging to improve food safety culture.

## T6-06 Impact of MLST Genetic Diversity on *Listeria monocytogenes* Growth

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**Introduction:** Advancements in DNA sequencing-based identification methods now allow for more accurate characterisation of *L. monocytogenes*. Its MLST genetic diversity is reflected by the different prevalences of the "clonal complex" (CC) in food or infections. Understanding *L. monocytogenes* growth variability is essential for quantitative risk assessment, and to ensure efficient detection of each CC.

**Purpose:** We studied the impact of MLST genetic diversity of the pathogen on its growth under selective and unselective conditions.

**Methods:** Using optical density measurements with Bioscreen C, we compared the growth rate and lag phase of 39 strains from 12 different CC and various origins (vegetables, dairy, meat and fish products), in three broths mimicking stressing food conditions (8°C,  $a_w$  0.95 and pH5) and in ISO Standard enrichment broths (Half Fraser and Fraser).

**Results:** Comparison of the mean using Student *t*-test showed a significant effect of CC or groups of CC on  $\mu_{max}$  as well as on lag time in some specific conditions. However, differences in values were always very limited and did not impact the total population attained after one or several days.

**Significance:** Despite limited differences highlighting natural intra-specific variability, it is interesting to note that growth performances can't explain higher CC "virulence" or prevalence. In conclusion, the growth performances of *L. monocytogenes* strains do not appear to be strongly correlated to CC.

## T7-01 Trends and Early Signals of Emerging Risks Identified in the Food Chain

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**Introduction:** In order to have resilient food systems, systematic assessment of short, medium and long-timescale trends and emerging risks is inevitable. However, there are several factors that make this process a complex, interdisciplinary task. Risks may arise from different emerging hazards, which are well defined, and yet to be examined, however, complex, driver-induced early signals and the increase of exposure to known hazards also must be considered.

**Purpose:** Identification of evolving trends in the food chain aids strategic planning and analysis, and decision-making processes; early identification of emerging risks helps to protect human, animal, and plant health and also contributes to surveillance planning, and serves as input for risk management, mitigation, and prevention measures.

**Methods:** A systematic process management approach for identification has been elaborated and used in practice. Data and information on possible emerging issues are gathered by data mining algorithms and expert knowledge. The identification system is based on a multi-step filtering procedure conducted by an expert group in order to select the relevant issues from the large amount of data collected. At the end of the multi-step procedure, emerging risks that need further measures are selected and forwarded to the relevant stakeholders (e.g., authorities, consumers, and academic communities).

**Results:** Recent and evolving future trends (e.g., sustainable packaging solutions, essential oils and nanoplastics for antimicrobial use) in the food chain and early signals of emerging risks (e.g., micro- and nanoplastics, migration from food contact materials) have been identified and to be presented in the lecture.

**Significance:** By timely identification of emerging signals, there is a possibility to execute the necessary risk-mitigating actions and thereby preventing the evolution of the risk. Adjusting food systems to the recent and evolving trends in innovation, technology, and consumer behaviours is key to success and sustainability.

## T7-02 Sym'Previus MAP: A Web Application for the Design of Food Packaging to Improve the Preservation of Food Products

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**Introduction:** The objective of this work is to develop a software application for predicting the growth of spoilage microorganisms during storage of 'non-respiring' food under modified atmosphere packaging (MAP), taking into account gas composition, packaging characteristics (e.g., materials, geometry), and food formulation.

**Purpose:** Design and optimise MAP and food packaging to prevent the growth of sporulated and non-sporulated bacterial species.

**Methods:** The overall mathematical model comprises three different modules. The first module consists of a set of predictive models for the growth rate of spoilage of microorganisms as a function of storage

temperature, gas composition (dissolved CO<sub>2</sub> and O<sub>2</sub>) and physico-chemical product characteristics (pH, a<sub>w</sub>). The second module consists in a mathematical model for diffusion and solubility coefficient of CO<sub>2</sub> as a function of nutrition information labels (e.g., water, protein, fat, carbohydrates, NaCl and fibers). The third module predicts the evolution of the headspace gas (O<sub>2</sub> and CO<sub>2</sub>) in the packaging.

**Results:** A computing program developed in R uses an ordinary differential method (ODE) to solve the system of differential equations for (i) microbial growth and (ii) gas exchanges during MAP storage. The program makes it possible to performed simulations for the growth of seven different microorganisms, including *Bacillus weihenstephanensis*, *Paenibacillus* spp., *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Carnobacterium maltaromaticum* and *Leuconostoc mesenteroides*. The user inputs properties of the packaging materials, gas composition, physico-characteristics and nutritional information of food and storage temperature. Outputs are predictions of the evolution of O<sub>2</sub> and CO<sub>2</sub> concentrations in the headspace and the probability to reach a threshold microbial concentration over storage time.

**Significance:** The application, embedded in the predictive tool Sym'Previus ([www.symprevius.eu](http://www.symprevius.eu)), will enable manufacturers to design and optimize food packaging to improve the preservation of 'non respiring' foodstuffs stored under MAP.

## T7-03 Implementation and Application of Quality and Predictive Microbiology Models of Strawberries and Tomatoes in Microhibro for a Holistic Approach for Shelf-Life Assessment

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**Introduction:** The shelf life of strawberries and tomatoes is greatly limited by changes in their quality attributes, which are mainly determined by storage conditions and microbial spoilage. The combined use of predictive microbiology models and models describing changes in the quality attributes of fruits and vegetables at different reasonably foreseeable conditions is crucial for shelf-life assessment, being tools aiding in the reduction of food waste. In this sense, the predictive microbiology software MICROHIBRO ([www.microhibro.com](http://www.microhibro.com)) has been recently updated to allow the implementation and application of food quality models.

**Purpose:** To search, develop and implement quality and microbiological mathematical models for strawberries and tomatoes in MICROHIBRO.

**Methods:** A literature review was performed by combining mathematical modelling key terms (e.g., kinetic model) with keywords related to quality (e.g., weight loss, texture, colour, spoilage) and the microbiological safety (e.g., foodborne pathogens) of strawberries and tomatoes. Models were directly extracted from articles for implementation, while kinetic data of changes on quality and microbiological parameters available in tables and graphs were extracted for further mathematical modelling using R.

**Results:** The literature search resulted in more than 20 quality models, encompassing more than six quality attributes. The most common model structures describing changes in quality attributes were the empirical Arrhenius, zero, first and second-order models. Regarding microbiological models, *Salmonella* and *Listeria monocytogenes* behaviour has been described by the Baranyi and Gompertz models. Spoilage microorganisms' models were also revised. The revised models available in the articles and the developed models were implemented in the MICROHIBRO database and were applied to assess and optimize the food production-distribution chains of tomato and strawberry based on real data obtained from Spain and Turkey in the framework of the European project biofreshcloud.eu.

**Significance:** The models implemented in the MICROHIBRO prediction module can be used for the safety and shelf-life assessment of strawberries and tomatoes.

## T7-04\* Comparing the Performance of Two Predictive Models When Fitting Noisy Data

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**Introduction:** Predictive modelling has become a well-established quantitative method to ensure the quality and safety of food. Commonly used models focus for example on the maximum specific growth rate that an organism can reach in its exponential growth phase. Among them, the mathematical formulae describing that rate as a function of temperature is the subject of many (and also this)

studies. Two models are especially popular as they have physiologically easily interpretable parameters: the minimum, optimum and maximum temperatures, and the maximum specific growth rate at the optimum temperature. This parametrization form is attractive because approximate values can be relatively easily provided, which is a great advantage for any nonlinear regression. Another advantage appears when developing the model in culture medium then applying it to food systems. Namely, it has been observed that the above cardinal temperatures don't change much in the latter case, and it is enough to calibrate the optimum specific rate only.

**Purpose:** Recommendations on the choice of model for fitting, when the data are noisy and poorly distributed.

**Methods:** The robustness of the parameter estimates will be studied when one model fits data simulated by the other model and vice versa. We perturb the predicted values from one model by realistic random errors and fit the data simulated this way by the other model and vice versa.

**Results:** We compared the regression performance of the two models simulating scenarios when the data are scarce, and the measurements are noisy. We also let the noise have inhomogeneous distribution, mimicking the well-known case when the rates measured at super-optimal temperatures are more uncertain than at sub-optimal temperatures.

**Significance:** By means of the results, we provide objective recommendations on the models' suitability for fitting data of limited quality in terms of their range, distribution, and uncertainty.

## T7-05 Method for Tick-Borne Encephalitis Virus Detection in Raw Milk Products

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**Introduction:** Tick-borne encephalitis is an emerging tick-borne zoonotic disease affecting the central nervous system of humans. The transmission of tick-borne encephalitis virus (TBEV) through food is rare but can occur through the consumption of raw milk products from animals infected by tick bites. In 2020, France faced a TBEV outbreak linked to the consumption of unpasteurized goat cheese underlining the need to develop a sensitive method for the detection of TBEV to ensure the safety of raw milk products.

**Purpose:** The aim of this study was to develop and characterize a molecular method for the detection of TBEV in raw milk products. This characterization was based on the recent international standard ISO 16140-4 according to the ISO 15216 recommendation in terms of controls (process control and external amplification control).

**Methods:** Raw dairy products (sheep, cow, and goat raw milk products) were randomly allocated to four settings and were inoculated with four TBEV inoculation levels. Samples were co-inoculated with murine norovirus (MNV-1), used as an internal control virus. Proteinase K-based method was used for the molecular detection of TBEV in raw milk samples.

**Results:** The TBEV recovery rates varied with the inoculation level and settings. The LOD<sub>50</sub> and LOD<sub>95</sub> of TBEV were 6.40 × 10<sup>3</sup> genome copies per g or per mL and 2.84 × 10<sup>4</sup> genome copies per g or per mL, respectively. The percentages of RT-qPCR inhibitions were lower than 75% and the MNV-1 was detected in all samples with a recovery rate higher than 1%, as recommended in ISO 15216.

**Significance:** To conclude, the molecular method characterized in this study successfully detected TBEV in raw milk products and this method is essential in routine diagnostic laboratories and to assess potential health risks.

## T7-06\* Zebrafish Embryo: A Simple and Robust Tool for the Cultivation of Human Noroviruses

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**Introduction:** Human noroviruses (hNoVs) are the leading cause of epidemic and sporadic acute gastroenteritis worldwide and are the primary cause of foodborne gastroenteritis. For almost 50 years, the lack of a cultivation system for hNoVs has been a major barrier to understanding the virus biology and the development of effective antiviral and viral inactivation strategies.

**Purpose:** Giving to its simplicity and robustness, the recent model of zebrafish (*Danio rerio*) larvae to study hNoV has been very promising. The current model, however, displays high variability in virus replication, likewise, the lack of sustainable viral passaging.

**Methods:** Here, we report an improvement to the existing model using zebrafish embryos that was evaluated with three globally prevalent hNoVs genotypes and P-types; GII.2[P16], GII.4[P16], and GII.17[P31]

**Results:** The new model has shown the following: i) significantly higher efficiency and robustness, by which the model could detect up to eight-log genome copies/10 embryos at 3-days post-infection (3 dpi), which is approximately three-log higher in comparison to the larvae model (up to five-log genome copies/10 larvae at 3dpi); ii) enabled continuous virus passaging; and iii) the generated viruses were with clear binding profiles to human histo-blood group antigens (HBGAs) in human saliva.

**Significance:** Use of the zebrafish embryo tool as developed in this study serves as an efficient way to support continuous virus replication. It is expected that this tool will not only benefit epidemiological research of hNoV but can also be used to generate hNoV inactivation parameters which are highly needed by the water treatment and food industry.

## T8-01 Potential of High Pressure Processing (HPP) to Inactivate Pathogens and Extend Shelf Life of Cold Brew Coffee

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**Introduction:** The popularity of cold brew coffee is increasing. However, the lack of preservation steps after the removal of the solids requires the implementation of control measures to mitigate the potential biological hazards that may be present in the beverage.

**Purpose:** To assess the potential of high-pressure processing (HPP) to inactivate *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* in cold brew coffee, and to preserve its physicochemical quality attributes during storage at 4°C or 23°C.

**Methods:** Commercial grounded coffee was mixed with mineral water to a final concentration of 7% (w/v). Cold brewing was performed at 4°C for 17 h. After filtration, 30-ml aliquots of the beverage were dispensed on PET bottles and inoculated in triplicate with separate five-strain cocktails of *L. monocytogenes*, *E. coli* O157:H7 or *S. enterica* to a final concentration of 10<sup>7</sup> CFU/ml. Half of the spiked samples were processed at 600 MPa for 3 min at 10°C, whereas the other half remained unprocessed. Each set of processed and unprocessed samples was further divided for storage 4°C or 23°C for 90 days. Overall antioxidant capacity and total polyphenols were determined.

**Results:** Processing cold brew coffee (pH 5.9) at 600 MPa for 3 min at 10°C achieved a >five-log reduction of *L. monocytogenes*, *E. coli* O157:H7 and *S. enterica*. This reduction was sustained during 90 days of storage at 4°C and 23°C, respectively. Unprocessed samples did not support the growth of the pathogens, but the inoculated species could be detected throughout the experiment. Antioxidant capacity and total polyphenols did not change as a consequence of HPP and remained stable regardless of storage conditions.

**Significance:** HPP effectively inactivated the tested foodborne pathogens in cold brew coffee during 90 days of storage at 4°C and 23°C. Additionally, physicochemical quality attributes remained stable. Hence, HPP emerges as a nonthermal technology to ensure safety and extend shelf-life of the beverage.



## T8-02\* Better Together? Effect of Isostatic High Pressure and Nutrients in Bacterial Spore Control

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**Introduction:** Industrially applied high-pressure (HP) processing (400 to 600 MPa,  $\leq 20^\circ\text{C}$ ) is an emerging non-thermal decontamination technology. Highly resistant bacterial spores can survive such processing conditions. However, HP combined with moderate temperature (37–60°C) can trigger spore germination, which allows subsequent spore inactivation under mild conditions with minimal thermal input. The bottleneck of this HP germination-inactivation strategy is that not all dormant spores germinate under HP.

**Purpose:** To implement a HP germination-inactivation strategy, possibilities to achieve complete germination under HP are investigated, e.g., addition of nutrients that are known to trigger germination.

**Methods:** *Bacillus subtilis* PS533 spores were treated at 150 MPa, 37°C for 2 min or at 550 MPa, 60°C for 2.5 min in ACES buffer with or without nutrients (L-alanine, L-valine, AGFK [L-asparagine, D-glucose, D-fructose, KCl]). As control, spores were treated with nutrients at same temperatures for same times under atmospheric pressure. Germination of 150MPa- or 550MPa-treated spores was quantified using phase-contrast microscopy ( $n_{\text{spores}} > 700$ ) after nutrient removal or using plate count after heat treatment (80°C, 20 min), respectively.

**Results:** A clear synergistic effect of HP and nutrients on germination was observed at 150 MPa but not at 550 MPa. At 150 MPa, HP treatment alone led to 80.4±5.7 % (n=7) germinated spores. Germination at 150 MPa was increased by an additional 12.0 ± 2.2 % (n=3) or 8.3 ± 4.2 % (n=4) by 10 mM L-alanine or 10 mM AGFK, respectively. At 550 MPa, HP alone led to 5.1±0.4 log<sub>10</sub> units (n=8) of germinated spores, similar to HP treatments with 100 mM L-valine (5.6 ± 0.2 log units, n=6) or 100 mM AGFK (5.4 ± 0.3 log units, n=6).

**Significance:** Increased but still incomplete germination can be achieved by adding selected nutrients to HP, depending on the HP level. Understanding effects of food components on HP germination is prerequisite for successful HP germination-inactivation of bacterial spores.

## T8-03\* Inactivation Variability of Food-Associated Microorganisms Following Ultrasound Treatment

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**Introduction:** Ultrasound (U.S.) is a promising non-thermal technology, known for its antimicrobial efficacy. However, the impact of strain variability on the efficacy of U.S. have not been fully addressed by existing studies.

**Purpose:** This study examines the resistance of common food-associated microorganisms to U.S. treatment on a strain level.

**Methods:** Wild type strains (10 each) of *Listeria monocytogenes*, *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae* and *Escherichia coli* were exposed to U.S. treatment to investigate inactivation variability. Bacterial strains were grown in Tryptone soy broth—without dextrose (*E. coli* and *L. monocytogenes*), De Man Rogosa and Sharpe broth (*L. plantarum*) and Malt extract broth (*S. cerevisiae*) until stationary phase. Thereafter, cells were washed and resuspended in the respective growth medium. U.S. treatment was performed at an intensity of 68 to 71 W/cm<sup>2</sup>. Samples were drawn before and after U.S. treatment to determine microbial concentration by spread plating on the respective medium.

**Results:** The four microbial species showed large intra-strain variability following ultrasound exposure ( $P \leq 0.05$ ). Strains L6 and NCTC 10357 emerged as the most resistant and sensitive strains for *L. monocytogenes*, having a log reduction of ~1.5 and 5 respectively. Similarly, WCFS1 was the most resistant *L. plantarum* strain (~2.5 log reduction), while the other strains exceeded four-log reduction. Furthermore, FAM 21845 (~1.8-log reduction) and FAM 22082 (~ six-log reduction) emerged as the most resistant and sensitive strains respectively amongst the *E. coli* strains. *S. cerevisiae* strains 0130.0014 and 077.0001 were the most resistant with ~0.3-log reduction. In contrast, *S. cerevisiae* strains 028.0404 and 028.0315 were the most sensitive achieving 4.5 to 5-log reduction.

**Significance:** Microbial inactivation by U.S. is heavily impacted by strain variability. This finding is crucial for food safety and will guide further research on microbial physiology to US stress, risk assessment, optimisation, and upscaling studies on U.S.

## T8-04\* Looking for the Most Appropriate Inoculum Pre-Culture Conditions for High-Pressure Processing Validation Studies

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**Introduction:** The efficacy of high-pressure processing (HPP), as a post-lethality treatment to inactivate *Listeria monocytogenes* in RTE meat products depends on the food and pathogen related factors, which need to be considered in validation studies.

**Purpose:** To study the impact of *L. monocytogenes* physiological state derived from pre-culture conditions on HPP inactivation in matrices differing in  $a_w$  and lactate, factors known to exert piezo-protection.

**Methods:** *L. monocytogenes* CTC1034 (stationary phase) grown at 8°C, 37°C and at 37°C and subsequently frozen at -80°C, were inoculated at 7 log CFU/g in ham with  $a_w$  of 0.98–0.88 or at 9 log CFU/g in cooked ham model medium with 0 or 2.8% lactate. Samples were pressurized at 400 and 600 MPa. *L. monocytogenes* counts were determined on chromogenic agar before and after HPP.

**Results:** Cultures adapted at 8°C were more piezo-sensitive at 400MPa than those pre-cultured at 37°C and subsequently frozen. Moreover, the piezo-protection of lactate was only observed for the later physiological state, being the inactivation of *L. monocytogenes* 1.25 log lower in the presence of lactate. On the contrary, when *L. monocytogenes* was adapted at 8°C prior to HPP, a significant piezo-protective effect could be observed for  $a_w$  with 7 log reduction at  $a_w$  0.98 compared to <1 log at  $a_w$  0.88–0.92. When *L. monocytogenes* was grown at optimal temperature (37°C), without and with a subsequent freezing, conferred a high resistance towards HPP at 400MPa, with reductions of *L. monocytogenes* <1 log even at high  $a_w$ . At 600MPa, the piezo-protective effect of  $a_w$  prevailed over that derived from the physiological status, which was not relevant.

**Significance:** The pre-culture conditions prior HPP have a high impact on *L. monocytogenes* inactivation. Accordingly, cultures grown at 37°C and subsequently frozen were found the most appropriate for challenge testing in HPP validation studies.

## T8-05\* Microbial Species and Strain Heterogeneity Affect Resistance to High Pressure Processing

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**Introduction:** High-Pressure Processing (HPP) is a mild preservation method for the production of safe and nutritious food. Species and strain variability may influence microbial resistance towards HPP and have not been extensively characterized.

**Purpose:** This study investigates the resistance of four microbial species and selected strains toward HPP.

**Methods:** Wild type strains (10) of *L. monocytogenes*, *E. coli*, *L. plantarum* and *S. cerevisiae* were exposed to hydrostatic pressure (200 to 400 MPa) for 10 minutes. *L. monocytogenes* and *E. coli* were grown in Tryptic Soy Broth without Dextrose (TSB-G), *S. cerevisiae* in Malt Extract broth (ME), while *L. plantarum* in De Man, Rogosa and Sharpe (MRS) broth. Biological triplicates of stationary-phase cultures were used, and viability of stress-exposed cultures was determined by plating onto the respective medium.

**Results:** *L. monocytogenes* strain variability was observed at 400 MPa with L6 being the most resistant, and NCTC 10357 the most sensitive, while two to four-log reduction was observed for the other strains. Comparable inactivation was detected for *E. coli* FAM 21805, FAM 21845, and O157 VT<sup>-</sup>, unlike the rest of the strains that reached or exceeded six-log reduction. Strain variability was also detected at 300 MPa with *E. coli* FAM 21845 and O157 VT<sup>-</sup> being the most resistant and FAM 21843 the most sensitive. *L. plantarum* strains exceeded the 5.5-log reduction. *S. cerevisiae* strain variability was noted at 200 MPa with five out of 10 strains showing no significant reduction, while strain 28.0315 showed the highest decrease of 3.5 log CFU/ml.

**Significance:** This study confirms the importance of species and strain variability in HPP. The results are relevant for the improvement of decontamination efficiency predictions, the design of validation studies, and the application of hurdle technology.

## T8-06\* Efficiency of Non-Thermal Plasma Treatment Against Food Pathogens and Spoilage Microorganisms

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**Introduction:** Non-thermal Plasma (NTP) has shown to be a promising sustainable technology for surface sterilization and microbial control in the food industry. It presents important advantages such as short processing times, treatment at room temperature, and low energy use. However, its decontamination efficiency is not well characterized yet.

**Purpose:** Here, the effects of NTP treatment against foodborne pathogens and spoilage microorganisms *Listeria monocytogenes*, *Escherichia coli*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* are presented. The inter- and intra-specific resistance variability will be assessed by measuring the inactivation of 10 selected strains for each species.

**Methods:** Plasma-processed air (PPA) was produced with a microwave plasma source (MidiPlexc) and used for NTP treatments. The strains selected per each species varied in origin and heat resistance. NTP inactivation was tested by spot-plating serially diluted cell concentrations on nutrient-rich agar plates and subsequent exposure to PPA. After plate incubation to allow cell recovery and growth, cell reduction is quantified using the Most Probable Number estimation.

**Results:** The resistance to NTP treatment was assessed after an exposure of 60 seconds for bacteria and 30 seconds for yeast. For the bacterial species, an inactivation between 4.6 and 6.8 log was reached. Surprisingly, the susceptibility to the treatment didn't differ between bacterial species and strains, despite being selected based on diverse origins and heat sensitivity. Subsequently, the inactivation kinetics were investigated using various treatment times. No statistical difference was encountered between strains or species. The yeast strains were significantly more sensitive to NTP treatment, though also in this case strain variability was limited.

**Significance:** NTP treatment resulted in an effective inactivation of different microbial species. More information is needed to understand the effects on food matrices and the molecular mechanism leading to cell death, but this novel, non-thermal technology showed promising results for possible applications for surface decontamination.

## T9-01\* Are Meal Kits Promoting Food Safety? A Transatlantic Comparison Study

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**Introduction:** Household food safety is critical for consumers worldwide. Foodborne illness presents a significant risk to human health, often resulting from improper storage, cooking, or unsafe food handling practices. Meal kits provide consumers fresh ingredients and a recipe card which are a global trend with increasing sales in both the United States and the United Kingdom. Food safety communication included in meal kits remains unknown, especially among kits provided in different regions.

**Purpose:** The objective of this study was to compare food safety messaging included in meal kits in the U.S., and UK and evaluate the adequacy of advice provided.

**Methods:** A total of 485 meal-kit recipe cards (UK = 359; U.S. = 126) were collected from provider websites and consumers via social media. A data extraction tool was designed in Qualtrics (Qualtrics, January 2023) to capture all textual and visual food safety information integrated into the recipes.

**Results:** Over one-half (50.9%, n=169) of UK cards included refrigeration advice, while most U.S. recipes (88.1%, n=111) omitted this information. Of all recipes that included fresh produce, 11.7% (n=42) of UK cards and 18.4% (n=23) of U.S. recipes omitted instructions on washing produce. No U.S. recipes included instructions on handwashing before food preparation, while almost one-half (46%, n=165) of UK recipes incorporated such instructions. After handling high-risk ingredients, one-half (50.1%) of UK meal kits included appropriate handwashing advice, while only 5.6% of U.S. recipes included such instructions. Nearly one-fourth (24%, n=170) of UK cards did not include cross-contamination advice; this information was omitted in most (64%, n=80) U.S. cards.

**Significance:** The findings of this study highlight the differences in food safety communication in meal kits in different regions. While UK meal kits included more food safety messaging than the ones marketed in the U.S., there is generally a need for improved food safety risk communication. Future research is needed to produce more precise recommendations to decrease the risk of foodborne illness

## T9-02 Development of a Food Safety Culture Course for U.S. Regulators

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**Introduction:** Food safety culture is a set of shared values and norms that guides all staff in a food business that produces and provides food. Maintaining a mature food safety culture means that all staff can identify the risks associated with their products, understand why managing risks is important, and effectively manage those risks. In mature food safety cultures, individuals are expected to enact practices that represent the shared values system and identify where issues may occur.

**Purpose:** The purpose of this work was to design an introductory educational tool on food safety culture for FDA field staff to demonstrate where food safety culture intersects with FDA day-to-day work.

**Methods:** The team developed a food safety culture course composed of multiple modalities to appeal to all learning types. The first module, which was delivered asynchronously online, covered foundational food safety culture concepts. The second and third modules covered signals and indicators of mature and immature food safety cultures, consequences of immature food safety culture, and the role of personal biases in assessing food safety culture. These two modules were delivered synchronously and featured interactive discussion, breakout sessions, and exploration of case studies.

**Results:** The team delivered 24 course sessions with 1004 FDA participants, followed by two additional sessions delivered to AFDO membership. Course evaluations were administered to course participants with 466 surveys completed by FDA participants. A total of 59% of participants reported that they knew a lot about food safety culture after taking the course compared to 18% before the class.

**Significance:** Course evaluations demonstrate this was an effective course in increasing FDA knowledge about food safety culture. This is the first food safety culture course of its kind to be delivered to a U.S. regulatory agency and points to an increased awareness of the need for consideration of food safety culture in food businesses.

## T9-03 "Pearls of Wisdom": Canada's Experience Investigating Outbreaks of Norovirus in Oysters from 2017 to 2022

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**Introduction:** As water-filtering organisms, oysters are able to bio-accumulate norovirus if present in contaminated waters. Oysters may be consumed raw or lightly cooked and are a risk for norovirus illness in Canada.

**Purpose:** To summarize three multi-jurisdictional norovirus outbreaks associated with raw oyster consumption in Canada and describe their impact on the development of risk mitigation measures.

**Methods:** Multi-jurisdictional outbreaks of norovirus were identified through passive surveillance of shellfish-related illnesses. Epidemiologic and food safety data were collected and assessed to identify oysters as a likely vehicle of infection. Traceback and environmental investigations were conducted, and food and harvest site samples were tested. Product-specific Health Risk Assessments were performed to guide risk management determinations, such as shellfish aquaculture facility closures and product recalls.



**Results:** Three multi-jurisdictional norovirus outbreaks associated with the consumption of oysters were investigated in 2017, 2018, and 2022. In total, the outbreaks resulted in 846 cases comprising 352 clusters. A total of 29 shellfish aquaculture facilities were temporarily closed (10 in 2017, 5 in 2018, and 14 in 2022); 11 food recall warnings for oysters (all in 2022) were also issued. The suspected sources of contamination for each outbreak likely involved multiple factors, including discharge from fishing vessels during commercial fishing openings, sewage seepage, and environmental factors such as coastal land development. A working group comprised of the relevant jurisdictional authorities was developed to investigate potential pollution sources, and to examine how enforcement, management actions, and policy/regulatory changes can mitigate risk moving forward.

**Significance:** Norovirus is one of the leading causes of foodborne illness in Canada, and contamination in oysters continues to contribute to this burden of illness. Learning from past outbreaks, and leveraging partnerships to address the challenges posed by oyster-related outbreaks, is necessary to mitigate and manage similar outbreaks in the future.

**Purpose:** Aims of the study were to characterize Cbp strains isolated from residual food involved in hospitalized for botulism, in Northern Italy, in the last 18 years. Were investigated: i) the toxin serotype produced (A-G), ii) the minimum growth temperature and lag time duration of strains.

**Methods:** 7 Cbp isolated from home-canned vegetables and 1 from honey were i) processed in accordance with the CNRB31 protocol and the BoNTs were detected and identified using a mouse bioassay, according to the CNRB30 method; ii) isothermal curves were determined by incubating anaerobically each strain (1000 spore/ml) in TPGY at 14°C, then 12°C and 10°C; at a fixed time, the inoculated medium was plate counted using ISO 15213:2003 method. Lag time biovariability was calculated by DmFit software based on Baranyi and Robert's primary model. The mean and standard deviation of lag time is reported.

**Results:** *bont/A* target genes were detected in honey and home-canned vegetable, *bont/B* target genes were detected in artichokes, asparagus, olive, pesto, grilled zucchini and vegetable preserve; mouse bioassay confirmed the presence of Bont/A and Bont/B. Cbp strains isolated from honey, artichokes, olive, vegetable preserve were able to grow at 14°C and 12°C, while no growth was observed in the other strains. Mean lag time was 35 h (+/- 7.8) at 14°C and 124.9 (+/-24.8) at 12°C, no growth was observed at 10°C.

**Significance:** Our study highlights that foodborne botulism continues to be a public health concern, with high variability in strain behavior. Consumer education has to be implemented to avoid hazardous botulism outbreaks.

## T9-04\* Do Polysorbate 80 and Sodium Nitrite Affect Differently the Gut Microbiota of Healthy Individuals and IBD Patients?

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**Introduction:** Intestinal fibrosis is a long-term complication of inflammatory bowel disease (IBD). Changes in microbial populations have been linked with the onset of fibrosis and some food additives are known to promote intestinal inflammation facilitating the induction of fibrosis. Most of these studies have been performed in murine models or in healthy donors while the effects of food additives on the intestinal microbiota of patients suffering from IBD is less understood.

**Purpose:** The aim of this work was therefore to determine how food additives affect the intestinal microbiota of both healthy and "IBD" donors.

**Methods:** Two food additives, polysorbate 80 (P80) and sodium nitrite, were tested in a short-term (72 h) *in vitro* model of human intestinal microbiota. Three groups of donors were investigated: healthy persons (H), patients in remission of IBD (R) and patients with an active period of IBD (A). Changes in microbiota metabolome (short-chain fatty acids) were assessed by SPME-GC/MS while the evolution of microbial populations positively or negatively correlated with health, inflammation and/or fibrosis was assessed by qPCR analysis.

**Results:** Polysorbate 80 significantly and deeply decreased propionate and butyrate production in the H group. Conversely, in the R group, an increase in both propionate and butyrate production was observed. Moreover, with P80, *Ruminococcus*, correlated with increased risk of fibrosis, was increased in the H group and *Oscillospira* related to reduced risk of fibrosis, was decreased in H and A groups. Regarding the supplementation with sodium nitrite, a significant increase in propionate production in the R group and a decrease in butyrate production in the A group were observed. Sodium nitrite also decreased *Oscillospira* in the A group.

**Significance:** This study demonstrates how strongly the human microbiota can be affected by some food additives. In addition, to our knowledge, this is the first human *in vitro* study focusing on the impact of food additives on the microbiota of IBD patients.

## T9-05 Characterization of Proteolytic *Clostridium botulinum* Isolated from Residuals Food Involved in Hospitalized Cases with Botulism Diagnosis

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**Introduction:** Proteolytic *C. botulinum* (Cbp) is a group of gram-positive, anaerobic, spore-forming bacteria, capable of producing botulinum neurotoxins (BoNT). Although it is a very rare illness in Europe, Italy has one of the highest incidence rates of foodborne botulism.

## T9-06 Management Attitudes and Perceptions Towards Factors That Influence Food Safety Culture in UK-Based Small and Medium-Sized Food-Service Establishments

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**Introduction:** Internationally, food-service establishments are associated with the largest incidence of foodborne illness (FBI) and in the UK, small-to-medium-sized enterprises (SMEs) account for 43% of this sector. Food safety compliance in food service SMEs is essential for public health and minimising the risk of FBI. Creating a positive food safety culture (FSC) to optimise food safety practices may improve food safety compliance.

**Purpose:** This study aimed to evaluate SME food-service managers' attitudes and perceptions towards factors influencing FSC to inform the development of a sector-specific assessment tool.

**Methods:** Previous qualitative food-service research-informed development of an online FSC questionnaire, tailored for the foodservice sector. The questionnaire was distributed using email, social media, and industry contacts. A descriptive statistical analysis was performed using SPSS.

**Results:** Cumulatively, the majority (95%) of food-service SME manager respondents (n=45) reported a positive attitude towards food safety as a priority in their business; however, all respondents agreed the quality was the top priority. Most expressed confidence in their knowledge of food-safety measures (86%) and adequate training to manage food-safety (93%). However, 59% of managers reported compliance with legislation to be challenging, and 45% admitted difficulty in following food-safety procedures during busy periods. Whilst the majority (91-96%) of managers expressed a high level of personal responsibility for developing and implementing procedures, 28% considered records to be unnecessary. Although 78% managers indicated adequate resources were available, affordability was reported to be a factor in completing structural repairs (83%), and 69% believed structure did not impact food safety. Ninety-seven percent of SME managers expressed a clear understanding of food safety risks; similarly, the majority also perceived their customers were unlikely to get FBI from their business.

**Significance:** Findings provide valuable insight into the attitudes and perceptions of SMEs towards factors influencing FSC. Data can inform and aid the development of targeted interventions and support to improve food safety compliance and FSC in the foodservice sector.

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**3–5 MAY 2023**

**POSTER  
ABSTRACTS**

# POSTER ABSTRACTS

\*Student Award Competitor

## P1-01 Identification of Sources, Risks and Control Measures for *Salmonella* Contamination in Poultry and Pig Slaughterhouses: Case Studies

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**Introduction:** Poultry and pig meat are still an important source of the foodborne pathogen *Salmonella*.

**Purpose:** *Salmonella* contamination was studied in poultry and pig slaughterhouses.

**Methods:** In poultry slaughterhouses, samples were taken after cleaning and disinfection, and also during production. To identify the major source of *Salmonella* contamination in a pig slaughterhouse, samples were collected from the clean and unclean area and *Salmonella* isolates were typed.

**Results:** In all poultry slaughterhouses *Salmonella* was still isolated after cleaning and disinfection. The prevalence in the plucking area was 10.4%; in the evisceration room 1.5% and in the cutting area, 2.0%. On neck skin samples from the first slaughtered flock, *Salmonella* prevalence was 16.1% after plucking, 16.0% after evisceration, 23.3% after chilling, and 10.0% on thighs. Nine *Salmonella* serovars were identified with *S. Infantis* as most common (53.8%). Two major contamination risks were identified: 1) the incorrect *Salmonella* negative status of a flock led to an enormous contamination of the neck skins and the slaughter line, 2) molecular typing revealed cross-contamination from flocks slaughtered one day prior to sampling due to insufficient cleaning and disinfection.

Concerning the pig slaughterhouse, carcasses entering the clean area showed a *Salmonella* contamination rate of 96.7% in the oral cavity and 55.0% in the rectum, indicating the unclean area as major source for contamination. In the unclean area, a limited number of oral cavity samples were positive after bleeding, while a dramatic increase of positives was observed after dehairing. *Salmonella* was detected in the recycled water of the dehairing machine. Genotyping showed that similar *Salmonella* pulsotypes were present in the oral cavity and recycled water. This confirmed that the recycled water used in the dehairing machine was the major source for the carcass contamination in this slaughterhouse.

**Significance:** Sources, risks, and control measures for *Salmonella* contamination in poultry and pig slaughterhouses were identified.

## P1-02 Comparing Risk Perceptions and Self-Reported Practices of Pet Owners Providing Raw Pet Food Versus Pet Owners Providing Conventional Pet Food in Slovenia

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**Introduction:** Commercially produced and home-made raw meat-based pet diets are becoming increasingly popular among pet owners. Frequent handling of raw meat products and close contact with raw-fed pets may increase the risk of transmission of pathogenic and antibiotic-resistant microorganisms to pet owners if proper food safety practices are not implemented.

**Purpose:** To determine the prevalence of raw meat-based feeding of pets among Slovenian pet owners and compare food safety perceptions and practices between owners who feed their pets raw meat and those who do not.

**Methods:** The anonymous online questionnaire was developed and distributed to the target population via interest groups on social media platforms. Ultimately, 750 respondents were included in the detailed analysis, divided into two subgroups. The "raw group" ( $n=382$ ) consisted of respondents who feed their pets raw meat, while the "conventional group" consisted of respondents who don't ( $n=368$ ).

**Results:** Respondents in raw (67.4%) and conventional (12.3%) group differ significantly in their agreement on whether raw meat-based diet is "appropriate" for pets ( $P \leq .001$ ). Self-reported practices of hand washing, and surface cleaning were higher in the raw group ( $P \leq .001$ ). However, one-third (29.2% in the raw and 29.3% in conventional group) reported using the same kitchen utensils to prepare food for pets and to prepare their own food ( $P = .914$ ). Rinsing raw meat before preparation (46.7%) and thawing frozen raw meat for pets on the kitchen counter (42.1%) were identified as core malpractices in the raw group.

**Significance:** The first study among Slovenian pet owners found that those who feed their pets raw meat are less risk-aware and report unsafe food handling practices. The results allow comparison with pet owners from other countries and provide a starting point for tailored education campaigns with a goal to prevent serious foodborne illness and to reduce the spread of antibiotic-resistant bacteria.

## P1-03 Meal Kits in the United States: Is Food Safety Appropriately Communicated?

Alicyn Dickman<sup>1</sup>, Naomi Melville<sup>2</sup>, Joseph Baldwin<sup>2</sup>, Elizabeth C. Redmond<sup>2</sup>, Ellen Evans<sup>2</sup> and Sanja Ilic<sup>1</sup>

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**Introduction:** Improper food handling practices at home puts many consumers at risk of contracting foodborne illnesses. Meal kit delivery services are gaining in popularity. While this innovation has the potential to improve cooking skills and may lead to healthier food choices, the food safety ramifications remain to be seen. It is unknown what kind of food safety messaging and risk communication is included with the recipes that commonly contain raw ingredients.

**Purpose:** The objective of this study was to evaluate food safety instructions and prompts included in meal kits sold in the U.S.

**Methods:** Recipe cards ( $n=126$ ) were collected from commercial meal kit suppliers in the US ( $n=11$ ). A data extraction tool was designed (Qualtrics, January 2023) to capture all food safety textual and visual information integrated into the recipes.

**Results:** The majority of recipes (88.1%) omitted refrigeration advice, although all recipes included at least one ingredient requiring chilled storage. One-fifth of the cards featuring fresh produce (99.2%) did not refer to washing produce. Almost one-half (44.7%) provided no minimum internal temperature to ensure cooking adequacy. While 7/126 recipe cards (5.6%) included instructions on handwashing after handling high-risk ingredients (raw meat, poultry, fish, etc.), none instructed handwashing prior to engaging in food preparation. Several recipes included incorrect information. For instance, 2.9% provided the incorrect cooking temperature, nearly half (48%) of recipes advised to pat raw protein ingredients dry with paper towels, and 2.9% included instructions to rinse and dry raw seafood.

**Significance:** Our research shows that food safety information is currently either lacking from the meal kits or suggests practices that can increase food safety risks in domestic kitchens. Additional research is needed to design recommendations for future improved meal kit food safety instructions.

## P1-04 Development and Assessment of an Asynchronous Course on Pandemic Preparedness and One Health: A Survival Toolkit for the Next Pandemic

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**Introduction:** Following the COVID pandemic, it is clear from global contemporary news media that society needs enhanced education and understanding on multiple topics related to navigating the next pandemic. One Health offers a collaborative and transdisciplinary approach to sharing information with the goal of achieving optimal health outcomes, recognizing the interconnection between people, animals, plants, and their shared environment. A course was developed to frame topics critical to a pandemic in a One Health context.

**Purpose:** The objective of this course is to train students at the college level about health and disease issues related to pandemic preparedness in a One Health context.

**Methods:** Undergraduate students (n=187) studying in a four-year degree program across various non-science and science majors enrolled in Pandemic Preparedness (ANFS 124) as a non-required elective course that was designed and offered in Fall and Winter semesters (n=4) beginning in the Fall semester of 2021. Each class contains 14 modules, each with readings and recorded lessons. Pre- and post-assessment was gathered on all 14 modules, including but not limited to these topics: peer-reviewed science, evaluation of science literature in the media, global science agencies, microbiology, immunology, disease diagnosis, transmission, vaccine development, epidemiology, public health, microbial contamination, chronic disease, and risk assessment. Pre- and post-assessments were analyzed using JMP Student's t-test.

**Results:** Students' self-reported increased competence as they progressed through the 14 modules. Six modules were analyzed using four semesters of students' responses (scientific literacy, microbes, vaccines, pandemic/epidemic, public health, and risk assessment). Overall, Fall 2021, Fall 2022, Winter 2022, and Winter 2023 students scored significantly higher in their post-quiz than the pre-quiz ( $P < 0.0001$  for all but Winter 2023 when  $P < 0.0012$ ).

**Significance:** Information on zoonotic disease and spillover, immunizations, information spread in an infodemic, and disease transmission were well received by students in an asynchronous Pandemic Preparedness course.

## P1-05 Food Safety Intervention for Direct Support Personnel (DSP), Assisting People with Intellectual Disabilities in Everyday Life

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**Introduction:** In Sweden, people with an intellectual disability (ID) have the legal right to live in their own housing with everyday support. The residences are often organized in a form of group homes with Direct Support Personnel (DSP). The support is individually tailored but influenced by DSPs' awareness, routines, and knowledge.

**Purpose:** To study DSPs' awareness, routines, and knowledge of food safety in general, and to follow up with how a hygiene lecture affected the study participants' awareness, routines, and knowledge immediately and over time.

**Methods:** An intervention study has been carried out within a small group of DSPs at a newly started group home. The intervention has been implemented via four different steps consisting of a hygiene lecture and three surveys, one before the lecture, after the lecture, and nine months after the lecture.

**Results:** Awareness of risks for food poisoning seemed to be low for the DSPs, although it was higher in close connection with the hygiene lecture, yet it decreased again after a while. Study participants kept the information about risks with food handling given during the lecture as facts related to authentic cases and authority information, which increased their awareness in general over time. Everyday food safety routines were rather poor and affected to a lesser extent by hygiene lecture. Colleagues were perceived as an important and credible source for food safety. The hygiene knowledge in general was improved in the group home by the COVID-19 pandemic, which might have health benefits for the residents over time.

**Significance:** Only one hygiene lecture can increase DSPs' food safety awareness and knowledge, but not in the longer term. A lecture with facts supplemented with examples from the real world increases the chance of permanent food safety knowledge. The lecture should be repeated periodically with updated facts.

## P1-06 Short Educational Food Safety Videos (Infotoons) are Preferred and Perform Better Than Plain Text

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**Introduction:** Foodborne illnesses are a concern in the U.S. and across the world. Numerous studies have posited that our food supply's safety depends on a "food safety culture" in the workplace. However, establishing such a culture is challenging. It depends on constantly educating food handlers about food safety basic concepts, such as exponential bacterial growth, that require cognitively taxing abstraction levels.

**Purpose:** To aid food safety educators, we created eight short videos (~2-3 minutes) that combine real-life scenarios and animations to make abstract concepts easier to grasp.

**Methods:** We named these videos "Infotoons," and two video concepts (exponential growth and cross-contamination) were presented to college students in either text or Infotoon format to evaluate their efficacy. Half of the survey participants were randomly assigned to

watch a video for one theme and read a text for the other theme, with a flipped text/video combination for the other half. Participants were asked how much they knew about each of the themes before and after watching the video or reading the text and their delivery method preference.

**Results:** Three hundred and seventy-eight students from five different classes from three U.S. public universities (UMaine, NCSU, and URI) answered the survey. The scores after watching the videos (7.75 points on a scale of 0 to 10) were significantly ( $P < 0.05$ ) higher than after reading the texts (7.07 points). The increase in knowledge was significantly ( $P < 0.05$ ) higher for videos (3.46 points) than for texts (2.43 points). On a preference scale where 0 was a preference for the text and 10 for the video format, the average score was 8.00, showing a strong preference for the videos.

**Significance:** This preference and better efficacy are consistent with the literature and indicate that short educational videos are a viable option for food safety training and a better understanding of harder-to-learn concepts.

## P1-07 Meal Kits in the United Kingdom: A Recipe for Food Safety?

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**Introduction:** Meal-kits are boxes of fresh, measured ingredients requiring further storage, preparation, and cooking by the consumer. Growing in popularity, meal-kits promote a healthier alternative by giving the opportunity to prepare home-cooked meals using recipe cards providing step-by-step instructions.

**Purpose:** To determine the provision of food safety communication in UK-based meal-kit providers' recipe cards and websites.

**Methods:** Citizen science methods were used to obtain images and physical copies of recipe cards. An evaluation tool was developed using Qualtrics and a framework from the Partnership for Food Safety Education 'Safe Recipe Style Guide'. Content analysis was performed on UK meal-kit provider recipe cards ( $n=359$ ) and websites ( $n=7$ ).

**Results:** Although 46% of recipes referred to handwashing at the start of recipe preparation, these stated 'wash hands' with no further advice regarding hand hygiene. There were 48% of recipe cards that did not refer to handwashing during recipe preparation. Most recipes (88%) referred to washing fruit and vegetables but were not observed as frequently for herbs (51%). Of the applicable recipes ( $n=332$ ), 50% referred to storing ingredients in the fridge, but only one recipe (0.3%) referred to recommended temperatures ( $\leq 5^{\circ}\text{C}$ ). When applicable ( $n=346$ ), cross-contamination prevention advice was present in 51% of recipes. Statements regarding the cooking adequacy of high-risk foods ( $n=1306$ ) included subjective advice with 35% relating to the visual assessment of colour and 26% referring to cooking duration. Websites reviewed indicated some food safety advice but requires increased online navigation from the consumer when searching for the advice, reducing accessibility.

**Significance:** Although all meal-kit providers provided some form of food-safety related information in reviewed recipes and websites, the information was often not deemed sufficient and accessible to enable consumers to ensure food safety in the domestic setting. There is a need to understand how consumers engage with such information with further exploration required through observational research.

## P1-08 "Is That Breastmilk in the Fridge?": Mothers' Experiences of Expressing Breastmilk in the Workplace and a Thermometry Study of Communal Workplace Refrigerators

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**Introduction:** Breastfeeding is the most effective ways to ensure child health and provides half the nutritional needs up to the age of two. If mothers continue breastfeeding beyond maternity leave, UK employers are required to provide a space to express breastmilk.

**Purpose:** To explore experiences of mothers when expressing breastmilk in the workplace and to establish the temperature performance of communal workplace refrigerators.

**Methods:** In-depth interviews were undertaken with mothers who expressed breastmilk ( $n=40$ ) to explore experiences of expressing in the workplace. A thermometry study using time-temperature data-loggers was undertaken to determine temperature performance of communal workplace refrigerators ( $n=25$ ).



**Results:** Reasons for expressing in work were to continue providing "best for baby" for as long as possible. A supportive workplace was said to have a positive impact on a woman's "breastfeeding journey". However, situations where no suitable spaces to express and store breastmilk, along with the lack of workplace support were discussed. A mother described she "didn't feel like a valued student" as her university did not provide a space for expressing, which resulted in her "having to express in a car." Concerns regarding temperature of workplace refrigerators were discussed, and the lack of facilities for storing breastmilk resulted in some storing breastmilk in cool-bags during the day. A lack of space to sterilise expressing-equipment and fears over hygiene resulted in some mothers disposing of expressed breastmilk. The thermometry study established unsafe temperatures to be widespread in communal workplace refrigerators, with 92% having average operating temperatures exceeding UK recommendations (0 – 5°C) and 96% not having thermometers to check the operating temperature.

**Significance:** This study has established that workplace support should enable women to transition back into the workplace after maternity leave and express breastmilk in a suitable and hygienic environment to ensure the safety of expressed breastmilk for their children.

## P1-09 The Complexity of Articulating In-House Food Safety Culture: Perspectives from Senior Managers in Food Manufacturing and Processing Facilities

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**Introduction:** It is widely acknowledged that congruous food safety culture (FSC) is central to delivering continuously safe food outcomes to protect consumer safety, brand reputation, operational viability, and legal compliance. Thus, food safety expectations communicated by senior management ultimately shape the FSC underpinning food safety governance company wide.

**Purpose:** To explore senior manager attitudes towards, and perceptions of, company food safety expectations and FSC strategy in multiple food manufacturing and processing facilities.

**Methods:** In-depth interviews ( $n=40$ ) with senior managers in high risk, high care, and low risk food manufacturing and processing facilities ( $n=4$ ) explored FSC characteristics encompassing vision, mission, and company strategy towards food safety expectations.

**Results:** In describing current company FSC, associations between food safety expectations and consumer protection were drawn. Ensuring food products were "not going to kill anybody" and that customers were satisfied "legally and safely" was suggestive of minimum compliance attitudes towards food safety. However, "responsibility" was mentioned frequently noting that "each individual needs to look after their own aspects of food safety" and consequently, "everyone knows what their responsibility is". Managers not directly involved in food production noted that they "don't always understand every aspect" of food safety and that they may have "not done any food safety training [...] for some time". Assumptions were made about food safety monitoring and accountability ("mostly supervisors observing") and that prior "mission statements" if not abandoned, were "just not heading in the same direction".

**Significance:** Although awareness around the importance of food safety was evident, no clear articulation of company food safety expectations to support the same was provided. At best, a basic understanding of food safety on a superficial level suggests that the culture aspect was less well understood by senior managers than was anticipated, which may have repercussions for food safety behavioural outcomes on the shop floor.

## P1-10 Bruneian Consumers' Attitudes, Knowledge and Self-Reported Practices Associated with Food Safety in the Home: Implications for Culturally Bespoke Food Safety Education

Nur Arina Hj Hamidun<sup>1</sup>, Ruth Fairchild<sup>2</sup> and Elizabeth C. Redmond<sup>2</sup>

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**Introduction:** Foodborne disease (FBD) is a global concern and safe food-handling in the home is known to be important in reducing the risk of consumer illness. Little is known about consumer food safety in Brunei and available data indicates a need for consumer food safety behavioural improvement.

**Purpose:** To explore/understand Bruneian consumers' attitudes, knowledge and self-reported practices associated with food safety in the home.

**Methods:** In-depth interviews with Bruneian consumers ( $n=20$ ) informed development of a culturally-applicable food safety questionnaire to determine quantitative cognitions associated with home-based food consumption, preparation, cooking and storage. Online questionnaire distribution to Bruneian consumers occurred using call-to-action posters on social media and in consumer groups. A statistical analysis of responses ( $n=143$ ) occurred using SPSS, structured using the World-Health-Organization (WHO) 'Five-Keys-to-Safer-Food'.

**Results:** Washing chicken was a commonly reported cultural food preparation practice, implemented by all participants to "make it [the chicken] *cleaner*"; 55% believed washing raw meat/poultry and seafood removes bacteria. Frying was reportedly a common cooking practice and methods of judging cooking-efficacy reported included observing "the colour change", or "cut it open" and "estimate [time] when it's done"; 74% indicated that looking at the meat colour is a reliable way to judge 'doneness'. Cooked rice left in a rice cooker for >4 h was reportedly "typical" as rice consumed in an evening was commonly used for cooking "in the morning" for "fried rice"; 84% believed cooling food at room temperature is acceptable. Hand-washing was perceived as "very important", however some consumers admitted they "do it quickly" if "in a rush"; 23% indicated that rubbing hands together for 20 s is too long.

**Significance:** Findings indicated variable cognitive influences associated with Bruneian consumers food safety in the home that were not aligned with WHO recommended practices. Data can be used to inform targeted, culturally appropriate food safety education approaches to reduce the risk of FBD among Bruneian consumers.

## P1-11 Consumer Understanding and Self-Reported Practices Related to Date Labelling and Household Food Waste

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**Introduction:** Household waste levels in the UK have increased in recent years to 9.5million-tonnes, 70% of which is reportedly edible food. Research suggests a lack of consumer understanding concerning date-labelling and its association with food-safety are important factors that contribute to domestic food-waste behaviours.

**Purpose:** The study aims to explore consumer understanding and self-reported practices associated with food date-labelling of raw and ready-to-eat meat products in the context of household food-waste practices in Wales, UK.

**Methods:** A review of consumer date-labelling and food-waste literature (2004-2022) informed development of a quantitative tool to assess related consumer cognitions. Post-pilot, distribution of an online survey using call-to-action posters occurred to consumers working in a meat-processing site and in the general population. Statistical analyses of responses ( $n=94$ ) in SPSS were undertaken.

**Results:** Findings indicated considerable gaps in consumers' understanding of date-labelling, for-example, 10-11% consumers failed to associate 'use-by-dates' with food-safety, however, more consumers (71%) with food-sector experience equated food quality with 'best-before' datemarking, compared with 59% 'general' consumers. Many (42%) consumers did not to agree that 'use-by-dates' were the best way to determine how safe food is to eat and concernedly, 69-77% reported consumption of food past its 'use-by-date'. More consumers with food-sector experience (53%), than 'general' consumers (36%), believed they are more likely to adhere to 'use-by-dates' than 'other people' indicating perceptions of optimistic bias. A positive attitude from all consumers towards the need to reduce food waste was determined and food planning/storage behaviours reported, as well as personal responsibility for minimising food waste.

**Significance:** Some consumers from the general population and those with experience in the meat-sector demonstrated awareness and appropriate self-reported use of date-labels, however, many indicated a lack of knowledge, misconceptions and unsafe practices that may increase the risk of illness. Provision of targeted information about datemarking use and management in the home may help to reduce domestic food waste.

## P1-12 Food Safety Information Provision in UK-Based Children's Cookbooks, Online Recipes and Audio-Visual Sources

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**Introduction:** Incidence of foodborne disease prevalence among children highlights the need for and importance of food safety education. It is vital that from a young age, children are taught about food safety, to embed safe food handling habits and encourage implementation of risk-reducing behaviours. To date, limited research has been conducted to understand food safety information provision a variety of formats utilised/accessed by children.

**Purpose:** This study aimed to evaluate food safety information provision in UK-based children's cookbooks, online recipes, and audio-visual sources.

**Methods:** UK-based children's cookbooks (n=33), cookbook recipes (n=108), online recipes (n=90) with webpages (n=10) and audio-visual sources (YouTube/television) (n=50) intended for children aged 5 to 10 yrs were selected for analysis using purposive sampling. Data was captured using a structured checklist to obtain inclusion/need for food safety information according to the UK Food Standards Agency's recommended 4C's (Cooking-Cleaning-Cross Contamination-Chilling).

**Results:** Cumulatively, all children's resources evaluated provided limited food safety information. In online recipes, colour was the most common (59%) indicator cited for cooking efficacy, whereas 82% cookbook recipes provided a cooking time/temperature and 64% indicated how to check for 'doneness' using visual/textural indicators. Forty-six percent of audio-visual sources did not demonstrate or instruct how to check if food was cooked adequately. Analysis indicated that hand hygiene information was lacking within cookbooks, with 36% providing information on how to handwash and 9% advising hand-drying; < 1% of cookbook recipes advised handwashing prior to food preparation, and handwashing was required a total of 130 times during food preparation but was advised twice. Observed audio-visual sources indicated that handwashing was implemented, practices were inadequately demonstrated or explained.

**Significance:** Findings from this study highlighted children's cooking resources in a variety of formats are under-utilised sources of food safety information. There is a need to optimise such educational opportunities using age appropriate and targeted approaches to encourage adoption of risk-reducing behaviours to children and parents alike.

## P1-13 Metagenomic Investigation of Artisanal Cheeses from the Mediterranean Area

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**Introduction:** Artisanal cheeses are highly appreciated for their genuineness and organoleptic peculiarities. However, unlike industrial dairy products, the lack of standardized production procedures can make artisanal cheeses a source of biological hazards.

**Purpose:** In this pilot study we compared the metagenomes of four different types of artisanal cheeses produced in Italy, Morocco, Spain and Portugal to check the presence of biological hazards relevant as zoonotic agents and foodborne pathogens, to characterize their virulence genes as well as antimicrobial resistant genes.

**Methods:** All tested cheeses were submitted to DNA extraction and shotgun metagenomic sequencing. The obtained sequences were analyzed using MG-RAST for taxonomy, VFDB for virulence factors, and RGI for antimicrobial resistant genes.

**Results:** *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* and several species of the genus *Shigella* were identified in the tested cheeses at high relative abundances, while *Yersinia* ssp., *Clostridium* ssp. and *Bacillus cereus* displayed abundances < 1% in all samples. Virulence and antimicrobial resistant genes clustered according to the origin of the cheeses.

**Significance:** Artisanal cheeses produced in different countries of the Mediterranean area can represent potential vehicles of zoonotic agents and foodborne pathogens. Their taxonomic and functional gene composition is country specific. This study was funded by the PRIMA project ArtiSaneFood.

## P1-14 Feasibility of Using pH and Water Phase Salt (WPS) for the Characterization of Cheddar Type Cheeses in Terms of Their *Listeria* Growth Potential

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**Introduction:** European Regulation 2073/2005 requires dairy companies to assess the growth potential of *Listeria monocytogenes* in their products and to classify them as supporting or not supporting growth. This assessment is based on scientific justification and may involve theoretical or lab-based data. Among theoretical data, predictive models can be used but they require a high level of expertise and data on parameters that are not regularly monitored by dairies (e.g. organic acid concentrations). pH and WPS are suggested as an alternative means of predicting the growth potential of *Listeria* in cheese but there is a lack of detailed studies to show the feasibility of this approach for different cheese categories.

**Purpose:** The aim of this study is to explore the use of pH and WPS for assessing the growth potential of *Listeria* in Cheddar type cheeses.

**Methods:** A literature search was performed for challenge tests of traditional Cheddar/Cheedar like cheeses and Cheddar variants (reduced salt, reduced fat, etc.). A total of nine challenge test studies containing 91 datasets of relevance for this product category were found.

**Results:** All studied challenge tests concluded that Cheddar and Cheddar-like cheeses do not support growth of *Listeria*. However, there was a wide variation on the survival rate of this pathogen based on product characteristics and spiking conditions (inoculum size and point of inoculation, storage temperature, etc.). Current literature shows that for a pH  $\leq$  5.5 and WPS in excess of ~1.3%, Cheddar and Cheddar-like cheeses do not support the growth of *Listeria*. This finding agrees with Danish guidelines for the control of *Listeria* (i.e., pH < 5.5 regardless of  $A_w$ , not supporting the growth of *Listeria* in cheese).

**Significance:** The risk of growth of *Listeria* in Cheddar-type cheeses with the studied pH/WPS combinations is negligible. WPS and pH can be used as an alternative to predictive models for this cheese category.

## P1-15 In-Situ Electrochemical-based pH Control for the Analysis of Milk

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**Introduction:** The effect of pH and heating on dairy products are essential parameters for product quality control in the dairy industry. The current approach to determine the effect of pH is to alter the pH manually, and then analyse the sample for stability and processability. This approach has a number of issues including: additional cost of reagents, associated safety risks with hazardous materials, and the potential of these reagents to act as a source of contamination. For this reason, an alternative analysis method would be highly beneficial for industry to allow for acidification and analysis to be conducted in unison.

**Purpose:** Controlling the pH using in-situ electrochemical methods enables adjustments to be temporally made in the vicinity of an electrode, which also acts as the sensor device enabling electroanalysis. This approach eliminates the extra steps of sample preparation required to adjust pH.

**Methods:** In our approach, the electrochemical gold oxide reduction peak was used as a probe to monitor local solution pH at the working electrodes across a range of pHs in both buffer and the solution of interest. A current is applied to an array of "protonator" electrodes, which splits water, resulting in an excess of protons. These diffuse to the "sensor" comb of electrodes, reducing the pH in the electrode vicinity, while the bulk solution pH remains unchanged.

**Results:** It was found that milk and other relevant dairy products could be acidified without the need for additional reagents in the range of pH 6.7 to pH 4.6 and below. The range most relevant for industry was pH 6.7 to pH 5 which can be acidified on the electrochemical chip to a resolution of 0.2 of a pH unit.

**Significance:** This research has huge significance for industry as it allows for a simpler objective analysis method in the determination of dairy product quality.

## P1-16 Surveillance of Udder Health Status of a Sheep Herd and Assessment of the Quality of Raw Milk by Performing Bulk Tank Milk Analysis and Statistical Process Control

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**Introduction:** Bulk tank milk (BTM) analysis has been widely accepted as a useful tool for evaluating milk quality and monitoring udder health in cows. Bacterial and somatic cell count (SCC) determination of BTM, when performed repeatedly over a period, can provide significant knowledge. Statistical process control (SPC) combines the process performance statistics of a herd with the process performance specification criteria (milk quality).

**Purpose:** To assess the use of BTM analysis and SPC as tools to monitor udder health of a sheep herd and the quality of raw milk as a replacement of the more expensive, less convenient, and time-consuming testing of milk samples from individual animals or groups.

**Methods:** Raw sheep milk samples were collected from BTM of a commercial dairy sheep farm (Lacaune) over a year. Four samples were collected at each visit and subjected to microbiological, physico-chemical, and SCC analysis.

**Results:** The BTM results revealed that mastitis prevention and control program need to be implemented immediately since a chronic infection with high rates of mastitis was present within the herd. The Capability Index (Cpk) value supported this observation and showed high SCC in raw milk ( $0.06 < 1.33$ ).

**Significance:** When BTM analysis and SPC are interpreted within the context of the farm management practices, this information constitutes a basis for assessing current and potential milk quality and mastitis problems in a sheep herd.

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## P1-17 Sfela, a Greek PDO Cheese and its Artisanal Variants: A First Study of their Microbial Composition and Safety as Assessed by Amplicon Sequencing and Shotgun Metagenomics

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**Introduction:** Sfela is a Greek PDO white cheese ripened in brine, made in the Messinia and Laconia regional units, from ovine or caprine milk and has rarely been investigated. Sfela cheese along with Sfela touloumotiri and Xerosfeli, which are two closely related variations, were selected for this study.

**Purpose:** To unravel the microbiota of Sfela cheese and its artisanal variants and the evaluation of their safety

**Methods:** 16S rDNA amplicon sequencing analysis and shotgun metagenomics were chosen to characterize the cheese microbiome. The first method enabled a genus-level characterization of the populations. The bacterial and yeast species constituting the microbial ecosystems of the examined cheeses were identified by shotgun metagenomics analysis including identification of metagenome-assembled genomes (MAGs).

**Results:** Lactic acid bacteria (LAB) dominated all cheese samples. Among them, *Streptococcus thermophilus*, *Lactococcus lactis*, *Levilactobacillus brevis*, *Latilactobacillus curvatus*, *Lactobacillus delbrueckii*, and other LAB species were prevalent in Sfela. The yeast *Debaryomyces hansenii*, *Tetragenococcus halophilus*, and *Lactococcus lactis* were the most widespread species in Sfela touloumotiri. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* were the two predominant bacterial species in Xerosfeli. Further analysis indicated the presence of partial MAGs of *Bacillus cereus*, *Acinetobacter baumannii*, *Klebsiella oxytoca* and *Pseudomonas putida* in specific samples.

**Significance:** These findings shed light into the microbiome of Sfela cheese and its variants while indicating that further research is needed concerning their hygiene.

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## P1-18 Assessment of the Microbial Ecosystem of the Greek PDO Cheese Anevato with Metagenomic Analysis

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**Introduction:** Anevato is a traditional PDO cheese produced in the Grevena region in Western Macedonia, Greece. It is a white soft cheese produced from sheep or goat milk or mixtures of them.

**Purpose:** The objective of the present study was the identification of the microbial populations of the Anevato cheese which has not been extensively studied in the past.

**Methods:** The samples of the cheese produced in Grevena region were provided from three different Anevato cheese producers. Apart from the culture-based microbiological analysis, shotgun metagenomics were also applied for an in-depth picture of the cheese microbiome.

**Results:** The main microbial populations were Lactic Acid Bacteria (LAB) and yeasts, while only in a few samples coliforms, *Enterobacteriaceae*, *Staphylococcus* spp., *Escherichia coli* and *Pseudomonas* spp. were identified. The shotgun metagenomics analysis allowed species level identification of the microbiomes analyzed. In these samples, the predominant identified species were LAB like *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactococcus raffinolactis*, *Lactobacillus helveticus*, *Lactiplantibacillus plantarum*, *Streptococcus parauberis* etc. The yeast species identified were identified at a low abundance. *Kluyveromyces lactis* and *Saccharomyces cerevisiae* were present. Also, metagenome-assembled genomes (MAGs) were predicted. In addition, several potential spoilage microbes were detected.

**Significance:** Shotgun metagenomics could provide us with crucial information about the microbial ecosystem of Anevato cheese. This information can help us to identify novel starters and adjuncts which could be appropriate for this type of cheese. Furthermore, it could be employed to standardize the production of this cheese and perhaps lead to an extended shelf life.

## P1-19 Precision and Data Analysis Approach for Increasing the Effectiveness of Rabbit Meat Production

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**Introduction:** In this paper, we describe – through the characteristics of industrial rabbit meat production – the digitalisation of agri-food sector, its tools and approaches. Precision farming coupled with data analysis enables the exploitation of the opportunities for increasing production effectiveness. Rabbits are susceptible to a wide range of pathological disorders, so timely identification of the different risk factors, causes and conditions is highly relevant from a health, safety, economic, and welfare perspective.

**Purpose:** Our aim was to find correlation between environmental parameters and animal performance and mortality data. These correlations in intensive farming are known in theory, but their effects are not so easy to detect and quantify.



**Methods:** The KNIME application was used for data analysis, using 32 fattening rabbit rotations (age groups), which is about 10,000 to 12,000 rabbits per rotation. For the analysis we used linear and rank correlation with  $P=0.01$ , and graphical visualisation to identify anomalies.

**Results:** One important factor was humidity. Based on the results, humidity needs to be kept within very narrow ranges. According to the analysis results, the annual losses can be reduced by 20 to 30%, which may be a more cost-effective approach compared to the current husbandry practices.

**Significance:** The environmental effects are not immediate but appear with a delay of several days or weeks, and in most cases, they do not take the form of mortality but of reduced performance. In the latter case, it is difficult to know when in a given rotation the animals have been affected by the environmental factors resulting in a performance loss. Continuous and detailed measurements of environmental parameters, in parallel with performance indicators (body weight gain, specific feed intake metrics, etc.), are very helpful in identifying the occurrence of an environmental effect and in predicting the possible outcomes.

have shown some progress in adulteration detection - however these rely on robust reference databases to represent the variation that can occur in honey. Molecular methods such as DNA barcoding have shown great promise in identifying plant DNA sources in honey and could be applied to detect plant-based sugars.

**Purpose:** We aim to investigate the suitability of using DNA markers for sugar adulterant detection in honey, with the goal of developing novel methodology for honey authentication.

**Methods:** DNA markers were designed for common sugar syrups (corn, rice and sugar beet) and tested for specificity using plant controls. DNA was extracted from sugar syrups and tested with qPCR to assess the presence of plant DNA. Pure honey was spiked with syrup at different levels of adulteration to evaluate the suitability of the method and determine LoD.

**Results:** Plant DNA was detected in all the sugar syrups tested using commercial DNA extraction kits. Furthermore, the markers for corn and rice were successfully amplified in syrup extracts. The rice marker was detected at a 10% adulteration level, with lower concentrations yet to be tested, showing that the method is highly sensitive.

**Significance:** We demonstrate that DNA barcoding can be used to detect common sugar adulterants in honey. This could be applied as a robust test to confirm the species origin of the sugar alongside current screening methods to improve existing honey authentication tests.

## P1-20 Monitoring of Genetic Diversity and Antimicrobial-Resistance of Shiga Toxin-Producing *Escherichia coli* (STEC) from Food in Northern Italy

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**Introduction:** Shiga toxin-producing *Escherichia coli* (STEC) are a subset of *E. coli* able to cause a spectrum of manifestations in humans, from mild diarrhoea to haemorrhagic uraemic syndrome (HUS), depending on the set of virulence genes harboured. In EU, STEC infections are notifiable, but since the relationship between serotype and disease potential is generally poor, it is fundamental to characterize isolates from possible sources at a deeper level, to support evidence-based control strategies.

**Purpose:** The aim of this study was to describe the genetic diversity of STEC isolated from food during routine monitoring in Northern Italy by reviewing virulence and antimicrobial resistance genes (ARGs).

**Methods:** Isolates identified as STEC by ISO/TS 13136:2012 were whole genome sequenced on the Illumina MiSeq platform. Using the tools available on ARIES public Galaxy server and on the Center for Genomic Epidemiology (CGE), sequence type, serotype, Shiga-toxins subtypes, virulence genes, and resistance genes were determined.

**Results:** A total of 56 isolates from dairy products (n=38, 68%), meat (n=16, 29%), barley and water (one sample each) were successfully sequenced. Forty-four serotypes were identified, with serogroup O26 accounting for 21% of isolates (n=12, only dairy products), followed by O100 and O2 (7%, n=4 each), and O157 and O177 (5%, n=3 each). Thirty-five isolates (63%) harboured *eae* gene, characterizing enterohaemorrhagic *E. coli* (EHEC), while 30% of the isolates (n=17) harboured haemolysin A (*ehxA*) gene. ARG prevalence was low, however, colistin resistance gene *mcr-9* was observed in three isolates (5%), while quaternary ammonium resistance genes *qac*, observed in four isolates (7%), were associated with the presence of sulphonamide-, tetracycline-, aminoglycoside-, and phenicol- resistance genes.

**Significance:** Gaining insight on the bacterial population from possible sources is crucial to the implementation of effective interventions against STEC. The inclusion of WGS generated data in source attribution models could refine the identification of the most burdensome sources.

## P1-21\* A Novel Method for Sugar Adulteration Detection in Honey Using DNA Barcoding

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**Introduction:** Honey is a valuable and nutritious food product, but it is susceptible to fraudulent practices such as dilution with cheaper sugar syrups. Honey authentication is of the utmost importance but the current accepted method for adulterant detection (isotope-ratio mass spectrometry (IRMS)) is unsuitable to detect all types of sugar, with C3 sugars such as rice and sugar beet going undetected. Additionally, IRMS is not able to distinguish the plant-based origin of the sugar. Other techniques such as nuclear magnetic resonance (NMR)

## P1-22 Impact of COVID-19 on Food Fraud and Mitigating Strategies of the UK Food Supply Chain

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**Introduction:** COVID-19 had shown the vulnerability of the food supply chain and fraudsters may have taken advantage of the pandemic whilst the population needed a continuous supply of safe and quality food.

**Purpose:** This study aims to investigate food fraud during COVID-19 and UK food supply chain's mitigating strategies against fraudulent activities.

**Methods:** A mixed-method approach including survey and semi-structured interviews were conducted among UK food businesses. Two hundred and two agri-food businesses responded to the survey and 15 semi-structured interviews were conducted. A two-step cluster analysis was conducted to classify food businesses according to food fraud mitigating strategies. Log-likelihood was used as a distance measure and independent t-tests uncovered statistically significant differences between clusters.

**Results:** Cluster 1 (n=94) was made up mostly of mixed food businesses, post-processing stage, and operated in more than one country. Cluster 2 (n=108) consisted of animal and plant-based food businesses operated within farm and processing stages. Cluster 1 was more likely to carry out preventative strategies such as increased testing (t[204]=6.28,  $P<0.001$ ), vulnerability assessments (t[204]=3.45,  $P<0.001$ ), and increased monitoring of the supply chain (t[204]=6.96,  $P<0.001$ ). The majority of the food businesses did not experience food fraud incidents during COVID-19. Two thematic domains and ten sub-themes were identified from the data set. There was a heightened sense of anticipation and preparation for increased fraudulent activities during the pandemic. The main risk-mitigating strategies included: horizon scanning, developing and maintaining supplier relationships and assurance, understanding product characteristics & testing capabilities, and conducting vulnerability assessments and training.

**Significance:** This is the first empirical study on food fraud and mitigating strategies of the UK food supply chain during the pandemic. Our findings provide evidence for food regulatory authorities and best practices to protect the UK food supply chain against food fraud during exogenous shocks like COVID-19.

## P1-23 One Health: From Human to Food Safety, Natural Sanitizer Could Spread the Difference

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**Introduction:** Chemicals represent a real issue in Human Health, Veterinary, Agronomics and Food Safety. Many ways have been explored to identify chemical substitutes. Nature leads us in a direction: animal and vegetable cells indeed produced natural hypochlorous acid, which is not dangerous for their bodies or tissue but extremely powerful against biological agents.



**Purpose:** One Health is the goal of future medicine. This case study involves human health and food safety that aims to use a natural sanitizer to prevent biological hazards while avoiding the use of common chemicals with environment impact.

**Methods:** On the human health side, hypochlorous acid was used in a Dental Cabinet in direct contact with sterilized dental surgical instruments and also with a contaminated set after used in a human mouth. Both tools were than tested with a swab for ambient bacteria (36°C) and ambient mold. The same practice was used on surgical gloves (clean and contaminated). On the food safety side, inside a hotel restaurant a swab was used after cleaning with hypochlorous acid a food contact surface used to prepare meat products and to test a clean steel surface, for the same biological agent.

**Results:** In both cases, 100% of clean tools (surgical instrument, gloves) and surfaces (food contact surface) in direct contact with hypochlorous acid resulted in ambient bacteria and mold <10 CFU. On contaminated tools, hypochlorous acid was able to reduce a known bacterial contamination of 200 CFU to < 10 CFU and on contaminated surface from 40 CFU to < 10 CFU. Mold was not detected (equal in both case < 10 CFU).

**Significance:** This case study demonstrates that alternative ways are available to manage the spread of biological hazards using a One Health approach, preventing bacterial resistance and at the same time respecting the environment.

**Methods:** We studied the guideline published and available on official website in USA, New Zealand, EU and also published by the GFSI technical working group or other GFSI standard. We also assessed a group of 10 major Food Industries in Italy with more than 250 employees and a maximum of 600 employees to discover their starting point.

**Results:** EU is the first country with the mandatory requirement of Food Safety Culture but with a strong lack in implementation experience. In US, the FDA launched The New Era for Smarter Food Safety, rich in guidelines and tools available for the stakeholders; Australia and New Zealand also released guidance tools for industries. In EU, a communication was released in September 2022 but no national guidance was released. Industries in touch with international noted having only one way: using the GFSI standard to try to understand how to plan and measure this topic.

**Significance:** EU countries have work at a different speed and many times are not coordinated to implement new topics. In Italy, lack of Officials and lack of their training represent a big challenge for industries to be firstly directed by them and then enforced.

## P1-24 Similarities and Differences between U.S., Canada and EU Food Safety Regulations

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**Introduction:** FDA FSMA introduced different regulations starting in 2015. Preventive controls and new GMPs characterized this new set of regulations for the major Domestic and Foreign Food Manufacturers; Canada answered through the CFIA Safe Food for Canadians Regulations that introduced new GMPs and one Preventive Control since January 2019; EU received all this news through the Codex Alimentarius with the EU Reg. 382/2021 in March 2021.

**Purpose:** Describe misunderstandings and false interpretations between European and North American Food Safety Law.

**Methods:** A set of different EU Food Industries based in Italy involved in export to North America was assessed to identify their difficulties regarding US FSMA and Canadian SFCR comprehension and requirement implementations. A Gap Analysis was conducted on two group of 10, one with GFSI standard and one with only EU HACCP in place, studying all non-conformities released to them by US FSVP Importers and Canadian Importers about their Food Safety Program.

**Results:** Versus USA, 99% of all Food Industries assessed (N. 20) had no knowledge that the new US GMPs introduced a mandatory training; in 80% of both groups, preventive controls was identified as equal to a common HACCP CCP; 80% of both groups do not know Hygienic design. Versus Canada, 100% of Industries do not know the new SFCR and new Importers role. Many food sectors (e.g., meat and cheese) offer issues regarding Customs. In EU, Industries are struggling to understand how to implement Food Safety Culture. In the EU, countries have different rates of comprehension and adaptation to new international law.

**Significance:** Training and updates of National Official Authority are needed in EU to help Food Industries comprehend different regulations. U.S. and Canada are working better in that way.

## P1-25 Food Safety Culture: Summary from West to East Experiences, Passing through the EU Mandatory Application.

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**Introduction:** With the EU Regulation 2021/382 published in March 2021, EU introduced by law a new mandatory requirement for Food Business Operators. This regulation was released after Codex Alimentarius Food Hygiene guideline was updated in September 2020. Food Safety Culture represents the major news, known by some Europeans only thanks to GFSI Standards, but it is not commonly implemented.

**Purpose:** Many examples are available on how to implement this new topic from mandatory regulations by US FDA, Australia, and New Zealand, and also through GFSI standard and Commercial Restaurant Standard.

## P1-26 Food Production Establishments' Financial Situation Associates with Food Control Inspection Grades in Finland

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**Introduction:** Many food business operators (FBOs) repeatedly violate food safety legislation and thereby compromise food safety and cause a health risk for consumers. It is therefore important to develop strategies to identify FBOs that are at risk of repeatedly violating food safety regulations. One option to target these FBOs could be the use of financial indicators.

**Purpose:** The purpose was to investigate the association between the financial situation of food production establishments and food control inspection grades in Finland. Our hypothesis was that a poor financial situation of the FBO is associated with poor compliance with food safety regulations.

**Methods:** Of all meat, fish and milk establishments inspected between 2016-2020 in Finland (n=612), establishments repeatedly violating food safety (n=142; case definition: non-compliances in recurring years) were identified from inspection reports, and randomized matched controls were chosen among establishments not violating food safety repeatedly. Due to missing economic data and stringent requirements for case-control pairs, 45 case-control pairs were formed. Economic indicators describing viability, liquidity, and solvency were created and used in a logistic regression model to explain odds of the FBO belonging to the case group.

**Results:** The analyses showed that when the FBO's operating profit describing viability was in the best-performing quartile, the odds for belonging to the case group were statistically significantly lower than the odds of FBOs in the weakest quartile (OR=0.22, P=0.04). Therefore, low viability of an establishment indicates an elevated risk of recurring violations.

**Significance:** Knowledge of the viability of the FBOs could be used for mapping FBOs that are in a higher risk of repeatedly violating food safety. This could be utilized in targeting food control inspections.

## P1-27 Front-of-Pack Nutrition Labelling: Global Outlook

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**Introduction:** Front-of-pack (FOP) nutrition labelling is among a suite of public health tools available to policymakers and regulators to combat obesity and diet-related non-communicable diseases (NCDs). From LATAM to Europe, from Canada to Middle East, India and Australia, public health agencies have proposed forms of FOP labelling to communicate nutrition values more effectively to consumers.

NCDs are intrinsically complex and multifactorial, often driven by stress, lack of time, limited budget, decision-fatigue and not necessarily by lack of information. Regulators have decided that the clear, immediate, and balanced expression of the main nutrients on the front-of-pack can help consumers to make better choices.

**Purpose:** If the answer is FOP labelling, the question we're left with is: what should FOP labelling look like?

To date, most countries that have FOP frameworks have created new ones. The multiplicity of FOP labels that have been proposed or enacted is raising the issue of the harmonization for industry and for consumers. In a global society, how can a consumer understand tens of different labelling schemes? And are these schemes damaging to international trade? Is it possible that certain schemes might arbitrarily discriminate against certain food categories?

**Methods:** The fight between colour-coded schemes and less suggestive ones is particularly harsh in Europe, where the EU Commission intended to propose new legislation by the end of 2022. Powerful countries are sponsoring different options. In Canada, a new scheme will be mandatory by 1st January 2026. The speakers will offer insights on the problems and opportunities with different schemes and practical comparative examples.

**Results:** The objective of the panel is to summarize the status quo and analyse possible ways forward.

**Significance:** This panel will be of interest to anyone working in public health and safety, food law, regulatory compliance, and industry.

## P1-28 Standardization of Two Real Time Methods to Detect *Alexandrium* spp. and *Alexandrium minutum* for Sea Water Controls in Italian Molluscs Breeding Areas

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**Introduction:** Diffusion of phytoplankton, potentially producing biotoxins, is becoming more concrete by the climate changes that have occurred in recent years. Molluscs in contaminated areas could accumulate toxic algae, becoming a serious risk for consumers. Many researchers are therefore focused on new and suitable analytical methods for algae and toxin detection.

**Purpose:** The aim of this work was the set up and standardization of two methods for the identification of *Alexandrium* spp. and quantification of *Alexandrium minutum*.

**Methods:** The set-up activities were focused on Real Time PCR (RT-PCR) based on sybr green technology, to verify the methods performances. For detection and quantification, performances were evaluated according to the ISO standards 16140-2:2016 and 22118:2011, and OGM Minimum Performances Requirements guidelines (2015) for the calculation of different parameters, including limits of determination (LOD) and quantification (LOQ). After the validations, both methods were applied to sea water samples, (aggregated by two geographic coastal macro-areas, named A and B) collected in Italy during the 2020-2022 period; seawater was collected by hose sampler (integrated sampling) or by net, only the first were pooled. Also reference microscopical method was applied and results were compared.

**Results:** The LOD for both methods was 10<sup>2</sup> cells/ml, and no cross-amplification was detected. For the *Alexandrium minutum* quantitative RT-PCR the LOQ was 10<sup>2</sup> cells/ml, R<sup>2</sup> for linearity evaluation was 0.98, and RSDr was > 35%. In aggregated tubes samples from both areas *Alexandrium* spp. were identified, according to the microscopical reference method; the species *Alexandrium minutum* was detected only in area B. In 100% of net samples were positive for *Alexandrium* spp.

**Significance:** The validated methods could support national competent authorities for official controls, in particular in mussel-farming areas most at risk of algal blooms. Also, they can be a starting point for exploiting new generation genotyping technologies useful for epidemiological studies.

## P1-29\* Biopreservation in Spanish Salchichón Artisanal Production: The Effect of Lactic Acid Bacteria on Microbial Hazards

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**Introduction:** *Salchichón* is a Spanish dry-cured fermented sausage especially linked to the artisanal production. The microbiological safety of this artisanal product has been mainly compromised by the presence of *Salmonella* spp. or *Listeria monocytogenes* (LM). The addition of microbial starter cultures in dry cured-fermented sausages can inhibit the growth of foodborne pathogens, where lactic acid bacteria (LAB) represent the main competitors.

**Purpose:** To investigate the fate of *Salmonella* spp. and LM, both in LAB and non-LAB added artisanal *salchichón* samples.

**Methods:** Formulated meat batter, with or without added commercial LAB cultures (ca. ≈ 6 log CFU/g), was stuffed into permeable plastic bags by vacuum-packaging (30-35mm Ø) for producing lab-scale *salchichón* samples (80 g). The samples were inoculated with a three-strain cocktail of *Salmonella* spp. and LM (ca. ≈ 6-7 log CFU/g), grown in modified TSB containing salt (25g/kg) and sodium nitrite (0.15g/kg), at 12°C for 5 d. The physicochemical parameters (pH, a<sub>w</sub>) and microbial counts (LAB and pathogens) of samples were monitored during fermentation and drying (1 5d, 15°C).

**Results:** *Salmonella* grew in non-LAB samples (mean population increase: 1.05 log CFU/g) reaching a maximum of 8.25 log CFU/g, but growth was inhibited in LAB inoculated samples. LM could also grow in samples, describing differences between LAB and non-LAB inoculated samples, both in population increases (1.96 and 1.70 log CFU/g, respectively), lag phases (36.32 and 150.02 h, respectively) and maximum growth rates (0.008 ± 0.003 and 0.011 ± 0.007 log CFU/g, respectively). Despite this, LAB final concentrations ranged from 8.71 to 9.18 log CFU/g in all evaluated samples. The a<sub>w</sub> of samples decreased over time reaching final values of 0.960, while the pH slightly increased from 5.22 to 5.84.

**Significance:** These findings may be of interest for implementing measures against potential microbial hazards during the artisanal *salchichón* manufacturing, which would increase the microbiological safety of these products.

## P1-30 Validation of Traditional Crust Pizza Baking Process and Thermal Inactivation Kinetics of *Salmonella* and Shiga Toxin-Producing *Escherichia coli*

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**Introduction:** *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) are known to survive in low water activity raw ingredients such as wheat flour under dry conditions for many weeks and months. Therefore, it is vital to validate the pizza baking process to mitigate harmful pathogens entering the finished food products and to prevent foodborne illness outbreaks.

**Purpose:** To validate traditional crust pizza baking process to control *Salmonella* and STEC contamination, and to determine the thermal inactivation kinetic (*D*- and *z*-values) parameters of *Salmonella* and STEC in traditional crust pizza dough.

**Methods:** Two independent laboratory-simulated experiments were carried out to validate pizza baking process to control *Salmonella* and STEC contamination, and to determine the thermal inactivation kinetic (*D*- and *z*-values) parameters of *Salmonella* and STEC in traditional crust pizza dough. To prepare the pizza dough, two batches of wheat flour were initially mist inoculated with a five-serovar *Salmonella* cocktail and a seven-strain STEC cocktail. The traditional crust pizza was baked at 500°F (260°C) for 12 minutes using a conventional kitchen oven followed by 15 minutes of ambient air cooling.

**Results:** Both studies validated that a typical pizza baking process with an internal temperature of 209°F (98.3°C) for 12 minutes results in 5 log reductions in the *Salmonella* and STEC population. The *D*-values for *Salmonella* in pizza dough at 56, 59, 62°C were 23.2 ± 1.82, 7.5 ± 0.32, and 2.0 ± 0.15 min, respectively. The *z*-value of *Salmonella* was 5.7 ± 0.27°C. Similarly, *D*-values for STEC in pizza dough at 55, 58, 61°C were 49.5 ± 4.10, 15.3 ± 0.68, and 2.8 ± 0.31 min, respectively and the *z*-value of STEC was 4.8 ± 0.16°C.

**Significance:** The *D*- and *z*-values determined in this study will help researchers and food processors in optimizing preventive controls to ensure the safety of finished food products.

## P1-31\* Antibiotic Resistance and Virulence of *Arcobacter butzleri* in the Large-Scale Poultry Slaughtering Chain

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**Introduction:** *Arcobacter butzleri* is a Gram-negative bacterium identified worldwide as a zoonotic pathogen. The ingestion of contaminated food is considered the main route of transmission to humans. *Arcobacter butzleri* has been isolated from several meat supply chains, including the poultry industry. Therefore, poultry products and their production chain represent the main transmission routes of this microorganism.

**Purpose:** The present study aimed to assess the antibiotic resistance and to characterize the virulence capacity of *A. butzleri* isolated from broiler carcasses during slaughtering and from the slaughterhouse surfaces.

**Methods:** One-hundred seventeen isolates were examined for their antimicrobial resistance to different antibiotic concentrations. After selection of the most resistant and susceptible isolates, infectivity on mucus-secreting human cells (HT29-MTX-E12) was tested, as well as their capability on forming biofilm.

**Results:** All isolates showed resistance to at least one antibiotic, highlighting a multi-resistance phenomenon. The greatest resistance was found to ampicillin (98/117 isolates). 73% of the isolates were resistant to more classes of antibiotics. The results showed that *A. butzleri* from slaughterhouse surfaces were more resistant to antibiotics than those from broilers. All the isolates were able to infect the HT29-MTX-E12 cells and displayed moderate biofilm production. The colonization and the biofilm production abilities were not correlated to the isolation sources.

**Significance:** The antibiotic resistance detected is of remarkable relevance considering the possible transmission of resistance factors to humans. Subsequent whole-genome sequencing analyses will be conducted to understand the genomic traits correlated to the high antibiotic resistance of strains and to their persistence. The results obtained highlight the importance of increasing optimization actions in slaughter processes to reduce the incidence *A. butzleri* considering the risk to which the population is subjected.

## P1-32 Temperature Level of Domestic Refrigerators: The Italian Experience

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**Introduction:** Temperature is considered one of the most important factors capable of determining and influencing the multiplication and survival capacity of microorganisms in food, in particular in those ready-to-eat (RTE). Compliance with temperatures during all stages of food production, transport, and marketing is a responsibility of the Food Business Operator (FBO), as indicated by the EC Reg. 852/2004 and 853/2004; on the contrary, compliance with the domestic storage temperature is due exclusively to the diligence and microbial risk awareness of the final consumer.

**Purpose:** The project aimed to define the average temperature level of domestic refrigerators related to the geographical, seasonal, and demographic characteristics of Italy; at the same time the project provided useful information to the competent Health Authorities, FBOs, and citizens.

**Methods:** On the basis of the number of households in Italy (approximately 16 million) we consider a list of 800 families (target population). For measuring the temperature inside the domestic refrigerators and outside we used specific dataloggers capable of continuously detecting and recording the temperature value.

**Results:** Between January 2019 and February 2020 1,516,325 surveys were carried out inside 761 refrigerators, and 505,440 surveys outside them. The average temperature was 7.4°C (sd 1.8°C). The results broken down by probe position showed that the temperatures recorded at the top and bottom of the refrigerator were very similar, averaging 7.0 and 6.8°C respectively, while the temperatures recorded in the refrigerator door were higher (average 8.3°C).

**Significance:** To determine the shelf life of RTE foods in a scientifically sustainable way, the storage conditions must reflect the reasonably foreseeable conditions in which the food will be stored also at home and up to consumption. The *Listeria monocytogenes* risk assessment in RTE foods indicates the inadequate storage temperature of food as a determining factor for the increase of listeriosis at the human level.

## P1-33 Evaluation of HACCP Implementation in Food Manufacturing Companies in the Middle Eastern Region

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**Introduction:** The way that food is produced and distributed has undergone fundamental changes in recent decades particularly in Dubai and the Middle Eastern region. The food safety area has become more complex, driven by widespread changes in methods of food production and processing, coupled with rapid increases in global food trade and increased tourism. Consumers today are demanding more meaningful information about food safety and quality. To meet this demand, some companies are engaging third-party audit bodies to provide greater assurance that their products meet quality and safety requirements.

**Purpose:** The purpose of the study was to evaluate the level of implementation and operation of hazard analysis critical control points (HACCP) and Prerequisite Programme (PRPs) as per the Codex Alimentarius commission protocol of 12 logical steps and Codex Good Hygiene Practices (GHP)

**Methods:** Both qualitative and quantitative analysis techniques of in-depth interviews, observations and review of documents were used in this study to complement each other. The triangulation method used in this research was to look at the problems from different angles. Five cluster random samples were collected from the sampling frame of 112 food manufacturing companies of Dubai Municipality Food Control Department (DM FCD) list.

**Results:** Research identified lower compliance rates of Good Hygiene practices (PRPs) which compromise 37.4% for the sampled factories and 31.8% compliance rate for HACCP protocol logical steps. A number of barriers exist to the successful implementation and operation of HACCP and also perceived benefits. Barriers included various aspects like difficulties in identifying hazards and inadequacy of knowledge.

**Significance:** Findings from this study provide insights into a fairly new but evolving research area of HACCP implementation in the food manufacturing sector in the Emirates. The outcomes of this study are expected to have national implications for the enhancement of food safety management system implementation through effective training and enforcement.

## P1-34 How European Food Processors are Responding to Regulatory and Environmental Changes

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**Introduction:** Many changes have occurred in the scientific and regulatory environment for food processors in the past decade. Processors have had to significantly change many of their daily procedures, including processing, testing, training, and reporting. This situation has created a new set of challenges and responses from food processors. Much has been written about what processors may be or should be doing, but it is important to find out what they have actually been doing.

**Purpose:** The purpose of this survey study was to better understand these changes and responses implemented by food processors and the areas in which they plan to focus their resources over the next few years.



**Methods:** *Food Safety Magazine* conducted a series of surveys over a three-year period ending in June 2022, involving more than 1,000 food processing locations throughout Europe. Survey questions were asked about current food safety practices, how these practices have changed, and where processors plan to make further investments over the next two to five years.

**Results:** Processors have been making numerous changes to their operations, including the development of Hazard Analysis and Risk-Based Preventive Controls programs, but they report prioritizing upgrades to their employee training programs, putting additional resources into microbiological and pathogen control programs, increasing outsourcing to commercial laboratories for their testing and enhancing supplier compliance and supply chain programs, especially with their international suppliers. The data also show that processors are investing more in the validation of processes than a reliance on analytical testing. Recent results have also included processors' responses to the disruptions brought on by the COVID pandemic.

**Significance:** These data suggest that regulatory and technical challenges have significantly changed food processing practices since the implementation of FSMA, with recovery from the pandemic accelerating many of those changes and creating new ones. A better understanding of these changes is important for other stakeholders, including processors, regulators, and the service companies who support the industry.

**Methods:** The occurrence of microbial pathogens including human enteric viruses (norovirus GI and GII, rotavirus, astrovirus and hepatitis E virus) was investigated along with viral indicators (somatic coliphages, crAssphage and Pepper Mild Mottle Virus), *Escherichia coli* and antibiotic resistance bacteria (ARB) in six WWTPs over one year. Viral detection was performed by RT-qPCR and qPCR. In parallel, culture methods were used for *E. coli*, ARB, and coliphage determination.

**Results:** Titers of coliphages ranged from  $5 \times 10^4$  to  $2.5 \times 10^6$  PFU/L in influent, from  $1 \times 10^2$  to  $8.6 \times 10^6$  PFU/L in effluent wastewaters, and from  $2 \times 10^1$  to  $6.5 \times 10^6$  to PFU/g in biosolids. In effluent samples used for irrigation, *E. coli*, ARB, norovirus GII and rotavirus levels showed ranges of  $5 \times 10^2$  -  $7.9 \times 10^8$ ,  $5 \times 10^2$  -  $6.5 \times 10^5$  CFU/100 mL,  $1.4 \times 10^5$  -  $1.1 \times 10^7$  and  $5.5 \times 10^5$  -  $4 \times 10^8$  gc/L, respectively. Biosolid levels of *E. coli*, ARB, norovirus GII and rotavirus ranged from  $3.5 \times 10^3$  -  $7.4 \times 10^6$ ,  $5.2 \times 10^2$  -  $6.5 \times 10^5$  CFU/g,  $2.7 \times 10^5$  -  $4.9 \times 10^7$  and  $1.6 \times 10^5$  -  $8.4 \times 10^8$  gc/g, respectively.

**Significance:** Despite of the microbial load reductions detected in effluent compared to influent wastewaters ( $2-3 \log_{10}$  on average), reductions of between upstream and downstream wastewater do not comply with the European regulation (EU) 2020/741 on minimum requirements for water reuse for irrigation or 86/278/CEE for sludge spreading to land.

## P1-35\* Validation of Interventions in Commercial Beef Processing Using the TEMPO® System

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**Introduction:** In-plant validation data of microbial interventions is required to support the Hazard Analysis and Critical Control Point (HACCP) system implemented in commercial beef facilities to evaluate the efficacy of CCPs put in place to reduce microbial contamination on the final meat product.

**Purpose:** The objective of this study was to validate two microbial interventions, lactic acid and Citrilow™, on hot and cold beef carcasses by quantifying microbial levels using the TEMPO® System.

**Methods:** Hot carcasses, exiting the harvesting floor, went through a lactic acid intervention at 2 to 5%, and cold carcasses, prior to entering the fabrication floor, went through a Citrilow™ intervention (combination of citric and hydrochloric acid) at a pH range of 0.5 to 2.0. A total of 240 carcass swabs were taken for each validation, over four different sampling events, using pre-moistened swabs. Carcasses were randomly swabbed before and after treatment ( $n = 30$  swabs/sampling point) over a 200 cm<sup>2</sup> area at the inside round and inside shank. Samples were quantified using the TEMPO® System for aerobic counts (AC), *Enterobacteriaceae* (EB), and *Escherichia coli* (EC). Statistical comparisons were done using a *t*-test analysis.

**Results:** Overall, results indicate that there was a reduction of indicator bacteria, after the use of interventions, for all three microorganisms validating the microbial interventions used on hot carcasses and cold carcasses in this commercial beef facility. AC counts were significantly reduced by 0.89 and 1.90 log CFU/cm<sup>2</sup> for hot carcass and cold carcass, respectively ( $P < 0.001$ ). EB counts were significantly reduced by 0.10 and 0.97 log CFU/Sample for hot carcass and cold carcass, respectively ( $P < 0.05$ ). Lastly, EC counts were significantly reduced by 0.47 log CFU/Sample for cold carcasses ( $P < 0.001$ ).

**Significance:** The TEMPO® System is a fully automated enumeration system that offers a fast turnaround time for accurate results, which makes it an effective tool for validating microbial interventions in commercial beef facilities.

## P1-36\* Monitoring of Microbial Emerging Pathogens Reveals Potential Risk for Treated Wastewater and Biosolids Reuse

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**Introduction:** Water reuse is necessary for saving water resources, reducing the costs and energy involved in water resource management, and lowering environmental impacts. Safe water reuse requires taking control of the pathogens, metals and other parameter levels in wastewater treatment plants (WWTP). The presence of microbial pathogens of emerging concern in these water systems is an issue due to their transfer to food-webs and the possibility of causing adverse effects on the environment and human health.

**Purpose:** This study aimed to assess the risk associated with microbial pathogen contamination of reclaimed wastewaters and biosolids.

## P1-37 Temperatures in Domestic Refrigerators: An Important Parameter to Consider in Shelf-Life Studies

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**Introduction:** *Listeria monocytogenes* (*Lm*) is a pathogenic bacteria able to grow at refrigerated temperatures, widely distributed in the environment and thus susceptible to contaminate various food products of which Ready To Eat (RTE) foods are a particular high-risk category.

Regulation (CE) 2073/2005 defines food safety criterion for RTE foods. To comply with these criterion, food business operators (FBOs) could conduct studies to evaluate the growth of *Lm* during shelf life under reasonably foreseeable storage conditions.

Thus, having knowledge of the temperature of domestic refrigerators (last part of the cold chain of refrigerated products) is a key factor for ensuring food safety.

**Purpose:** The goal of this study was to gather available data on domestic refrigerator temperatures and to update the European Technical Guidelines for assessing shelf life of RTE foods related to *Lm*.

For this purpose, the European Union Reference Laboratory for *Lm* launched an inquiry in 2019 to National Reference Laboratories in order to collect data from national surveys or studies conducted on domestic refrigerator temperatures in the EU. In parallel, a review of scientific literature published from 2002 to 2020 was performed.

**Methods:** The 75th and 95th percentiles of refrigerator temperatures were determined from raw data using R software, obtained directly from the person in charge of the study, estimated from graphical representations of the temperature's distribution or calculated using numerous simulations based on a normal distribution of the temperatures (mean and standard deviation).

**Results:** Data collected from nine national surveys and 16 scientific publications, show that in EU, domestic refrigerators operate with a mean of 6.2°C and that the mean of the 75th and 95th percentiles were respectively 7.6°C and 9.8°C.

**Significance:** Based on these outcomes, the temperature at consumer level integrated in the European technical guidelines to assess shelf life of RTE food was revised and decreased from 12°C to 10°C.

## P1-38 A Review of Food Safety and Quality Improvement Mechanisms: Implications for Food Safety Culture in the Food Drink Manufacturing and Processing Industry

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**Introduction:** As prevalence of foodborne disease in the global population continues, food safety management is a priority for key stakeholders. Food safety culture (FSC) is reported to underpin a robust food safety management system; therefore, businesses within the food sector have a need to assess and improve FSC. To achieve this, effective improvement mechanisms are required. However, practical implementation studies have been limited.

**Purpose:** To evaluate the development, implementation, and evaluation of food safety/quality interventions within the food sector.

**Methods:** PRIMSA methodology was adopted to systematically search for literature to determine: interventions utilised for food-safety cognitive and behavioural improvement, evaluation of food safety intervention effectiveness, application of academic/psychological theories that have been used to improve food safety within the food sector, and consideration of the extent that key FSC parameters have been addressed in relation to food-safety/quality improvement interventions.

**Results:** Overall, 47 food-safety/quality/hygiene-related intervention studies were identified for review. Studies (2002-2022) were undertaken in 22 countries, with the majority from the United States (28%) and United Kingdom (8%). The majority of studies (42%) were undertaken in foodservice establishments, with few (12%) in food manufacturing businesses. Most studies reported intention to achieve behavioural change (39%), however, many of these were designed to improve food safety cognition. Indeed, questionnaires assessing food safety cognitions were used to evaluate effectiveness within 71% of studies. Most common interventions reportedly utilised and evaluated for food safety improvement included 'training' (37%), 'posters' (23%) and noticeboards (7%). Some studies reported that combining multiple, tailored intervention approaches proved to be effective. Overall, few FSC dimensions/components were addressed in food-safety intervention studies; the majority focused only on training and communication dimensions.

**Significance:** There is a need to develop/evaluate targeted food safety interventions that address specific FSC components within food manufacturing businesses, including cognitive and behavioural improvements. Evaluation of intervention effectiveness may benefit from a mixed-method approach, combining questionnaires for cognitive-based improvements and objective evidence for behaviour-based improvements.

was lacking. Responsibility for monitoring/maintaining FS-behaviour rested with "supervisors observing" during production, but no global mechanism for improvement existed.

**Significance:** Improved communication streams would enable practical improvements to FS training programmes. Tailoring of training to job role and learning style, including developing objective-based training with measurable impact areas may increase knowledge retention. Improved training practices may lead to improved, sustained FS-practices that positively influence FDMP businesses' FSCs.

## P1-39 Perceived Effectiveness of Food Safety Training in UK Food and Drink Manufacturers: Implications for Food Safety Culture

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**Introduction:** Improved food-safety (FS) practices within food/drink-manufacturing/processing (FDMP) businesses can lead to decreased instances of foodborne illness which remains prevalent globally. FS-training is an important aspect within FDMP and is consistently identified as key within various food-safety-culture (FSC) frameworks.

**Purpose:** Obtain a qualitative understanding of factors influencing FS-practices within FDMP businesses in the UK.

**Methods:** In-depth interviews (n=47) were undertaken within FDMP businesses (n=3) to capture qualitative data relating to FS practices. Interview schedules were developed using key FSC frameworks; digital interview recordings were transcribed, coded and thematic analyses performed.

**Results:** Operative-employees of business-1 reported negative attitudes towards delivery of training, specifically towards the large number of people attending training at once whilst management-employees considered training logistics efficient as they could assign trainee groups based on production schedule. Within business-2, management-employees agreed that FS training was sufficient in keeping the company compliant with relevant legislative requirements, similarly operative-employees positively regarded training as an imbedded, ongoing process, however this sometimes interfered with their responsibilities and content was not specifically relevant to their role. Employees reported training materials were clear/concise, whereas others reported rushed and insufficiently detailed training. Within business-3, although some management-employees received regular FS training/updates, seldom did senior management-employees, therefore awareness regarding company-wide FS practices

## P1-40 Quantitative Bio-Mapping of *Salmonella* and Indicator Organisms in Modernized and Conventional Commercial Pork Processing Facilities for Optimizing Food Safety Management Decision Making

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**Introduction:** Bio-mapping provides a useful tool for risk-based food safety decision-making by determining what processing locations are at higher risk for pathogenic contamination.

**Purpose:** The purpose of this study was to utilize quantitative bio-mapping strategies to compare indicator organisms and *Salmonella* in modernized and conventional pork processing facilities throughout the processing chain. The modernized facility implemented an updated sanitary design, including the utilization of a bio-furnace, for enhanced microbial control performance.

**Methods:** Swab samples from the round, side, and shoulder of the carcass were taken at processing steps on the harvest floor, and 2-pound trim and ground product samples were collected by trained employees and shipped overnight to Texas Tech University. Samples were analyzed for Aerobic Count, Total *Enterobacteriaceae*, and generic *Escherichia coli* with the bioMérieux TEMPO<sup>®</sup> enumeration system. *Salmonella* presence and quantity were evaluated using the BAX<sup>®</sup> System Real-Time *Salmonella* SalQuant<sup>™</sup> methodology. Microbial counts were converted to log CFU prior to statistical analysis using R.

**Results:** The conventional plant showed a higher prevalence of indicator organisms at all sampling points and displayed an overall *Salmonella* prevalence of 19%, while the modernized facility showed an average of 5.38% *Salmonella* prevalence. *E. coli* enumeration indicated an average of 2.24 log CFU/g of *E. coli* in the conventional plant that was reduced to less than 1 log CFU/g after the PAA cabinet, however, *E. coli* detected in the modernized plant was reduced from an average of 2.8 log CFU/g to less than 1 log CFU/g post snap chill, and was not detected in further processed products.

**Significance:** Carcasses processed in both facilities displayed an increase of *Salmonella* prevalence in further processed products, revealing a U-shaped bio-mapping curve, emphasizing a need for *Salmonella* mitigation strategies in further processed products. This study provides evidence for the utilization of bio-mapping methodologies for indicator and pathogen quantification within commercial pork processing operations.

## P1-41\* Biofilm-Forming and Multidrug-Resistant *Bacillus* Species Isolated from Artisan Bakery Environment

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**Introduction:** The presence of *Bacillus* in baked products cannot be completely eliminated as the crumb temperature seldom exceeds 100°C, which is not effective against their thermotolerant spores. Consequently, European Food Safety Authority (EFSA) considers *Bacillus* species in bakery production chain as a significant public health risk.

**Purpose:** This study investigated the biofilm-forming ability and resistance profile of *Bacillus* isolates from artisan bakery environment in addition to the feasibility of hyperspectral imaging in determining real-time biofilm development.

**Methods:** The *Bacillus* isolates (n=21) from artisan bakery environment were investigated for their biofilm-forming ability at 30°C, 37°C and 45°C between 18 and 96 h using Congo Red-Calcofluor assay for extracellular matrix components and Crystal Violet biomass analysis, susceptibility to a panel of 24 antibiotics, and whole genome

sequencing (WGS) using HiSeq Illumina platform to identify the underlying genetic determinants. The potential of hyperspectral imaging in visible and near-infrared (VNIR, 406-997 nm) and short-wave infrared (SWIR, 951-2496 nm) regions to determine real-time biofilm development was explored on industry-grade stainless steel SS<sub>316</sub> and aluminum surfaces.

**Results:** All *Bacillus* isolates (n=21) displayed biofilm-forming ability with persistence and dispersal capabilities at 30°C, 37°C and 45°C. The stress adaptive RDAR (red, dry, and rough) morphotypes suggested strong adherence potential in food manufacturing environments. All isolates demonstrated multidrug resistance (MDR) including resistance to the drugs of last resort (vancomycin, carbapenems, and 3<sup>rd</sup> generation cephalosporins). These findings were validated with the presence of genetic determinants for antimicrobial resistance (*tetL*, *mphK*, *aadK*, *fosB1*), MDR (*ebrA*, *ebrB*, *blt*, *bmr*), and biofilm formation (*epsA-O*, *bslA*, *sinR*, *sinI*, *abrB*, *tapA*, *tasA*, *spo0A*) relevant to food safety. Finally, the preliminary hyperspectral imaging studies using Fourier-transform infrared spectroscopy (FTIR, 4000-675 cm<sup>-1</sup>) showed positive results for biofilm detection in food production environments.

**Significance:** The findings of this study will contribute significantly towards development of decontamination strategies and inspection automation in relevant food processing industries.

## P1-42 How Food Facilities Can Meet Preventive Controls Requirements: A Comparative Approach in Food Safety and Food Defense between U.S. Regulation and Europe

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**Introduction:** A comparative approach was conducted between Preventive Controls and Food Defense under the FSMA-FDA regulation and HACCP requirements under European current rules.

**Purpose:** To analyze the similarities and differences between Preventive Controls and HACCP, connected regulations that impact suppliers (such as Foreign Supplier Verification Programs), requirements of Preventive Controls Qualified Individuals (PCQIs), and FSPCA programs (including training curricula and Lead Instructor requirements) and the European approach.

**Methods:** Using a comparative approach of the food safety systems for manufactured food, the similarities and differences between Preventive Controls and HACCP were examined and documented. An analysis was conducted resulting in communicative ways to relate how HACCP and Preventive Controls work together so that companies exporting to the U.S. can meet FSMA requirements.

**Results:** To understand the changes to be implemented by the European entities, associations, and other stakeholders interested in the US market, it was determined that companies can modify existing HACCP plans to meet Preventive Control requirements. However, more work will be needed during the Hazard Analysis phase to identify non-process controls.

**Significance:** The consequences of suppliers not documenting Preventive Controls for food safety can prohibit export and result in costly supply chains in the food system.

## P1-43 Heterogeneity in Biofilm Formation Capability of *Listeria monocytogenes* Food-Associated Isolates

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**Introduction:** The capability of *Listeria monocytogenes* to form biofilms is considered to be one of the major factors contributing to the organism's persistence in the food processing environment (FPE).

**Purpose:** This study aimed to examine the genetic underpinning of biofilm-forming capacities in *L. monocytogenes* isolated from foods and food processing environments.

**Methods:** A collection of 150 *L. monocytogenes* strains isolated from a range of food products, food-processing environments, and clinical sources were available from the Teagasc Culture Collection and were screened for their ability to form biofilms using the crystal violet method.

**Results:** Of the 150 strains evaluated, 16.67% exhibited strong biofilm formation ( $P < 0.05$ ), in particular isolates sources from seafood, serogroup 1/2a, 1/2b-3b-7 and Clonal Complex (CC) 101.

Pan-genome-wide association analysis identified 524 candidate genes that are associated with strong biofilm formation, many of which (78.05%) were of unknown function (hypothetical). Comparative analysis of the genome sequences of the isolates for a complement of genes previously shown to have a role in biofilm formation (i.e. *actA*, *lmo0435*, *lmo0673*, *luxS*, *inlL*, *lmo2504*, *prfA* and *recO*) revealed that all 150 of the isolates carried *actA* and *recO*. However, the presence of the remaining genes, namely *lmo0435*, *lmo0673*, *luxS*, *inlL*, *lmo2504* and *prfA*, were found to not be statistically significant in the ability to form biofilm.

**Significance:** The intricate mechanisms behind biofilm formation will be better understood with further research on the genes highlighted to have unknown functions by pan-genome analysis.

## P1-44 Association of Virulence, Biofilm, and Antimicrobial Resistance Genes with Specific Clonal Complex Types of *Listeria monocytogenes*

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**Introduction:** Precise classification of the foodborne pathogen *Listeria monocytogenes* is a necessity in efficient foodborne disease surveillance, outbreak detection, and source tracking throughout the food chain.

**Purpose:** The aim was to perform comparative genomic analysis of *L. monocytogenes* isolates to determine specific genetic markers associated with presence of genes previously shown to have a role in virulence, stress tolerance, biofilm formation, and antimicrobial resistance.

**Methods:** A total of 150 *L. monocytogenes* isolates' Whole Genome Sequences (WGS) from various food products, food processing environments, and clinical sources were investigated for variation in presence of specific genes and their possible link to genetic markers namely (Clonal Complexes (CC)), serogroups, as well as phylogenetic relationships.

**Results:** Pan-genome-wide association analysis by Scoary using Fisher's exact test identified 11 genes specifically associated with clinical isolates. Screening for the presence of antimicrobial and virulence genes using the ABRicate tool uncovered variation in presence of *Listeria* Pathogenicity Islands (LPI) and other known virulence genes. Specifically, the distribution of *actA*, *ecbA*, *inlF*, *inlJ*, *lapB*, LPI-3 and *vip* genes across isolates were found to be significantly CC dependent while the presence of *ami*, *inlF*, *inlJ*, LPI-3 was associated with clinical isolates specifically. In addition, Roary-derived phylogenetic grouping based on Antimicrobial Resistant Genes (AMRs) revealed that thiol transferase (*FosX*) genes were present in all of lineage I isolates. The presence of lincomycin resistance ABC-F type ribosomal protection protein (*lmo0919\_fam*) were also genetic lineage dependent. More importantly, the genes found to be specific to CC-type were consistent when a validation analysis was performed with fully assembled, high quality complete *L. monocytogenes* genome sequences (n = 247) extracted from the National Center for Biotechnology Information (NCBI) microbial genomes database.

**Significance:** This work highlights the usefulness of MLST based CC typing using Whole Genome Sequence as a tool in classifying isolates.

## P1-45\* The Evolution and Evaluation of Multimedia Used in Food Safety Training in LMICs

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**Introduction:** Food Safety Education is an important tool for spreading food safety awareness, and hence help reduce the foodborne disease burden. A variety of training techniques have been reported in LMICs in the past two decades.

**Purpose:** This study examines the design of training interventions and multimedia used for communication of important food safety messages, with the aim of identifying the most effective and sustainable training strategies.

**Methods:** A multi-vocal, systematic approach was taken to search relevant academic and grey literature including NGO reports. Authors of selected studies were requested to provide access to educational materials used during research. The training components were analysed using a combination of quantitative parameters derived from the marketing industry, like image-text ratio and readability, and qualitative parameters such as attractiveness and acceptability.

**Results:** A total of 28 authors provided access to the educational materials (out of 75 contacted). The most common form of training was observed to be lecture style with add-on multimedia & activities. Thirteen studies displayed a satisfactory combination of spatial and numerical image-text ratio. The readability of training material ranged from 'fairly easy' to read to 'difficult' to read. Interactive training components in food safety education have increased across the last decade. Use of local language and media displaying local people and practices is the most common factor contributing to acceptability of trainings. Results show a wide range of methods, media, and practices being implemented to impart training and to measure its success.

**Significance:** A reporting framework highlighting the concepts critical to intervention efficacy and sustainability would help identify best practices and understand how these vary with geographies, culture, and digital literacy.

#### P1-46\* Empirical Investigation of the Link between Food Safety Culture Maturity and Cost of Quality in Food Processing Companies

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**Introduction:** A more mature food safety culture is linked to achieving higher levels of food safety. However, the impact of food safety culture on the economic performance of a food business remains unclear.

**Purpose:** This research explores the association between food safety culture maturity and cost of quality.

**Methods:** A cost of quality questionnaire determining prevention, appraisal and failure costs, was developed based on a literature review and pilot tested. Subsequently, the maturity of the prevailing food safety culture was assessed in a convenience sample of five food processing companies by a validated food safety culture mixed-method assessment. The same companies provided cost of quality data using the developed questionnaire. The relation between cost of quality and food safety culture was investigated descriptively and statistically (Pearson correlation), using the percentages of total annual sales each cost accounted for and the number of food safety culture gaps (i.e., underdeveloped dimensions).

**Results:** Collecting cost of quality data is not yet standard practice in the food industry. This is especially true for failure costs: three out of five companies were unable to specify all types of failure costs, which made it impossible to investigate its relationship with food safety culture maturity. For prevention and appraisal costs, results showed descriptively and statistically that more investments in these costs are linked to a more mature food safety culture and vice versa (however not statistically significant). This result coincides with the basic assumptions of cost of quality models, which state that as appraisal and prevention costs increase, quality enhances.

**Significance:** The discovered positive relationship suggests that financial investments in prevention and appraisal might foster a more mature food safety culture. Alternatively, this might reflect that maturing food safety culture might fuel investments in prevention and appraisal costs or that the described relationship is bidirectional.

#### P1-47\* Trends in Scientific Publications on Food Safety Management in Türkiye

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**Introduction:** Türkiye lacks a coordinated foodborne disease surveillance system. However, foodborne outbreak data from Turkish news reports and research papers, as well as foodborne disease occurrence data from hospital and forensics medicine directorate databases, indicates issues with how food businesses manage food safety. This is coupled with the fact that accreditation is not mandatory for third-party certification bodies operating in Türkiye. Thus, there is a need to better understand food safety management practices in Turkish food production.

**Purpose:** The aim is to explore to what extent scientific publications enable an understanding of food safety management practices in food production companies in Türkiye and identify ways in which research might facilitate their improvement.

**Methods:** A systematic literature review was conducted on food safety management research within the food production sector in Türkiye. Inclusion criteria covered research papers in both international and national databases and in English and Turkish languages.

**Results:** From 46 studies meeting the review criteria, 40 were published in national journals, of which 19 were theoretical papers on developing the HACCP system for different product types. Common research methods were microbiological hygiene evaluations (ten studies), surveys (ten studies) and case studies to identify CCPs (five studies). Finally, one study explored the food safety management system in one company based on the ISO 22000:2005 requirements and only one study included qualitative methods, like interviews.

**Significance:** Taking into consideration that Food Safety Culture has been determined to be the key factor in improving food safety practices in food businesses, the results show a need to shift the focus of Turkish food safety management research from a solely production-oriented approach to also include culture and management-related aspects. Furthermore, the use of qualitative research methods is not a common practice in Turkish food safety management research, which impairs an in-depth understanding of food safety management practices.

#### P1-48 Evaluation of Suitability of *Enterococcus faecium* B-2354 as a Nonpathogenic Surrogate for *Salmonella* sp. to Evaluate Antimicrobial Oils and Water-in-Oil Emulsion Sanitizers in the Food Processing Environment

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**Introduction:** Destruction of *Salmonella* sp. without water is challenging due to the increase in heat resistance in low-water activity environments, such as in chocolate, peanut butter, or pet food processing environments. Our lab has developed antimicrobial oils and water-in-oil (W/O) emulsions which are highly effective against desiccated *Salmonella* sp. and *Listeria monocytogenes* in laboratory testing.

**Purpose:** This study aimed to evaluate the dry processing surrogate *Enterococcus faecium* for scale-up studies to study antimicrobial efficacy on pilot plant scale equipment.

**Methods:** Inoculums of *Salmonella* Enteritidis BAA-1045 and *E. faecium* NRRL B-2354 were inoculated onto stainless steel coupons at a final concentration of 7 log CFU and desiccated at a constant relative humidity (RH, 33% and 75%). Treatments were acidified oil with surfactant (AOS 200 mM acetic acid and 3% w/w PGPR surfactant), water-in-oil emulsions (W/O, 200 mM acetic acid, 1% v/v water, 3% w/w PGPR), or water-in-oil emulsions with glycerol (WG/O 200 mM acetic acid, 1% v/v water, 1.5% v/v glycerol, 3% w/w PGPR) with a contact time of 30 min at 22°C. Bacterial survival was determined using plate counts and Most Probable Number (MPN), and microbial log reduction (MLR) was calculated.

**Results:** Desiccated *Salmonella* sp. is highly susceptible to W/O emulsions with > 6.5 MLR after a 30 min treatment at both 33% and 75% RH. The effectiveness of this treatment was 10,000x lower when glycerol (WG/O) was present, indicating differential osmotic pressure may be contributing to microbial kill. By comparison, W/O emulsion against *E. faecium* produced a significantly lower MLR (2.12 ± 0.23 and 1.36 ± 0.14 MLR at 33 and 75% RH, respectively), 10,000x – 100,000x more resistant than desiccated *Salmonella*.

**Significance:** These results show *E. faecium* is not an appropriate surrogate for validating *Salmonella* destruction using W/O formulations due to dramatically higher resistance to treatments.

#### P1-49 Safety and Quality Assessment for Ready-to-Eat Meat-Based Meals Commercialized in Kigali City, Rwanda

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**Introduction:** Poor hygiene, improper handling and inadequate heat treatment of meat are one of major risk factors contributing to increased food borne illnesses associated to the consumption of meat.

**Purpose:** This study was conducted to assess the microbiological quality of ready-to-eat meat based meal commercialized in Kigali City and determine the risk factors associated to their microbiological contamination.

**Methods:** A total of 95 meat samples (beef, goat and pork) and accompanying vegetable salads were collected from bars and restaurants of Kigali City (n=19) and their microbiological quality assessed through the enumeration of hygiene indicator bacteria (total aerobic bacteria and total coliforms) by using conventional culture methods. The hygienic meat handling practices and preparation pattern in the studied establishments were assessed by using a structured questionnaire.



**Results:** Preliminary findings showed a mean contamination of  $4.37 \pm 0.34$  log CFU/g aerobic plate count (APC) and  $1.73 \pm 3.44$  MPN/g total coliforms in beef meat;  $4.23 \pm 0.97$  log CFU/g APC and  $2.2 \pm 4.92$  MPN/g total coliforms in salad accompanying beef meat;  $3.87 \pm 0.51$  log CFU/g APC and  $1.80 \pm 1.72$  MPN/g total coliforms in goat meat,  $4.35 \pm 0.82$  log CFU/g APC and  $3.16 \pm 4.55$  MPN/g total coliforms in salad accompanying goat meat, and  $3.63 \pm 0.44$  log CFU/g APC and  $1.92 \pm 2.19$  MPN/g total coliforms in pork meat. The microbiological contamination of fried pork meat served without vegetable salads was found to be significantly ( $P < 0.05$ ) lower than other ready to eat meat based meals served with vegetable salad indicating a post-cooking contamination of meat by vegetable salads.

**Significance:** Despite the thermal destruction of microorganisms during the ready-to-eat meat preparation process, the re-contamination of cooked meals from uncooked vegetable salads appears to be an issue hampering the quality of commercialized meals. There is therefore a need to enhance hygiene in the preparation of meat based meals and corresponding accompanying salads.

**Methods:** Three batches of 20 kg minced pork meat, were prepared as meat itself (M), adding salt (2.0%) (MS), and adding salt, sodium ascorbate (0.05 %), and sodium nitrite (0.015 %) (MSNA). Meat was inoculated with 1 % v/w of spore suspension (mix of three strains). Samples (100 g of vacuum-packed meat or broth) were cooked (75°C for 20 min), quickly cooled and then stored at 12°C for six weeks (isothermal conditions). Sampling was performed every two weeks or adding an abuse of 20°C per 24 hours before the sampling (dynamic condition). The clostridia enumeration was performed according to ISO 15213 (2003). The growth potential ( $\Delta$ ) was calculated according to ISO 20976-1 (2019).

**Results:** Starting from an initial contamination level of about 3.5 Log CFU/g after the cooking step, during six weeks of isothermal storing, the  $\Delta_{max}$  of *Cb* was 2.6, 1.84, -1, and -2.1 log CFU/g in broth, M, MS, and MSNA respectively. Adding an abuse of 20°C for 24 hours, the  $\Delta_{max}$  was 3.7 and 2.8 Log CFU/g in broth and in M respectively, after two week of shelf life. Conversely, *Cb* growth was not observed in MS and MSNA.

**Significance:** Although the consumers' demand is for meat products with low or no additives, Food Business Operators must consider the possible increase of risk of illness caused by clostridia.

## P1-50 Novel Clean Label Formulations of Hams: A Study of their Microbiological Safety and Impact on the Human Gut Microbiome

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**Introduction:** Clean label is an emerging trend in the food industry. As consumer awareness of the ingredients used in day-to-day products increases, clean labeling arises from the need for healthier and more nutritious food products that have a short, simple, and known ingredient list and undergo minimal processing.

**Purpose:** This study aimed to assess if natural sources of nitrate as a substrate for nitrate reductase-producing starter cultures are able to assure microbiological safety and confer a protective role against *Clostridium* spp. in four novel ham formulations. The impact of these novel formulations on the human gut microbiome for potential consumers was also evaluated.

**Methods:** Challenge testing was performed by artificial contamination of different ham formulations with *Clostridium sporogenes* spores to assess their germination throughout 28 days at two different temperatures, 4°C and 10°C. The impact of these formulations on the human gut microbiome was assessed through HPLC quantification of short-chained fatty acids.

**Results:** No significant differences ( $P < 0.05$ ) in spore germination between ham formulations and the control were found for both temperatures. Cycle differences of 2 log and 2.5 log were observed between days 0 and 28 at 4°C and 10°C, respectively. Also, no significant differences ( $P < 0.05$ ) in the concentration of short-chained fatty acids was observed throughout 48 h of colonic fermentation in relation to control, suggesting the absence of a negative impact of these novel products on the human gut microbiome.

**Significance:** In this context, the clean label movement is trending to offer natural products while having their safety in sight. Both studied parameters support a promising application of this technology in the meat industry, keeping in mind consumers' wishes and demand for more nutritious and natural ingredients in day-to-day foods.

## P1-51 Studying the Growth Potential of Proteolytic *Clostridium botulinum* in Cooked Ham, Simulating Thermal Abuse during Shelf Life

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**Introduction:** It is not realistic to rely on temperature control to ensure microbiological safety of cooked or processed meat products, where proteolytic *Clostridium botulinum* (*Cb*) growth is usually inhibited by conventional additives such as nitrites.

**Purpose:** The objective of this study was to evaluate the growth potential of *Cb* in a cooked ham model simulating conditions of thermal abuse during the shelf life.

## P1-52 The Chilling Stage as a Potential Lever at the Slaughterhouse for Controlling *Campylobacter* in Broilers

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**Introduction:** *Campylobacter* is the most common bacterial food-borne pathogen in Europe, mainly associated with poultry meat consumption. To mitigate the campylobacteriosis risk and reduce the contamination level of *Campylobacter* in poultry carcasses, measures can be taken on the farm but also at the slaughterhouse.

**Purpose:** Based on two real industrial situations where the levels of *Campylobacter* contamination at the end of slaughtering were contrasted, the objective of this study was to identify the critical slaughtering stages which could explain these differences.

**Methods:** Two French slaughterhouses were selected because of their difference in *Campylobacter* prevalence not complying with the EU Regulation, while their slaughter process was otherwise similar. Between October and December 2022, nine batches over three slaughter days were sampled at each slaughterhouse. Caeca, as well as neck skins at the end of plucking, final rinse after evisceration, and chilling stages, were collected for *Campylobacter* enumeration.

**Results:** From caecal contents with not significantly different levels of *Campylobacter* contamination in the two slaughterhouses, lower levels of *Campylobacter* on the neck skin at the end of slaughter were found in slaughterhouse A (SA) ( $1.9 \pm 1.1$  log CFU/g) than in slaughterhouse B (SB) ( $2.8 \pm 1.1$  log CFU/g). The highest decrease in *Campylobacter* contamination level on neck skins from the plucking to the chilling stage in SA ( $-0.89 \pm 0.54$  log CFU/g) than in SB ( $-0.05 \pm 0.39$  log CFU/g), could be mainly attributed to chilling, resulting in a decrease of  $0.68 \pm 0.54$  log CFU/g in SA, compared to only  $0.18 \pm 0.29$  log CFU/g in SB.

**Significance:** This study highlights that acting at the slaughterhouse and more specifically at the chilling stage could represent a potential lever to reduce *Campylobacter* levels in broiler carcasses. The influence of factors influencing survival of *Campylobacter* under cold and dry stress still needs to be investigated for chilling optimization.

## P1-53\* The Incidence and Distribution of Antibiotic-Resistant *Salmonella* in a South African Poultry Processing Plant

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**Introduction:** *Salmonella* is a causative agent of foodborne illness globally and is commonly associated with poultry products. Knowledge about the occurrence during poultry slaughtering can be used to develop alternative control methods and review current practices.

**Purpose:** This study aimed to determine the occurrence and distribution of antibiotic resistant *Salmonella* isolates throughout the poultry slaughtering process.

**Methods:** A total of 156 samples from all stages of poultry slaughtering were taken in a processing facility in South Africa. Neck skin samples and cloacal vent swabs of at least three carcasses at each stage were taken. Water samples were taken from the scalding tank and the spin chiller, and environmental surface samples were taken throughout the facility. The Food and Drug Administration's BAM



method for the detection of *Salmonella* was used, and presumptive positive *Salmonella* were confirmed using the VITEK® 2 Compact system and were further tested for antibiotic susceptibility using this system.

**Results:** During slaughtering, 100 presumptive positive *Salmonella* isolates were detected at bleeding, plucking, evisceration, chlorine spray, ozone application, and chilling. Thirty-two isolates were further confirmed as *Salmonella* isolates and showed resistance to cefuroxime, cefoxitin, cefuroxime axetil, amikacin, and gentamicin. Additionally, eight of the isolates showed multi-drug resistance and were obtained from bleeding, pluckers, evisceration, chlorine spray, and ozone application.

**Significance:** Antibiotic-resistant salmonellae pose major public health risk. Cross-contamination and transmission of *Salmonella* throughout the poultry food chain, and the development of antibiotic resistance, remain a concern for the health of the consumer and warrants reconsideration of the use of antimicrobials and a review of current practices and control methods.

## P1-54 Prevalence of Foodborne Pathogens in Raw Chicken, Pork, Beef and Vegetables in Thailand: A Systematic Review

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**Introduction:** Thailand is a major food export nation, ranking in the top 15 of food exporters in the world. However, little is known of the prevalence of foodborne pathogens in Thailand.

**Purpose:** This study reviews the literatures for the prevalence of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* to identify the potential risk level in foods.

**Methods:** A systematic literature review was performed using three scientific databases (PubMed, Scopus and ThaiJO). Inclusion criteria include the publications concerning the foodborne pathogens prevalence in food samples in Thailand. Exclusion criteria include papers that are not concerning the prevalence, not in Thailand, human, animals, or farm samples, clinical and antimicrobial studies. The median values and range were identified, and Kruskal-Wallis H Test and Mann-Whitney U Test were performed.

**Results:** After the screening process, 47 articles (out of 1667) were used in analyses. In chicken, significant differences in the prevalence among the pathogens were observed, whereby *E. coli* and *Salmonella* significantly exhibited higher median values than *L. monocytogenes* ( $P < 0.05$ ), but not from each other. In pork, *E. coli* prevalence (median prevalence at 91.9% with a range from 3.6-96.7%) was significantly higher than *Salmonella*, *L. monocytogenes*, and *S. aureus* ( $P < 0.05$ ). In beef, there was no significant difference in the prevalence among the four pathogens (i.e., *E. coli*, *L. monocytogenes*, *S. aureus* and *Salmonella*) ( $P > 0.05$ ). In vegetables, there was no significant difference in the median prevalence values among the studied pathogens ( $P > 0.05$ ). Certain pathogens were not reported in certain foods, thus they were not included in the analyses.

**Significance:** This study identified the median values and the most prevalent foodborne pathogens in chicken, pork, beef, and vegetables. The findings provide quantitative assessment of the risk.

## P1-55 Microbiological Quality Assessment of Pre-Cut Melons and the Need for Food Safety Practices

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**Introduction:** Due to the increasing consciousness of a healthy diet and pursuit of convenience, the market for pre-cut fruit is on the rise, and melons, such as watermelons and cantaloupes, are one of the welcomed fruits for their sensory attributes and nutritional properties. However, the safety of consumption of pre-cut fruits remains one of the concerning issues that affects public health.

**Purpose:** This work aimed primarily to study the microbiological quality of melons, which were cut, wrapped in plastic cling film by retailers and exposed to room temperature in fruit shops in Porto, Portugal. Secondly, the possible passage of pathogens from the peel to the interior of the melon was evaluated after slicing and their growth over storage time.

**Methods:** A total of 26 pre-cut melons, *Cucumis melo* L. var. Piel de Sapo, were characterized microbiologically. Artificial contamination of melon peels with cocktails of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* was carried out and survival was evaluated over 0 h, 24 h and 48 h of storage.

**Results:** No *Listeria* spp. or *Salmonella* spp. were found in all the samples, while *E. coli* and *Staphylococcus aureus* were enumerated. After artificial contamination of melon peels, all the pathogens were transferred from the contaminated peel to the flesh of the melons and an increase of about 4 log CFU/g was observed among melon slices immediately cut (0 h) and after 24 and 48 h at 20°C.

**Significance:** Pre-cut melons classified as microbiologically unacceptable or unsatisfactory are being sold in local fruit shops in the Metropolitan Area of Porto, highlighting that effective practices to prevent contamination, cross-contamination, and bacterial growth on cut fresh melon must be adopted. This study clearly demonstrates the need for education campaigns to alert the local sellers and consumers to good food safety practices.

## P1-56 Antibacterial Activity of *Piper betle* L. Ethanol Extract in Beer Brewing

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**Introduction:** A rancid taste is produced during the beer brewing process due to the contamination of *Levilactobacillus brevis*, lactic acid bacteria. *Piper betle* L. has been found in past studies to have a variety of biological activities including antibacterial, antiulcer, antioxidant, anti-inflammatory, caries prevention, free radical scavenging, intestinal protection, and liver protection. However, its antimicrobial activity toward *L. brevis* has not yet been researched extensively.

**Purpose:** This study investigated the antibacterial ability of *Piper betle* L. extract against *L. brevis*.

**Methods:** *Piper betle* L. was extracted using ethanol followed by purification of the compounds using HPLC and confirmation of the compound structures using NMR. The inhibition ability of *Piper betle* L. against *L. brevis* was investigated by the disk diffusion method, minimum inhibition concentration (MIC), and minimum bactericidal concentration (MBC). The mechanism of bacterial inhibition was studied by observing the integrity of the cell membrane, the permeability of the cell membrane, and the cell shape.

**Results:** The inhibition zone diameter of the ethanol extract was 17.0 mm and the MBC energy was 2.0 mg/ml. The inhibition zone diameter of the ethyl acetate extract was 33.0 mm and the MBC energy was 1.0 mg/ml. Three compounds were identified as 4-allylcatechol, 4-diallylcatechol, and 5-allylbenzene-1,2,3-triol. The protein content of *L. brevis* fractions with cell membrane integrity was analyzed by incubating the *Piper betle* L. extracts for 24 hours at 37°C; ethanol extract increased by 79.2 ug/ml and EA extract increased by 178.4 ug/ml.

**Significance:** This study showed that ethyl acetate extract of *Piper betle* L. can inhibit *L. brevis* and further reduce the production of a rancid taste in beer brewing.

## P1-57 Performance Assessment of the Neogen Soleris® NF-105 Vial for Commercial Sterility in UHT Treated Milk and Dairy Alternative Plant-Based Drinks

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**Introduction:** Commercial sterility testing of products using methods described in EU regulation No 1662/2006 and Codex can take 18 days to achieve a result; Neogen's Soleris® NF-105 can reduce time to detection significantly.

**Purpose:** Validate the Soleris® commercial sterility testing method in UHT treated milk and plant drinks as an alternative to traditional methods according to the ISO 16140-2:2016 protocol.

**Methods:** NF-105 vials utilize the growth of the organisms to enable detection. As microorganisms grow and respire, the CO<sub>2</sub> produced diffuses down into the soft agar plug generating a colour change. This colour change is read by optical sensors in the Soleris.

The performance of Soleris® NF-105 Vial was compared to EU regulation No 1662/2006 and Codex (ref: CAC/RCP 57-2004). All samples were tested after the required pre-incubation for reference and alternative methods.

**Results:** During the ISO 16140-2 validation study scheme, 60 samples in total were analysed by Campden BRI to determine the sensitivity of the Soleris NF-105 Vial versus the Codex method. Results observed were successfully below the acceptability limits (AL) required for both pre-incubation times. Sensitivity of the alternative method was 100% and sensitivity of the reference was 93.3%.

The relative limit of detection for the alternative method was 1.315 at 48 hour pre-incubation and 0.553 at 72 hour pre-incubation for the category tested, which is below the acceptability limit of 2.5 for unpaired studies. During the inclusivity study the 50 target strains gave an expected result with the Soleris method. In conclusion, the Neogen Soleris commercial sterility testing detection method is selective and specific.

**Significance:** The Soleris NF-105 test vial is equivalent to the Codex reference method for commercial sterility in UHT treated milk and plant drinks. This is the first MicroVal certification of a commercial sterility method using the independent method validation protocol ISO 16140-2 (2016).

## P1-58 Flash Frying to Reduce *Salmonella* on Cacao Beans

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**Introduction:** *Salmonella* contamination is a concern in the cocoa and chocolate industry. The inclusion of a process control step earlier in the supply chain could improve safety. Insertion of a process control step after fermentation, but prior to export, could create value-added differentiation for this commodity and increase the profitability of smaller entities in or near cacao farming communities.

**Purpose:** There were two objectives for this project: 1) quantify the lethality of *Salmonella* on cacao beans by a low-technology, small-batch frying process that has been used in cacao production regions, and 2) verify the suitability of *Enterococcus faecium* as a surrogate for *Salmonella* in the frying of cacao beans.

**Methods:** Cacao bean (454 g) samples were inoculated with a 7-strain *Salmonella* cocktail and *E. faecium* (ATCC 8459). Cocoa butter (5-10 g) was melted in a large wok on a small portable stovetop. Inoculated cacao beans were stirred continuously for the 3 minute frying time. Samples (3/timepoint) were immediately transferred to bags and chilled. Surviving bacteria were enumerated using standard dilution and spread plating methods on Tryptic Soy Agar (TSA). Plates were incubated at 37°C for 4 hrs, then overlaid with either Hektoen Enteric agar (*Salmonella*) or m-*Enterococcus* agar (*E. faecium*) with continued incubation (37°C, 48-72 hrs) prior to enumeration.

**Results:** A 2-minute frying treatment resulted in reductions of  $3.54 \pm 0.71$  and  $3.38 \pm 0.37$  log CFU/g for *Salmonella* and *E. faecium*, respectively. Further frying (3-minute total frying time) resulted in an average reduction of more than 5 log CFU/g for both organisms (*Salmonella*:  $5.32 \pm 0.31$  log CFU/g reduction; *E. faecium*:  $5.38 \pm 0.62$  log CFU/g reduction).

**Significance:** Low technology, small batch frying of cacao can achieve significant lethality of *Salmonella*. *Enterococcus faecium* lethality was comparable to *Salmonella* and could be used on-site for biological verification of unique frying equipment, environmental conditions, and commodity variability.

## P1-59 Identifying Potential Spoilage Organisms in New Beverage Products

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**Introduction:** Consumer interests related to “super foods”, “clean label”, and “sustainability” are driving innovation in the non-alcoholic beverage category. Success of new products is dependent on meeting retailer expectations for shelf life and positive new customer experiences. Microbial spoilage is a significant risk to success of new products; however, our knowledge of spoilage organisms is lacking.

**Purpose:** To identify potential spoilage organisms for two beverage products: a non-alcoholic (NA) beer and a plant-based (PB) beverage.

**Methods:** Individual ingredient, packaging, and final products were supplied by processors. Combinations of methods were used to identify potential spoilage organisms, including: 1) finished product analysis throughout refrigerated shelf life for changes in aerobic plate count (APC; 30-35°C, 24-48 hrs), 2) ingredients analyzed for APC, lactic acid bacteria, and spore count, 3) ingredients were enriched in non-selective broth at 7°C for 10-28 days and isolated on non-selective media (25-30°C, 72 hrs), 4) 16S metabarcoding of enriched

ingredients and finished product at high APC. Isolates with diverse morphology were inoculated into fresh product and growth was monitored at 7°C for 20-60 days. Isolates with spoilage potential were identified by 16S rRNA Sanger Sequencing.

**Results:** Psychrotrophic bacterial taxa isolated from ingredients that grow in NA beer during cold storage included members of *Bacillus*, *Corynebacterium*, *Enterobacter*, *Leuconostoc*, *Peribacillus*, and *Pseudomonas* genera. Sporeformer *Paenibacillus lautus* was isolated from finished NA beer, which presents an enumeration challenge. *Aerococcus*, *Paenibacillus*, *Pseudomonas*, *Sphingomonas*, and *Streptococcus* were identified as important spoilage taxa in the finished PB beverage. All spoilage taxa were also identified in psychrotrophic enrichments of wax board packaging.

**Significance:** This approach proactively identifies diverse spoilage organisms earlier in the product lifecycle to support decisions about processing, formulation, and packaging.

## P1-60 The Growth Potential and the Thermal Resistance of Bacterial Spores Under Conditions Relevant for Ambient Acid Dairy Products

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**Introduction:** The spoilage of acidic foods (e.g. fruit juices and canned fruits and vegetables) caused by spore-forming bacteria has been well studied. However, there is a lack of understanding of the spoilage of other acidic foods such as ambient/shelf-stable acid dairy-based products.

**Purpose:** The aim of the current study is to understand more about spore-forming bacteria relevant to ambient acid dairy-based products and their heat resistance.

**Methods:** The present study investigated the germination and growth of spores from sixteen different bacterial species present in milk, dairy, or non-dairy ingredients under conditions relevant to the ambient acid dairy-based product for a period of 12 weeks. Additionally, the heat resistance of spores from four strains of *Alicyclobacillus acidoterrestris* was evaluated in a yoghurt-based medium.

**Results:** The growth experiments showed that the spores of seven bacterial species out of sixteen exhibited the ability to germinate and grow at a pH of 4.6 and temperatures of 25°C and/or 40°C. However, the presence of 0.4% or 0.8% LA in the growth medium at a pH of 4.6 resulted in the inhibition of growth for all species at both temperatures except *A. acidoterrestris*. The spores of *A. acidoterrestris* were capable of growth in the presence of both LA concentrations at a pH of 4.6 and a temperature of 40°C. The thermal inactivation kinetics of *A. acidoterrestris* spores revealed that all four strains possess higher thermal resistance profiles in yoghurt-based medium at pH 4.6, indicating that traditional pasteurization treatment may not be effective in controlling them in ambient acid dairy-based products.

**Significance:** This study highlights the significance of considering *A. acidoterrestris* as a potential spoilage hazard in ambient acid dairy-fruit products and the need for further research on effective control measures.

## P1-61 Clean Label Ropiness Control in Bakery Applications

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**Introduction:** Outgrowth of *Bacillus subtilis* spores during storage of contaminated bread results in the formation of rope spoilage. This causes deterioration of bread by off-odors and sticky bread crumb, leading to economic loss.

**Purpose:** The purpose of this study is to show the efficacy of conventional organic acid salts and clean label preservation solutions in baking to prevent spoilage by *Bacillus subtilis* spore outgrowth.

**Methods:** A  $6 \log_{10}$  CFU/mL spore solution of *Bacillus subtilis* ATCC 8473 was used to inoculate bread dough (100% wheat flour, 0.62% instant yeast, 1.62% salt, 3.78% sugar, 2% sunflower oil and 70% tap water (baker's percentages)). Control breads without preservatives were compared with bread containing 0.3% Probake CP (calcium propionate), 0.5% Progusta CA (calcium acetate) or 0.5% IsoAge Ca (natural vinegar) (based on flour weight). The doughs were mixed, divided into 70g portions and baked for 20 minutes at 210°C in a convection oven, after which breads were cooled for one hour, transferred to plastic bags and incubated at 30°C for a maximum of 14 days, during which buns were inspected on smell and texture and *Bacillus subtilis* was enumerated in triplicate by plating on BHI agar plates after 4, 7, 10 and 14 days of storage.

**Results:** Growth of *Bacillus subtilis* in control breads reached  $8.2 \pm 0.2 \log_{10}$  CFU/g after 14 days. Addition of 0.3% Probake CP, 0.5% Progusta CA and 0.5% IsoAge Ca reduced counts significantly ( $P < 0.05$ ), to respectively  $2.4 \pm 0.4$ ,  $2.7 \pm 0.7$  and  $2.9 \pm 0.6 \log_{10}$  CFU/g after 14 days. None of the treated buns showed any sign of spoilage, while the control treatment showed texture loss and clear off-odors.

**Significance:** Incorporating conventional organic salts or natural vinegar in bread can significantly reduce the growth of *Bacillus subtilis* during storage, decreasing the economic loss due to ropiness.

## P1-62 Clean Label *Listeria* spp. Control in Plant Protein-Based Foods

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**Introduction:** Refrigerated plant protein-based foods are susceptible for safety issues like psychrotolerant microorganisms, similar to other cold stored foods such as meat. The refrigerated environment will select for *Listeria monocytogenes*, an opportunistic pathogen with a relatively high mortality rate.

**Purpose:** Reducing food safety risks due to *Listeria* sp. outgrowth in plant protein-based foods by incorporating a clean label vinegar gar-based preservative, IsoAge DV250.

**Methods:** Three treatments of a plant protein model application (82% cooked chickpeas, 0.1% garlic powder, 1% NaCl, 3% lemon juice, water 13.25-13.9% and pH 6.2-6.4;  $A_w$  0.977-0.982), and a control without antimicrobials and different concentrations of IsoAge DV250 (0.5% and 0.75%). The fresh application was prepared by mashing and mixing, and then inoculated with  $3-4 \log_{10}$  CFU/g *Listeria innocua* (NCCB 100510). The inoculated product was vacuum-packed in portions of approximately 30g in sterile plastic bags and stored at 4°C and 7°C up to 45 days, during which *Listeria* sp. were enumerated in triplicate by surface plating on PALCAM agar at regular time points.

**Results:** Growth of *Listeria* sp. to 7-8 log CFU/g was observed during 45 days on the control treatment at both 4°C and 7°C, with an average increase of respectively  $1 \pm 0.1$  and  $1.5 \pm 0.2 \log$  CFU/g per week. All other treatments showed significant growth reduction ( $P < 0.05$ ) of *Listeria* sp. and 0.75% IsoAge DV250 reduced growth to less than a 2 log CFU/g increase in concentrations during 45 days at 4°C as well as at 7°C.

**Significance:** This research demonstrated the possibility to increase safety of plant protein-based foods using a vinegar-based preservation system that meets current food trends, like sodium reduction and natural origin.

## P1-63 16S rRNA Metabarcoding: A Potential Tool in Food Safety for Novel Plant-Based Ingredients

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**Introduction:** Increases in consumer awareness of the environmental impact of food production has contributed to a greater demand for plant-based foods. The development of such products creates new safety challenges to produce foods that mimic those of animal origin. 16S rRNA metabarcoding combined with a culture-based approach enables a better understanding of the microbial ecology of plant-based proteins as raw ingredients and in final product. A case study using different hold temperatures and acidic levels was used to understand critical safety boundaries for manufacturing.

**Purpose:** This study investigated the use of these molecular tools to determine if they can be used to enhance food safety during manufacturing. The results allowed us to determine additional critical steps the manufacturer must take to keep the product safe.

**Methods:** 16S rRNA metabarcoding was used to determine the microbial ecology of faba bean protein concentrate followed by statistical analysis using ANOVA. A culture-based method was used to determine growth levels of specific bacterial groups during manufacturing.

**Results:** Molecular analysis clarified which target bacterial group needed to be followed using different manufacturing parameters. Mesophilic and thermophilic spore-formers microorganisms were observed at different levels. Samples post-pasteurisation stored at 60°C for up to 16 hours without antimicrobial inhibitors or food preservatives showed a level of bacterial growth that reached unsafe levels. Samples stored at a pH 4.5 reduced or slowed the growth of microorganisms in the aerobic plate count, stopping the sporulation of the thermophilic bacteria.

**Significance:** Manufacturing food product using novel plant-protein needs to be carefully considered and must not introduce additional risks to the food producer and consumer. Using molecular analysis enabled us to determine the current true microbial ecology of these ingredients as well as the impact of different process parameters on the final product.

## P1-64 Extended Shelf Life of Modified Atmosphere Packed Raw Chicken Meat: A Microbiological and Sensory Evaluation

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**Introduction:** One way to reduce food waste of perishable meat products could be to extend shelf life, e.g., by using dynamic food labelling. An important step for this innovation is to find the point of spoilage based on bacterial counts as the gas levels produced by these bacteria will be used to decide when the meat is no longer good to eat.

**Purpose:** The aim was to evaluate if the current shelf life of cooked chicken breast fillet packaged in modified atmosphere can be extended by analysing bacterial populations and sensory characteristics of the meat.

**Methods:** Chicken breast fillets were analysed at use by date, two days past use by date and four days past use by date (at 4°C/8°C) to evaluate populations of total aerobic counts (TAC), *Enterobacteriaceae* and *Lactobacillus*. A consumer preference test was performed to evaluate flavour, odour and texture at these time points. In addition, a discrimination triangle test with trained panelists was performed.

**Results:** The highest population of TAC (8 log CFU/g) was found in chicken breast fillets stored at 8°C for 4 extra days after use by date. Chicken breast fillets stored at 4°C and analysed at use by date had the lowest TAC levels (6.5 log CFU/g). However, the sensory evaluations showed that none of the chicken breast fillets tested were significantly different than the others.

**Significance:** Extended shelf life had no significant effect on sensory parameters of cooked chicken meat compared to when the meat was consumed at use by date. The results indicate that the current shelf life can be extended but further research is needed to establish a point of spoilage and evaluate the usefulness of dynamic food labels for chicken meat.

## P1-65 Investigation of Antifungal Properties of *Lactobacillus plantarum* Metabolites Concentrated by Lyophilisation

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**Introduction:** *L. plantarum* from unique Kaunas University of Technology collection is known for producing plantaricin, which has potential antifungal properties. The aim of this investigation is to find the correlation of different growing conditions for producing maximum amount of plantaricin by changing temperature and pH of the media.

**Purpose:** Find correlation between growing conditions and qualitative inhibition ability of fungi mostly found in food products.

**Methods:** PDA media was enriched with different quantities of a concentrated metabolites mixture. On the hardened medium, mycelial discs (10 mm in diameter) of each isolate, taken from the edge of cultures of fungi isolates grown for seven days, were placed mycelially downwards in the centre of each Petri dish. The plates were incubated in a thermostat at  $25 \pm 2^\circ\text{C}$  for 7 days. Every 24 hours, the growth of the isolate was measured with a ruler in two perpendicular directions until the growth of the test isolate in the control plate reaches the edge of the plate. The suppression coefficient (SC) was calculated according to the formula.

**Results:** The results also showed that metabolite concentrates at the original pH (3.5 to 4.19) showed higher antifungal activity compared to those neutralised to pH=7.

**Significance:** Potential antifungal activity will be further investigated in order to use it as wheat or other grain protection against fungal growth after harvesting.

## P1-66\* Growth of Foodborne Pathogens *Listeria* and *Salmonella* and Spore-Forming *Paenibacillus* and *Bacillus* in Commercial Plant-Based Milk Alternatives

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**Introduction:** An increase in vegan diet preference, lactose intolerance, calorie concern, and environmental awareness has led to a rise in the popularity of plant-based alternatives to bovine milk. However, there are still gaps in understanding how known bacterial food contaminants behave in plant-based beverages.



**Purpose:** The present study is the first to compare the growth of food pathogens *Listeria monocytogenes* and *Salmonella enterica*, food spoilage *Bacillus subtilis* and an industrial milk product isolate, spore-forming *Paenibacillus* in commercially available ultrahigh temperature processed bovine milk and plant-based milk alternatives (coconut, almond, cashew).

**Methods:** Beverage samples were inoculated with a strain cocktail or individual strains of either *Listeria*, *Salmonella*, *Bacillus* or *Paenibacillus*, respectively (approximately  $1 \times 10^3$  CFU/mL) and stored at chilled and ambient temperatures (4°C, 8°C or 20°C).

**Results:** Bacterial strains used in the study were capable of proliferating in plant-based beverages at higher rates than in bovine milk at 8°C and 20°C for *Listeria* and 20°C for *Salmonella* and *Paenibacillus*, respectively. *Bacillus subtilis* grew equally fast in bovine milk and plant-based milk at 20°C. No statistically significant difference ( $P > 0.05$ ) in growth rates between different types of tested beverages was observed at 4°C and at 8°C for *Listeria* and *Salmonella* cocktails, respectively.

**Significance:** These data suggest that plant-based beverages may present a significant risk for listeriosis and salmonellosis, and post-opening recommendations should be carefully considered.

**Methods:** From proven solutions to challenging new applications, food manufacturers can rely on Pall's food and ingredient processing filtration and separation expertise and collaboration, helping our customers increase their benefits by reducing their environmental impact.

Our membrane systems process recovers added value from side streams, turning "waste" into valuable products. Upcycling helps customers reduce product loss and waste; Pall has many experiences in this area, such as protein recovery from spent grains in breweries or from potato juice.

**Results:** It generates high profits because protein ingredients can be valued for their nutritional and/or functional properties.

Our unique Membralox GP (permeability gradient) membrane can achieve >95% protein transmission, which helps justify Capex and Opex.

**Significance:** Pall contributes to recycling because in addition to the recovery of proteins, the treatment of secondary streams makes it possible to reduce CO<sub>2</sub> footprint, solid waste, water consumption, product losses

## P1-67 Enzymatic Production of Vegetable Protein-Derived Peptides from Red Kidney Bean Protein and Its Antioxidant Capacity

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**Introduction:** In recent years, the awareness of environmental protection and sustainability has become more and more important. Vegetarianism is considered to be an environmentally friendly way. With the increase in the population of flexible vegetarians, people tend to choose plant-based products, and red kidney beans are common beans. They are rich in protein and can be used as plant-based protein raw materials to produce peptides. Peptides have many physiological functions, such as anti-oxidation, anti-inflammation and anti-bacterial, and can be obtained through chemical, microbial fermentation and enzymatic hydrolysis of proteins.

**Purpose:** The purpose of this report is to use red kidney beans as raw materials to produce plant-based protein-derived peptides by enzymatic method, measure the degree of hydrolysis, and measure antioxidant capacity.

**Methods:** After the isolated protein was obtained from red kidney beans by alkali extraction and isoelectric precipitation, the isolated protein was hydrolyzed by enzymes to obtain peptides. After measuring the degree of hydrolysis and SDS-PAGE, the DPPH free radical scavenging ability of the hydrolyzed peptides was measured.

**Results:** After the isolated protein is hydrolyzed by enzymes, the macromolecular bands disappear in the SDS-PAGE graph, and the color intensity of the bands decreases, while the degree of protein hydrolysis is 11.6%. The DPPH free radical scavenging rate of the hydrolyzed peptide solution is 80%.

**Significance:** Peptides after hydrolysis of soybean protein have good DPPH free radical scavenging ability, and can be used as plant-derived peptides for development and application.

## P1-68 Sustainable Food Protein Supply Reconciling Human and Ecosystem Health

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**Introduction:** The food ingredient manufacturing industry is increasingly challenged to create innovative products that are high quality, safe, healthy and convenient to use.

**Purpose:** Faced with rising raw material and energy costs, producers are focusing on value, measuring the cost of ownership and implementing sustainable production methods to achieve their economic goals.

We treat a wide array of process fluids, such as: natural, alternative and high-intensity sweeteners, gelatin, industrial enzymes, amino acids, acidulants, flavors, extracts, seasonings, hydrocolloids, fermentation products, and many others. Creating value-added ingredients from traditional fluids is increasingly made possible by cutting-edge separation and purification technologies.

We provide countless food and ingredient filtration, separation and purification solutions; this enables food manufacturers to achieve their goals and protect their brands.

## P1-69 TITAN Transparency Solutions for Transforming the Food System

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**Introduction:** Food goes through many hands before it reaches our plate, from grower, processor, transporter, wholesaler and finally to retailer. Processed food has longer and more opaque chains, with more opportunities for breakdown of trust. Creating transparency from farm to fork can strengthen trust and create a healthier food system.

**Purpose:** The TITAN project will demonstrate the latest transparency-related solutions to help drive the formation of a demand-driven European economy predicted on the production and consumption of healthy, sustainable, and affordable food.

**Methods:** Comprising 27 partners from 14 countries (10 MS and 2 associated countries), TITAN will provide an extensive platform for the development of a wide range of innovations that aid transparency and address key challenges identified in the EGD. The project comprises a mix of technology providers and research centres linked to agri-food actors and businesses through an interactive co-creation approach that was initiated prior to the submission process.

**Results:** The TITAN innovations, all transparency-related, address the following themes: enhancing transparency in agri-food businesses with a focus on SMEs; improving food choices by providing more transparent information to the consumer; using improved transparency to enhance food safety and authenticity of products; and providing improved information on the health and sustainability of food products. TITAN has included a substantial policy element within the project that will function in two ways: 1) identifying policy changes/levers that are needed to aid transparency, 2) identifying the implications for policy resulting from the TITAN innovations.

**Significance:** By bringing together business strategy, the latest technology innovations, policy and the consumer, TITAN will provide the blueprint of a demand driven economy that provides healthy, sustainable, and accessible food for its citizens.

## P1-70 Giant Leaps Towards Healthy and Sustainable Future Diets by Filling Knowledge Gaps on Alternative Proteins

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**Introduction:** The major impacts of the current food system on biodiversity, land and water use, and animal welfare, could be mitigated by a shift from traditional animal-based towards more sustainable protein sources.

**Purpose:** The ambition of the Giant Leaps project is to develop improved, fast, and animal-friendly methodologies for the health, safety and environmental assessment of alternative proteins to arrive at future diets that are accepted by consumers and optimized for health and environmental impacts within the scope of the project.

**Methods:** The proposed research will focus on a broad set of nine alternative proteins which are not yet commonly applied in foods (lentils, faba beans, oat, quinoa, rapeseed, microalgae, single cell bacteria, insects, cultured meat) to fill existing knowledge gaps as well as on a selected set of novel alternative proteins for specific safety-related aspects in an exploratory way. This allows identifying the highest potential alternatives for specific cultures and target groups in the different regions of Europe.



**Results:** All missing elements of an integrated framework to evaluate protein sources are developed to enable the shift towards a sustainable food system and healthy nutrition: (i) innovative prototypes that unlock the potential of sustainable protein sources in foods, products and diets, (ii) predictive in silico and in vitro methodologies for safety and health parameters, (iii) datasets and cloud platform for data integration, analysis and comparison, and (iv) models to estimate and optimize the environmental and health impacts of future diets and the shift towards alternative protein foods.

**Significance:** Around 60 to 70% of total protein intake is derived from animal sources in European diets. The ambition of the GIANT LEAPS consortium is to achieve 50% of total protein intake derived from alternative protein sources, representing a decrease in absolute animal protein intake of 20 to 30% under the assumption that increased alternative protein intake fully substitutes traditional animal protein intake.

## P1-71 The Conjugative Transfer of Plasmid-Mediated Mobile Colistin-Resistance Gene, *mcr-1*, to Shiga-Toxin Producing *Escherichia coli* during Mung Bean Sprout Production

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**Introduction:** Fresh produce crops are at high risk of contamination with foodborne (enteric) pathogens which can cause acute and even fatal illnesses. The spread of the mobile colistin-resistant gene (*mcr-1*) in *Enterobacteriaceae* represents a global public health concern. Conjugation of bacteria (commensal and pathogenic) associated with food may facilitate global dissemination of the *mcr-1* gene through the food supply.

**Purpose:** The present study investigated the transfer frequency of the *mcr-1* gene among *Escherichia coli* during the production of mung bean sprouts.

**Methods:** Isolates used in the study included the donor strain *E. coli* NCTC 13846 (*mcr-1* positive) and recipients *E. coli* O157:H7 and *E. coli* O104:H4. The donor strain was recovered from a human clinical case and the recipients were associated with outbreaks of foodborne illness. *In vivo* mating experiments (growing sprouts) were conducted in a sprout growth chamber with irrigation of 1min/h under controlled temperature of 25°C over six days following inoculation of mung bean seeds with the donor and a recipient. Donors, recipients, and transconjugants were evaluated for susceptibility to colistin.

**Results:** Transfer frequency (5.68E-05 per recipient) of *mcr-1* was greater during mung bean sprout growth for *E. coli* O104:H4 compared to *E. coli* O157:H7 (1.02E-05 per recipient) days three to six. The MIC value for colistin of transconjugants was between 2 µg/ml to 8 µg/ml; most transconjugants demonstrated a MIC (4 µg/ml) to colistin consistent with the donor strain, although a few transconjugants showed an increased colistin resistance (up to 8 µg/ml).

**Significance:** This study suggests that the transfer of antibiotic-resistant gene(s) among bacteria during mung bean sprout production may facilitate the spread of antibiotic-resistant bacteria in the environment and to humans. Consumption of food harboring antibiotic-resistant bacteria and antibiotic-resistance gene(s) may increase the potential for the exchange of antibiotic-resistance genes with gut bacteria in humans and animals.

## P1-72\* Novel Mycoremediation Technique Utilizing White-Rot Fungi, *Pleurotus ostreatus* and *Phanerochaete chrysosporium* to Inhibit Pathogenic *E. coli* for Pre-Harvest Food Safety

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**Introduction:** Through activation of ligninolytic activity, white-rot fungi can be useful in degrading zoonotic pathogens in biological soil amendments of animal origin through mycoremediation within continuous-flowthrough systems.

**Purpose:** The objective was to assess the inhibitory effects of *Pleurotus ostreatus* (PO) and *Phanerochaete chrysosporium* (PC) grown on a ligninolytic activating matrix of woodchips (WC) on *Escherichia coli*.

**Methods:** Controlled continuous-flow tests with PO- and PC-treated WC (300g) were performed in sterile 1L bioreactors (n=26), housed in a biosafety cabinet. WC were inoculated with PO and PC millet-spawn (at 60%) and incubated at 22°C for 14 d. Next, PO- and PC-treated WC were subjected to a nitrogen limiting solution for 7d followed by overnight culture of *E. coli* TVS355 (50L PBS of 5.9 log CFU/mL) pumped through each reactor at a flow rate of 0.5 mL/min. Effluent (3mL) was sampled every 5 days to 50 d post-inoculation (dpi) and WC (30g) sampled on 0, 25, and 50 dpi. *Escherichia*

*coli* was enumerated on MacConkey agar with rifampicin. Controls included bioreactors without fungi and/or bacteria. Chemical analysis included pH, moisture, and ergosterol for each bioreactor. Data were analyzed using one-way ANOVA and student *t*-test across three trials (n=26 per treatment).

**Results:** Bacteria remained undetectable in uninoculated controls. *Escherichia coli* inactivation was observed first at 10 d and 20 d for PC and PO treatments ( $P<0.0001$ ) respectively. *Escherichia coli* detection was corroborated with metrics used for ligninolytic activity, determined through ergosterol analysis, indicative of fungal integrity and concentration. Ergosterol concentration for PC-treatments was significant ( $P<0.001$ ) between 0 (18.5µg/g) and 50 dpi (8.1µg/g) but not for PO-treatments. This is indicative of induced ligninolytic activity in PC in a starved environment, where *E. coli* reduction was  $>1.0$  log ( $P<0.0001$ ); however, *E. coli* reduction was not significant in PO-treatments.

**Significance:** PC is more useful than PO for food safety mycoremediation. PC inhibited *E. coli* growth accompanied by increased ligninolytic activity.

## P1-73 Leafy Green Vegetables: A Comprehensive Review on Cultivation, Risk Assessment, and Food Safety Interventions from a Global Perspective

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**Introduction:** Globally, leafy green vegetables (LGVs) are important for human health, due to their high nutritional value and health benefits. However, continued increase in the demand for LGVs coupled with hazards and health risks associated with the consumption of LGVs reveals an urgent need for improved and reliable sanitation methods that assure food safety and better consumer health.

**Purpose:** This study seeks to make recommendations to current food industry problems and issues of public health concern by providing an overview on current issues relating to the safety of LGVs in the food supply chain.

**Methods:** A comprehensive literature search was conducted using three databases (Scopus, PubMed central, and Web of Science) to identify relevant studies on LGVs within the last decade (2013 to 2022).

**Results:** This study highlights the hazards (which include heavy metals, pesticide residues, antibiotic residues, antimicrobial-resistant bacteria (ARB), antimicrobial-resistant genes (ARGs), and microbial toxins) harboured by LGVs from farm- to-fork, and the impact of various cultivation methods on food safety.

**Significance:** Maintaining the safety of LGVs requires a systematic approach that encompasses all aspects of the food value chain. Therefore, combination treatment strategies for the eradication of different hazards in LGVs can be explored while implementing and scaling up existing food safety intervention strategies.

## P1-74\* Foodborne Pathogen Presence from Unprocessed Leafy Greens and Processed Leafy Greens in South Africa

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**Introduction:** Leafy greens (LG) are increasingly linked to foodborne pathogen outbreaks globally.

**Purpose:** This study aimed to determine if a difference exists in pathogen presence and microbial load between unprocessed and processed RTE samples from a facility in the Western Cape, South Africa.

**Methods:** Sixty samples of whole LG, as received from farms, and 60 RTE products, cut and washed, along with chlorine wash-water samples (30 to 70ppm) were collected over a three-week period. Samples were screened for the presence of *Listeria* spp. (ISO 11290-2:2017), *Salmonella* spp. (ISO 6579:2002) and aerobic plate counts (APC) determined (ISO 6887-1:1999, ISO 4833:2003). Pathogen confirmation was done using the Vitek®2 compact automated system.

**Results:** Total APC ranged from  $<10,000$  CFU/g to 6 log CFU/g for the wash water, 5 to 8 log CFU/g for unprocessed LG and  $<10,000$  CFU/g to 8 log CFU/g for RTE products. Subsequently, numerous samples exceeded acceptable limits. The mean APC for the unprocessed LG was 6.7 log CFU/g and 6.8 log CFU/g for processed RTE products. Three of the 120 samples (0.025%) tested positive for *Listeria monocytogenes*, all three of which were processed RTE products,

and none for *Salmonella* spp. Upon confirming presumptive positives for *L. monocytogenes* from chromogenic agar *Listeria* pathogens such as *Enterococcus gallinarum*, *Enterobacter cloacae* complex, *Klebsiella pneumonia pneumonia*, and the emerging foodborne pathogen *Kocuria kristinae* were identified.

**Significance:** Results indicate there is a risk of foodborne pathogens contaminated on commercially available RTE leafy greens which may be linked to processing practices and facilities. The isolation of pathogens from a processed RTE product which is consumed raw poses significant risks to human health in developing countries.

## P1-75 *Bacillus thuringiensis* on Vegetables

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**Introduction:** *Bacillus thuringiensis* (*Bt*) is an important bioinsecticide applied in crop production. However, due to the presence of genetic prerequisites for producing enterotoxins and its close phylogenetic relationship with *B. cereus* sensu stricto (an opportunistic foodborne pathogen) the safety of commercial *Bt* strains is continuously under discussion. One knowledge gap that hampers the risk assessment of biopesticide *Bt* strains is their prevalence in foodstuffs.

**Purpose:** The aim of this work was to generate prevalence data for *Bt* on foods in order to assist *Bt* related risk assessment.

**Methods:** Bell pepper samples (n = 99, in 2017) from supermarkets in Berlin were tested for *B. cereus* (s.l.). In addition, the competent food control laboratories of the federal states in Germany tested tomato samples (n = 426; in 2016), pre-packaged leaf lettuce (n = 420; in 2021) and head lettuce (in 2022; data processing is ongoing) and submitted *B. cereus* (s.l.) isolates to the laboratory for spore formers at the BfR. All isolates were screened for their ability to produce parasporal crystals by microscopy. Subsequently, a representative number of *Bt* isolates underwent whole genome sequencing and SNP analysis, which included the sequences of seven commercial strains.

**Results:** In tomato and bell pepper, *Bt* was the dominating species in samples tested positive for *B. cereus* (s.l.) (99% and 98%, respectively) whereas in leaf lettuce the *Bt* proportion was only 22%. Overall, between 9 and 41% of samples from the different vegetables contained *Bt*. Most *Bt* isolates differed by less than 10 core SNPs from a respective biopesticide *Bt* strain. These strains were detected at the critical level of  $\geq 10^5$  CFU/g in one tomato sample and seven leaf lettuce samples.

**Significance:** These data provide an improved basis for exposure assessment as an element of the risk assessment of biopesticide *Bt* strains.

## P1-76 Fate of *Salmonella* Newport on Tomato Plants When Challenged with Bacteriophage Cocktails

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**Introduction:** Bacteriophages have emerged as a promising agent to reduce bacterial pathogens on foods and could be used to reduce *Salmonella* on tomatoes.

**Purpose:** The objective of this work was to determine the effectiveness of two bacteriophage cocktails in reducing *Salmonella* inoculated onto tomato plants.

**Methods:** Phage cocktails consisting of either three (HER19, 06, SE13) or five (Felix 01, HER19, 03, 06, SE13) phage were sprayed to deliver 6 log PFU/cm<sup>2</sup> onto a 4 cm<sup>2</sup> marked area of two varieties of tomato fruit or leaf in a greenhouse one day before or one day after a challenge with *Salmonella* Newport (5 log/cm<sup>2</sup>). *Salmonella* and phage populations were measured on days 0 and 1. The treated area of leaves was excised and processed by adding 20 ml water (phage) or TSB (*Salmonella*) in conical tubes and vortexed for one min. Whole fruit were placed in 50 ml sterile water (phage) or TSB (*Salmonella*) in whirl pak bags, rubbed by hand for 1 min and rotary shaken for 30 min. *Salmonella* samples were diluted and enumerated onto XLD. Phage samples were passed through a 0.22 µm filter for analysis by plaque assay. Three trials were completed with duplicate samples (n=6).

**Results:** The largest *Salmonella* population reductions occurred when the challenge of *Salmonella* preceded the phage application regardless of phage cocktail, plant variety, leaf or fruit. The population reduction on fruits was significantly higher ( $P < 0.05$ ) when *Salmonella* application occurred prior to a five phage cocktail (1.0 log CFU/cm<sup>2</sup>)

than after (0.0 log CFU/cm<sup>2</sup>). Population reductions on leaves were significantly higher ( $P < 0.05$ ) when *Salmonella* application occurred prior to a 3-phage cocktail (0.3 Log CFU/cm<sup>2</sup>) than after (0.1 Log CFU/cm<sup>2</sup>).

**Significance:** Bacteriophage applied following *Salmonella* contamination on the surfaces of tomato fruits and leaves significantly reduced pathogen populations; further investigations of its commercial potential are warranted.

## P1-77 Food Safety Culture Excellence through Implementation of GFSI-Benchmarked Schemes in the Fresh Produce Sector

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**Introduction:** Access to safe and quality food is of paramount importance and an essential requirement for consumers to maintain their health and wellbeing. The meticulous efforts of food producers to demonstrate their commitments to food safety and fulfil customers preferences and expectations can gain more attention if organizations demonstrate well established quality and food safety cultures. Top management commitment and involvement is mandatory to imbibe positive food safety culture at all levels in organizations.

**Purpose:** The purpose of this study was to depict adoption of innovative ideas and reflection of collective attitude, beliefs and behaviours of organizations' top management, managers, supervisors and food handlers towards resolving food safety and hygiene issues and setting contemporary trends. This leads towards transforming existing food safety practices into a more sophisticated and regimented food safety culture.

**Methods:** In the present study, a survey of fresh produce facilities and distribution centres on a quarterly basis of Dar al Fadhil Group was conducted in kingdom of Saudi Arabia.

**Results:** Results of this study showed that appropriate trainings, empowering the employees to share their ideas and motivations, and strong top management commitment are ways which lead towards transforming existing food safety practices into a more sophisticated and regimented food safety culture.

**Significance:** This study is quite helpful for fresh producers and storage facilities, by showing how their objectives turn into reality when they successfully attain certification of their food facilities against GFSI benchmarked schemes by a prestigious GFSI-approved certification body.

## P1-78 Screening for the Antibacterial Compound in Carrot Roots against *Listeria monocytogenes* Using Mass Spectrometry

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**Introduction:** The antibacterial activity of carrot roots against *Listeria monocytogenes* (*Lm*) has been described previously. However, the chemical responsible for the antilisterial effect of carrots has not been fully elucidated.

**Purpose:** This study is to characterize the mass spectra of different carrot samples and to identify a prevalent compound correlated with the observed antilisterial effect.

**Methods:** Carrots were cut into 1.5 g slices with the same diameter using an apple corer. Half of the slices were boiled in water for 10 min for inactivation of the antimicrobial compound. Carrot slices were then inoculated with 7 log CFU/mL *Lm* FS2025. After 30 min incubation, *Lm* was recovered from carrot slices by sonication and plated on Harlequin agar. Additionally, different carrots were cut into 1.5 g pieces and soaked in water for 30 min. One part of this carrot water was boiled. The carrot water samples were also inoculated with 7 log CFU/mL *Lm* for 30 min and their plate counts determined.

Slices of cut and boiled carrots, as well as carrot water samples were measured using Direct Analysis in Real-Time Mass Spectrometry (DART-MS). Mass spectra of different carrot samples were compared. In order to identify a prevalent compound of interest, the carrot water samples were extracted with hexane and measured via Gas Chromatography coupled with MS (GC-MS).

**Results:** Carrot water showed a higher antilisterial activity than cut carrots indicating that the antibacterial compound is water-soluble. Boiling of carrots and carrot water abolished the antilisterial activity and led to a decrease in intensity for some mass spectral peaks. Hexane extracts of the carrot water allowed identification and quantification of enriched peaks with potential antilisterial activity.

**Significance:** These data will help to identify the antilisterial compounds in carrots which could be used as natural antimicrobials against *Lm* in the fresh produce industry.

## P1-79 Compare the Efficacy of Hydrogen Peroxide-Peroxyacetic Acid Delivered by Conventional Garden Sprayer, Electrostatic Sprayer, and Dip Methods on the Reduction and Mitigation of Cross-Contamination of *Listeria monocytogenes* and the Surrogate *L. innocua* on Apples

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**Introduction:** *Listeria monocytogenes* has been recognized as an emerging, under-researched pathogen by the United States Department of Agriculture-National Institute of Food and Agriculture due to the multi-state outbreaks on apples from 2015-2019. The need for research regarding antimicrobial application strategies, such as this current study, is evident by looking at the results from past apple storage studies.

**Purpose:** To compare conventional garden spray (GS), electrostatic spray (ES), and dip procedures for their efficacy of reducing and preventing cross-contamination of *L. monocytogenes* and the surrogate *L. innocua* on Fuji apples.

**Methods:** Cross-contamination was made possible by treating eight clean, uninoculated apples and four apples inoculated with 200 ppm nalidixic acid-resistant *L. monocytogenes* or *L. innocua* together. The treatments included water or 0.05, 0.10, and 0.25% of a sanitizer containing 23% hydrogen peroxide ( $H_2O_2$ ) and 5.3% peroxyacetic acid (PAA). The treatments were applied using GS, ES, or the dip method for 45 s, respectively. A modified most probable number (MPN) method was utilized to enumerate microbial populations on apples. Data were examined using JMP software mixed model analysis ( $n=15$ , 4 repeats,  $P=0.05$ ).

**Results:** The greatest ( $P < 0.05$ ) reductions of 3.63  $\log_{10}$ MPN/g (*L. monocytogenes*) and 3.81  $\log_{10}$ MPN/g (*L. innocua*) were achieved using 0.25%  $H_2O_2$ -PAA delivered by the dip method. The cross-contamination rate was minimized by GS using 0.25%  $H_2O_2$ -PAA for both *L. monocytogenes* ( $-0.35 \log_{10}$ MPN/g) and *L. innocua* (0.19  $\log_{10}$ MPN/g). For both reduction and cross-contamination rates, *L. monocytogenes* and *L. innocua* behaved similarly ( $P > 0.05$ ) using GS and dip method with 0.1 and 0.25% of  $H_2O_2$ -PAA.

**Significance:** Results of this study indicate *L. innocua* is an appropriate surrogate for *L. monocytogenes* for antimicrobial treatments on apples. The results also indicated that the GS method along with 0.1 and 0.25%  $H_2O_2$ -PAA was the most efficient method for reducing and preventing microbial cross-contamination on apples.

## P1-80 Prevalence, Virulence Genes, and Antimicrobial Resistance of *Vibrio parahaemolyticus* Isolated from Seafood

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**Introduction:** *Vibrio parahaemolyticus* naturally inhabits estuarine and marine environments worldwide. It possesses two main pathogenic factors including thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin (*trh*). *V. parahaemolyticus* causes acute gastroenteritis after consumption of contaminated seafood.

**Purpose:** The purpose of this study was to isolate *V. parahaemolyticus* from seafood as well as presence of *tdh* and *trh* genes and antimicrobial resistance of the isolates to be assessed.

**Methods:** 180 raw seafood samples included mussels ( $n=20$ ), sea snails ( $n=20$ ), oysters ( $n=20$ ), salmon ( $n=20$ ), gilt-head bream ( $n=20$ ), horse mackerel ( $n=20$ ), bluefish ( $n=10$ ), seabass ( $n=20$ ), squid ( $n=20$ ), and shrimps ( $n=10$ ) were tested for the presence of *V. parahaemolyticus*. MALDI Biotyper<sup>®</sup> (Bruker, Germany) and a PCR method with a target *toxR* gene were applied to identify *V. parahaemolyticus*. It was tested for the presence of *tdh* and *trh* genes by means of PCR. Antimicrobial resistance of *V. parahaemolyticus* was determined by the Kirby-Bauer Disc Diffusion Method, and MAR-index was calculated as well.

**Results:** It was found that 44 (43%) out of 103 isolates were positive for *V. parahaemolyticus*. None of the isolates exhibited *tdh* gene, while *trh* gene was found in 1 (2%) isolate from sea snails. All isolates were susceptible to Co-trimoxazole, Tetracycline, Gentamycin, Amoxicillin+clavulanic acid, Amikacin, Ciprofloxacin, and Levofloxacin. Intermediate resistance was found to Ampicillin (25%; 11/44), Cefepime (21%; 9/44), and Ceftazidime (2%; 1/44). The results showed 9% (4/44) of the isolates were resistant to Cefepime and Ampicillin, while 5% (2/44) of the isolates were resistant to Ceftazidime. MAR-index values ranged from 0.1 to 0.3.

**Significance:** This is the first study demonstrating pathogenic *V. parahaemolyticus* in seafood intended for human consumption in Bulgaria. MAR-index indicates excessive usage of antibiotics in aquaculture fields.

## P1-81\* Genetic Diversity of *Listeria monocytogenes* from the Fish Industry in the Western Cape, South Africa Using Whole Genome Sequencing

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**Introduction:** Several outbreaks have been reported worldwide linked to fish (smoked trout, smoked salmon, smoked mussels, and raw fish and molluscan shellfish). *Listeria monocytogenes* is a problem in fish and the fish food processing environment (FPE). With no, or improper heat treatment and the ability of *L. monocytogenes* to survive and grow in refrigeration temperatures, fish products can pose a significant risk to the health of consumers. However, there is limited information in South Africa regarding *L. monocytogenes* isolated from fish and the FPE.

**Purpose:** The aim of this study was to investigate the genetic diversity of *L. monocytogenes* isolated from fish and the fish FPE in Western Cape, South Africa.

**Methods:** Twelve *L. monocytogenes* isolates from fish (salmon, smoked trout, fresh hake, oysters), from five different fish FPE were included in this study. *L. monocytogenes* were isolated using culture-based methods (RAPID<sup>™</sup>L.Mono<sup>™</sup> Chromogenic Media and 2% blood agar (for purity)), screened for the virulent *hly* gene by PCR, and categorised into lineage groups using PCR-restriction fragment length polymorphism (RFLP). The WGS was performed by CosmosID using Illumina technology and data files were analysed for serotype and sequence type (ST) information.

**Results:** The isolates examined belonged to two lineage groups and three serogroups. Lineage I ( $n=4$ ) with serogroups 1/2b (ST87) and 4b (ST54 and ST515) were identified. And lineage II ( $n=8$ ) consisted of serogroup 1/2a (ST121, ST204 and ST155).

**Significance:** The majority of human listeriosis cases are linked to serotype 1/2a, 1/2b and 4b. There has been an increase of listeriosis cases linked to 1/2a during the last decade in Italy, Finland, Denmark, Great Britain, Switzerland, and Canada. Serogroup 4b is typically associated with listeriosis outbreaks. All ST's, except ST515, have been associated with clinical cases around the world, highlighting the public health significance of these serogroups and ST's isolated.

## P1-82 Growth Variability of *Vibrio parahaemolyticus* Strains Isolated from Seafood

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**Introduction:** *Vibrio parahaemolyticus* is a major foodborne pathogen involved in seafood poisoning incidents. Microbial exposure assessment models require accurate characterization of the microbial growth of the studied strains. One of the most important parameters is that of the maximum growth rate ( $\mu_{max}$ ).

**Purpose:** The objective of this study was to quantify the growth and assess the strain variability of *V. parahaemolyticus* strains isolated from seafood.

**Methods:** A PCR method with a target *toxR* gene was applied to identify *V. parahaemolyticus* strains previously isolated from mussels ( $n=6$ ), sea snails ( $n=2$ ), oysters ( $n=2$ ), salmon ( $n=2$ ), gilt-head bream ( $n=2$ ), horse mackerel ( $n=2$ ), bluefish ( $n=2$ ), and seabass ( $n=1$ ). Additionally, the pandemic *V. parahaemolyticus* O3:K6 was used as a reference strain. The optical density of 2-fold serial diluted bacterial cultures grown in alkaline saline peptone water with pH 8.6 and 37°C was measured at 630 nm (Microplate Reader Rayto RT-2100C, China). The  $\mu_{max}$  was computed by regression analysis using the generalised reduced gradient algorithm. Each isolate was assessed in triplicate, and the mean values of  $\mu_{max}$  were calculated.



**Results:** In total, 19 *V. parahaemolyticus* strains were molecularly characterised. The mean  $\mu_{max}$  ( $h^{-1}$ ) of *V. parahaemolyticus* ranged from 0.8 to 1.78 for mussels, 1.55 to 1.74 for sea snails, 1.17 to 1.23 for oysters, 2.18 to 2.38 for salmon, 1.20 to 1.21 for gilt-head bream, 1.99 to 2.35 for horse mackerel, 1.9 to 2.69 for bluefish, while it was  $2.08 \pm 0.26$  for seabass and  $2.1 \pm 0.55$  for the pandemic strain O3:K6. The strain with the highest growth was isolated from bluefish, and the slowest grower was isolated from mussels.

**Significance:** This study provides useful information for the quantitative characterization of *V. parahaemolyticus* growth which can be a main input for microbial exposure assessments as part of risk analysis of food-borne pathogens.

### P1-83 Efficacy of Chlorine and Non-Chlorine Sanitizers in Process Water from the Produce Industry to Control Viral Contamination

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**Introduction:** Process water (PW) used in the produce industry needs to be maintained at elevated microbiological quality by properly dosing disinfection agents. Viral inactivation capacity results are currently underexplored.

**Purpose:** This study evaluates the antiviral disinfection efficacy of chlorine (FC), chlorine dioxide (ClO<sub>2</sub>) and peracetic acid (PAA) at recommended operational limits in PW of baby leaves, bell peppers, and a vegetable mix by experiments in batch (BS) and dynamic (DS) systems.

**Methods:** In BS experiments, chlorine and non-chlorine sanitizers were mixed with PW to target 20 mg/L FC, 80 mg/L PAA, or 3 mg/L ClO<sub>2</sub>. Changes in the levels of infectious viruses were measured by cell-culture (HAV, MNV and TV), plaque assay (MS2), and human intestinal organoid cultures (human norovirus). For DS inactivation experiments, a constant entrance of PW and disinfectant solution was maintained.

**Results:** In BS experiments, 20 mg/L FC and 3 mg/L ClO<sub>2</sub> completely inactivated human norovirus, MNV, TV and MS2 after 1 min contact time regardless of the PW type. On the contrary, FC and ClO<sub>2</sub> reduced the infectivity of HAV by less than 2 log after one min. For PAA, residual MNV and TV infectivity was observed despite the 2 log reductions. Using DS, FC (5 mg/L) and ClO<sub>2</sub> (2-3 mg/L) prevented the accumulation of detectable MS2, while PAA (80 mg/L) did not regardless of the type of PW.

**Significance:** Recommended operational limits for FC and ClO<sub>2</sub> effectively control human enteric viruses.

### P1-84\* Absolute Quantification of Viral Indicators via Digital Polymerase Chain Reaction Wastewater Surveillance through the COVID-19 Pandemic

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**Introduction:** Reliable detection of pathogens is critical to prevent foodborne outbreaks and to respond when they occur. Viruses in particular pose a unique threat in their ease of transmission, as well as the challenges faced with detection.

**Purpose:** The objective of this study was to determine the correlation between viral human fecal indicator Pepper Mild Mottle Virus (PMoV) and SARS-CoV2 to evaluate efficacy of wastewater monitoring for pathogens in agricultural matrices.

**Methods:** Composite wastewater samples (24 hr) were collected from 13 sites within Northern Delaware using ISCO samplers. Sites included public schools, municipalities, residential areas, and a hospital. Sub-samples (50 mL) were manually homogenized in triplicate and inoculated with Bovine Corona Virus (BCoV) as a process control and incubated at 60°C for 30 min to inactivate live virus. Samples were filtered in duplicate through a 0.22 µm PES membrane, 120 mL of each filtrate was then loaded into 4 Amicon Ultra-15 centrifugal filters (30 mL/filter) and centrifuged at 3000xg for 45 minutes at 20°C to concentrate the virus. Concentrate was collected and RNA extracted using Qiagen RNeasy Power Microbiome kit. Extracted RNA was analyzed for SARS-CoV2 N1 and N2, BCoV, and PMMoV using triplicate multiplex dPCR. Samples were collected over a period of 33 months (N=1809). Data were analyzed using bivariate distribution and Pearson's correlation coefficient.

**Results:** Across all sites, quantities of PMMoV and SARS-CoV2 had a significant ( $P < 0.01$ ) positive correlation. Samples collected from the hospital catchment showed a strong positive correlation (0.806) between PMMoV and SARS-CoV2 levels.

**Significance:** The strong positive correlation between these viruses proves the potential of wastewater surveillance as a useful tool for monitoring important foodborne pathogens such as norovirus and hepatitis A, with potential for detection of protozoa as well.

### P1-85 Occurrence of Indicator Genes of Antimicrobial Resistance Contamination in the North Sea and English Channel Seawaters

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**Introduction:** The marine environment is a potential natural reservoir of antimicrobial resistance genes, subject to anthropogenic effluents (wastewaters, industrial, domestic) and known as a final receiving system. The *tetA*, *bla<sub>TEM</sub>*, *sul1*, and *int1* genes have been proposed as indicators of contamination to assess the state of antimicrobial resistance in the environment. Yet, there is no information on their prevalence and abundance in large marine environments far from the coast, such as the English Channel and the North Sea.

**Purpose:** To investigate the abundance and geographical distribution of the *tetA*, *bla<sub>TEM</sub>*, *sul1*, and *int1* antimicrobial resistance indicator genes in the English Channel and the North Sea seawaters.

**Methods:** Bacterial DNA was extracted from 36 seawater samples collected during the IBTS oceanographic campaign in the English Channel and the North Sea. The absolute abundances of the indicator genes and the bacterial *tuf* gene (to evaluate the abundance of the bacterial population) were determined by qPCR and were analyzed in association with environmental variables and geographical locations to determine potential correlations.

**Results:** The *bla<sub>TEM</sub>* and *tetA* genes were quantified in 0% and 2.8% of samples, respectively. The *sul1* and *int1* genes were detected in 42% and 31% of samples, respectively, with an apparent co-occurrence in 19% of samples confirmed by correlation analysis. The abundance of these genes was correlated with the microbial population and environmental variables such as dissolved oxygen and turbidity. The highest abundances of the three *tetA*, *sul1*, and *int1* genes concerned the same sample that was collected from the West Netherlands coast area.

**Significance:** For the first time, we have shown the impact of anthropogenic inputs (rivers, man-made offshore structures, maritime activities) and environmental variables on the occurrence of indicators of environmental contamination by antimicrobial resistance in the North Sea and the English Channel seawaters.

### P1-86 Characterisation of Carbapenem-Resistance Profiles Derived from Different AST Methods in Gram-Negative Bacteria from River Water in the Western Cape, South Africa

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**Introduction:** Antimicrobial resistance (AMR) in natural environments has gained traction recently. The prevalence of carbapenem resistance (CR), specifically carbapenemase-producing Enterobacterales (CPE), is severely understudied in water environments in South Africa, posing significant risks to healthcare and likely food safety.

**Purpose:** The purpose of this study was to analyze CR profiles obtained from phenotypic and genotypic antibiotic susceptibility testing (AST) of environmentally-isolated Enterobacterales, *Aeromonas*, and *Pseudomonas* species from nine rivers in the Western Cape, South Africa.

**Methods:** Enriched water samples (N=33) were subjected to AST using two clinically relevant antibiotics, ertapenem and meropenem (10 µg/disc). Two phenotypic screening methods, (1) direct-plating disc diffusion, and (2) a two-step broth enrichment, were used to isolate CR Gram-negative bacteria. Isolates within the "resistant" zone (EUCAST guidelines) were screened for presumptive CPEs using CHROMID<sup>®</sup> CARBA SMART agar. VITEK<sup>®</sup> 2 COMPACT automated technology was used for bacterial identification and AST profiles (CLSI guidelines). PCR analysis was used for the detection of carbapenemase genes (KPC, NDM, VIM, IMP and OXA-48). WGS of four isolates (using VITEK<sup>®</sup> AST outputs) followed.

**Results:** In total, 87 GN isolates displaying a resistant phenotype to ertapenem and meropenem were recovered from phenotypic AST, 43% of which were identified as presumptive carbapenemase producers. Species of interest were confirmed using VITEK®. Only ten isolates displayed a resistant profile on VITEK® to carbapenems, showing resistance to imipenem (4), meropenem (4), one combination of both, and one resistant to ertapenem. None of the isolates harboured any of the five carbapenemase genes, but WGS revealed genes conferring CR.

**Significance:** This is the first study confirming the presence of environmental CR in river water in the Western Cape. Accurate identification of CR (by any mechanism) is essential for ensuring water sources are not posing a risk to food safety, thus minimising the spread of AMR, and maintaining antibiotic effectiveness for human medicine.

## P1-87\* Antibiotic-Resistance Profiling of Ultraviolet (UV)-Resistant Bacteria from Rivers Used for Irrigation in the Cape Winelands Region (South Africa)

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**Introduction:** The presence of antibiotic resistant bacteria in irrigation water after UV- treatment poses a threat to consumers of fresh produce. The profiling of UV-treated irrigation waters may aid understanding of the dissemination of antibiotic resistance through irrigation of fresh produce.

**Purpose:** The study aimed to establish antibiotic resistance profiles of UV-resistant bacterial isolates found in rivers used for fresh produce irrigation in the Cape Winelands region.

**Methods:** River water samples, originating from two rivers, were subjected to three consecutive doses of medium pressure UV irradiation (3x20 mJ.cm<sup>-2</sup>), followed by a three-hour recovery period. *Escherichia coli*, coliforms, and heterotrophic plate counts were monitored. The presence of *Salmonella* species (ISO 19250:2010) and *Listeria monocytogenes* (ISO 11290-1:2017) was also determined. Twenty-three UV-surviving isolates were identified using the VITEK®2 Compact Automated System and underwent antibiotic susceptibility testing (AST) towards 19 antibiotics, as well as phenotypic screening for extended-spectrum beta-lactamase (ESBL)-production.

**Results:** Identification of UV-surviving isolates revealed a high prevalence of *Escherichia coli* (32%) and *Enterobacter cloacae* complex (29%). All isolates screened for ESBL-production were ESBL negative. Of the 23 isolates which underwent AST, 65% were resistant to at least one antibiotic, 48% were resistant to two or more antibiotics, and 13% were identified as being multidrug resistant. Resistance to both cefalotin and amoxicillin/clavulanic acid (39% of isolates), and ampicillin (26% of isolates) was prevalent. Intermediate resistance to chloramphenicol was found in 39% of isolates.

**Significance:** This study demonstrates the presence of UV- and antibiotic resistant bacteria in irrigation water following UV-treatment. Notably, certain bacteria were found to be resistant to antibiotics classified by the World Health Organization as 'critically important' and 'highly important' antimicrobials for human medicine. With a predicted rise in outbreaks of untreatable disease related to antibiotic resistance, this study highlights the need for improved effectiveness in UV-treatment of these waters.

## P2-01 Replacement of Potassium Sorbate in Cultured Dairy with a Plant Extract and Fermentate Combination

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**Introduction:** Consumer label awareness has resulted in food manufacturers to seek clean-label alternatives for extending product shelf life. Cultured dairy products are susceptible to yeast and mold spoilage, with few clean-label alternatives to sorbate available.

**Purpose:** To evaluate the performance of a plant extract and fermentate (C1) on yeast and mold growth at 4°C in a cultured fresh cheese while maintaining live culture survival and pH stability within the product.

**Methods:** A commercial cultured fresh cheese (pH 4.4, 76.51% moisture) containing no preservatives (PC) was supplemented with 0.04% sorbate or 0.275% C1 prior to inoculation with 3 log CFU/g yeast (*Debaryomyces hansenii*, *Candida parapsilosis*) or mold (*Mucor janssenii*, *Eupenicillium javinivum*, *Penicillium roqueforti*, *P. camemberti*) cocktails. Yeast samples were assayed weekly by pour-plating with PDA for 49 days, while mold samples were visually inspected every 2-3 days. Uninoculated samples were assayed for pH and live

cultures (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*) by plating weekly on acidified MRSA and LM-17 for 49 days. Additionally, growth curves of *Zygosaccharomyces rouxii*, *Z. bailii* and *Saccharomyces cerevisiae* were generated in PDB (pH 4.5) with 0.275% C1, sorbate (0.04%, 0.1%) or benzoate (0.1%) (BioTek LogPhase600, 48 h, 25°C).

**Results:** PC supported >2 log CFU/g yeast growth by day 35, while 0.275% C1 inhibited yeast growth (<1 log) for 49 days. Time to mold was increased 35% with 0.275% C1 compared to the PC (from 21 to 28 days). The addition of 0.275% C1 did not impact pH (pH 4.40±0.05) nor live culture survival over 49 days, with cultures at >8log CFU/g throughout the test period. 0.275% C1 in PDB inhibited growth of *Z. rouxii*, *Z. bailii* and *S. cerevisiae*.

**Significance:** This work supports the use of fermentate and plant extract combination as a suitable clean-label antimicrobial against yeast and mold in cultured dairy while maintaining product pH and active cultures populations.

## P2-02 Targeting Sipa Protein with NAM-Aptamers as an Innovative Approach to Control Salmonellosis in the Poultry Industry

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**Introduction:** Salmonellosis is considered one of the main bacterial infections in the poultry industry having an important negative economic impact worldwide. Several measures have been implemented by competent authorities as an attempt to control salmonellosis, however this is still the second leading cause of foodborne zoonosis reported per year. Therefore, the prevention and control of *Salmonella* infections must be done by combining all available resources and covering the entire food production chain. Aptamer-based technologies are an innovative approach to treat infection with different pathogenic microorganisms and to prevent the infection process. Regarding salmonellosis, one way to do this is by blocking the adhesion, thereby preventing *Salmonella* from attaching to the intestinal epithelial cells.

**Purpose:** Blocking the adhesion protein SipA from *Salmonella* using SipA-specific nucleic acid mimic (NAM)-aptamers.

**Methods:** The Apt17 that recognizes SipA protein was selected based on current literature. Bioinformatic tools were used to predict possible tertiary structures of the NAM-aptamers. The structure of the aptamer-SipA complex was also analyzed and the contact residues between them were identified to improve the aptamer by replacing specific nucleotides with non-natural nucleotides. In addition to the full SipA protein also a specific domain (SipA426-685), that plays an important role in the entry of *Salmonella* into host epithelial cells, was analyzed. The K<sub>d</sub> constants were determined using a real-time PCR methodology and estimated directly from the experimental results through the binding curve using a non-linear regression analysis.

**Results:** The binding assays confirmed the binding capacity of the Apt17 to the SipA protein, both to the full protein (with K<sub>d</sub> values 2.7 nM) and the specific C-terminal domain (with K<sub>d</sub> values 2.1 nM).

**Significance:** This work aims to develop the first aptamer-based solution able to prevent/control *Salmonella* infection in the poultry industry, as an alternative approach to avoid the side effects associated with the use of antibiotics.

## P2-03\* The Effect of Ionophore Use and Essential Oil Compounds in the Diet of South African Calves on the Prevalence of Antibiotic-Resistant *Escherichia coli* and *Salmonella* spp.

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**Introduction:** Subtherapeutic antibiotics, such as the ionophore monensin, are used in calf rearing systems to aid in the growth and health of the animal. However, recent concerns of increased antibiotic-resistant foodborne pathogens linked to the subtherapeutic use of antibiotics in animals led to the investigation of alternatives to ionophores, such as essential oil compounds.

**Purpose:** The purpose of the study was to determine whether a blend of essential oil compounds could yield similar health and growth benefits compared to monensin when included in the diets of pre-weaned calves. The diets were investigated with the aim of influencing the



prevalence of antibiotic resistant *Escherichia coli* and *Salmonella* spp. found in the faecal matter of the calf, to hopefully limit the farm-to-fork spread of antibiotic resistant pathogens.

**Methods:** Garlic extract and a combination of carvacrol, capsaicin, and cinnamaldehyde were compared to monensin inclusion in the calf diet. Its cumulative effect on antibiotic resistant *E. coli* and *Salmonella* spp. was investigated during two animal trials.

**Results:** Antibiotic-resistant *Salmonella* spp. and *E. coli* was present across all treatment groups, where all *Salmonella* spp. isolates were resistant to two or more antibiotics tested. Ionophore inclusion in the diet resulted in an increased abundance of multidrug resistant *E. coli* isolates (71%) compared to that of the control (36%) and to calves fed a blend of essential oil compounds (46%;  $P = 0.02$ ). Interestingly, therapeutic antibiotic use did not result in the increased prevalence of antibiotic-resistant *E. coli* isolates ( $P = 0.09$ ).

**Significance:** The increase in multidrug-resistant *E. coli* associated with added ionophores to the diet confirms that ionophore use in calf rearing systems contribute to the increased risk of antibiotic resistance associated with subtherapeutic antimicrobial use in calves that could spread to the food chain and be harmful to human health.

## P2-04 Efficacy of Peracetic Acid (PAA) in Combination with a PAA Booster Against Bacterial Biofilm

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**Introduction:** Peracetic acid (PAA) is one of the most commonly used sanitizer/disinfectants in the food industry due to its efficacy, low cost, lack of toxic residues and easy application. Nevertheless, when used alone, undesirably high levels of PAA may be needed to achieve activity against difficult to control microbial targets such as biofilm and endospores.

**Purpose:** A formulation containing a mixture of organic acids, chelants, surfactants and biodegradable was developed and evaluated for its ability to enhance PAA performance against bacterial biofilm and endospores.

**Methods:** The MBEC Assay was used to screen various concentrations of PAA (50 to 400 ppm) alone and in combination with the booster to determine efficacy against biofilm formed by *Pseudomonas aeruginosa*. Additional testing was performed using a CDC biofilm reactor and the Single Tube Method at various concentrations of PAA +/- booster. Sporicidal activity against *Bacillus subtilis* and *Clostridium sporogenes* was determined using a modified version of EN 13697.

**Results:** In the MBEC Assay, the combination of PAA and booster achieved consistent  $\geq 6$  log kill at 100 ppm PAA whereas PAA alone required  $> 400$  ppm. In the Single Tube Method, the combination of PAA and booster achieved  $> 6$  log kill at 300 ppm PAA compared to PAA alone which required  $> 700$  ppm. Against endospores dried on stainless steel carriers, the booster increased the sporicidal activity of PAA as much as 1 to 3 log reduction values compared to PAA alone.

**Significance:** The results indicate that the booster can increase the performance of PAA against bacterial biofilm and endospores.

## P2-05 Low Temperatures Alter the Antimicrobial Activity of Silver Ions in Treated Surfaces

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**Introduction:** *Listeria monocytogenes* is a foodborne pathogen able to survive in food processing environments over a long period in spite of the repeated implementation of stringent cleaning and disinfection procedures. Silver ions are broad-spectrum antimicrobials. Therefore, some new materials impregnated with encapsulated silver ions are now proposed for use in food industries in order to control microbial proliferation on surfaces. These solutions may then contribute to a better control of *L. monocytogenes* in food industries.

**Purpose:** This study aimed at evaluating *in vitro* but also *in situ* the ability of a plastic film containing silver ions to inactivate *Listeria* cells in seafood manufacturing conditions.

**Methods:** The antimicrobial activity against *Listeria* of the treated plastic film was assessed according to the ISO 22196:2011 standard. Plastic films whether or not containing silver ions were also stuck on walls located in critical areas of seafood processing environments. Mesophilic aerobic microorganisms were enumerated, and *Listeria*

searched every month on installed films. The impact of temperature, bacterial concentration, mode of inoculation, the quantity of organic matter on the measured antimicrobial activity of silver containing materials was also evaluated *in vitro*.

**Results:** Films impregnated with silver ions had an antilisterial activity according to the ISO 22196:2011 standard. However, there was no evidence of antimicrobial activity in industries. To better understand these contradictory results, we further investigated the impact of the main parameters differing between the ISO 22196 standard *in vitro* conditions and industrial conditions. Among the tested parameters, temperature had the highest impact. Silver ions were ineffective at 8°C and 20°C against *L. monocytogenes* but effective at the temperature used in the ISO 22196 standard, i.e. 35°C.

**Significance:** Low temperature in seafood processing environments may explain the lack of activity of silver ions impregnated plastic films installed in industries.

## P2-06 Phenotypic Characterisation of Enterobacterales Species Isolated from UK Retail Food

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**Introduction:** Food systems can serve as reservoirs of antimicrobial resistant Enterobacterales, which are frequently associated with live-stock gastrointestinal tracts or horticulture production systems. They may cross-contaminate associated foods, presenting an important transmission vector to consumers. Antimicrobial resistance (AMR) in pathogenic species significantly complicates medical interventions and increases patient mortality, particularly when resistance to critical antimicrobials such as carbapenems are present.

**Purpose:** This study isolated and characterised 300 bacterial isolates from meat (n=70) and horticulture (n=40) food products from UK retail stores.

**Methods:** Food products were homogenised, incubated for 6h at 37°C in Buffered Peptone Water and plated on MacConkey and mSuperCARBA agar plates. Isolates were speciated using 16S Sanger sequencing. Kirby-Bauer tests were employed to determine AMR profiles and Triton modified Hodge tests to detect carbapenemase production. Biofilm production was characterised using Crystal Violet assays and Congo Red agar plates.

**Results:** Of the 300 isolates collected, 84% were identified as Enterobacterales (n=252). Species include *Proteus* (20.24%), *Escherichia* (18.65%), *Enterobacter* (11%), *Hafnia* (10.33%), *Serratia* (9%), *Citrobacter* (8%), and *Klebsiella* (2.33%). AMR was observed at the following rates: amoxicillin-clavulanic acid (44%), chloramphenicol (13%), cefotaxime (10%), ertapenem (9%), aztreonam (8%), ceftazidime (7%), trimethoprim-sulfamethoxazole (7%), ciprofloxacin (6%), tobramycin (3%). Six percent of Enterobacterales possessed resistance to five or more antimicrobials, one *K. pneumoniae* isolate resisted all tested antimicrobials; 65% resisted at least 1 antimicrobial screened. Carbapenemase-production was detected amongst 32% of ertapenem-resistant Enterobacterales (n=7). *Hafnia* species were associated with highest biofilm formation capacity, while *Klebsiella* species were the weakest biofilm producers, on average.

**Significance:** This study shows diverse AMR phenotypes among Enterobacterales within meat and horticulture food products; notably, with 44% of isolates resistant to amoxicillin-clavulanic acid and 9% resistant to ertapenem. These observations indicate that antimicrobial resistant Enterobacterales, some resistant to carbapenems, are present within the UK food chain and may present a risk to public health.

## P2-07 In-Vitro Antimicrobial Potential of a White Grape Skin Seasoning Against Food Pathogens

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**Introduction:** Due to trends toward more gentle processing and less intense cooking, prepared chicken products may represent a health risk to the consumer as a vehicle for *Campylobacter jejuni* and *Listeria monocytogenes*. Natural alternatives are currently being sought to reduce these risks.

**Purpose:** The aim of this work was to study the antimicrobial effect of a seasoning derived from white grape skin against *Listeria* spp. strains from various origins (due to its relevance in the food industry and high mortality) and *Campylobacter jejuni* (due to its high incidence), as well as to establish the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in both pathogens.

**Methods:** The broth microdilution method was used, testing concentrations of 4, 3 and 2% of the seasoning at 4°C for 15 days for *Listeria* spp. strains and at 37°C for 48 h and in concentrations of 2, 1 and 0.5 % for *C. jejuni* strains.



**Results:** The effectiveness of this seasoning as an antimicrobial on both pathogens was verified. The MBC for all *C. jejuni* strains was 2% and the MIC was 1%. The MBC for *L. monocytogenes* would be higher than 4%, which was the highest concentration tested and it did not eliminate this microorganism. Its MIC was 4% for all strains of *L. monocytogenes*.

**Significance:** This seasoning would be a good option to be added to poultry products, helping to improve food safety against the pathogens studied. In this way, the food industry can be helped both in the incorporation of new natural additives and in the use of by-products from the wine industry.

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## P2-08 Improving Poultry Burger Safety

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**Introduction:** Given the great influence of chicken meat consumption in the development of campylobacteriosis and the rejection of synthetic additives by consumers, many meat industries are investigating new sources of natural preservatives.

**Purpose:** The main objective of this work was to study the effect of a white grape pomace seasoning on *Campylobacter jejuni* and the shelf life in vacuum-packed chicken burgers stored at 4°C for 19 days.

**Methods:** Four different batches of burgers were produced: uninoculated control (C); 3% seasoning (S); control inoculated with 3 log CFU/g of a cocktail of four strains of *C. jejuni* (J); 3% seasoning and inoculated with 3 log CFU/g of *C. jejuni* (SJ). During the 9 days of sampling, microbiological analyses were carried out to determine the total mesophilic aerobic microorganisms (TMA), psychrophilic aerobes (PS), lactic acid bacteria (LAB) and *C. jejuni* using the ISO methodology. In addition, pH and water activity ( $a_w$ ) were also analyzed in the uninoculated batches.

**Results:** Regarding the physicochemical analyses, the addition of the seasoning did not modify the  $a_w$  values and decreased the pH values. On the other hand, the seasoning had no antimicrobial effect on LAB counts, but did significantly slow down PS and TMA counts for eight and 12 days, respectively, increasing the product shelf life by four days regarding the control batch. In inoculated patties (SJ), the seasoning reduced *C. jejuni* below the limit of quantification (< 1 log CFU/g) and, moreover, the pathogen was completely eliminated in the presence of the seasoning in batch S that was naturally contaminated.

**Significance:** The results obtained suggest that white grape skin seasoning could be used in poultry products as a sustainable functional ingredient to improve shelf life and ensure food safety against *C. jejuni*.

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## P2-09 Impact of Silver-Containing Surfaces on *Listeria monocytogenes* Biofilm

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**Introduction:** *Listeria monocytogenes* (*Lm*) is a foodborne pathogen that can persist on surfaces in food processing environments. The *Lm* persistence could be due to its capacity to form biofilms. The complex, multicellular structural characteristic of biofilms could offer bacterial cells protection during cleaning and disinfection procedures.

**Purpose:** We evaluated the impact of non-nanometric-sized silver ions encapsulated in smooth and hydrophobic surfaces on the mono-species and mixed-species *Lm* biofilm formation. The impact on the efficiency of the treatment with biocides was considered too.

**Methods:** Five surfaces containing non-nanometric-sized silver ions encapsulated or without silver ions were tested to prevent the mono-species and mixed-species (with *Carnobacterium*) biofilms of *Lm* in conditions close to the seafood environment (cultured at 8°C with the conditioning of the surfaces with salmon juice). After 24 hours of incubation, biofilms were observed by epifluorescence microscopy after live/dead staining. Additional tests were realized after treatment with biocides containing i) sodium hypochlorite ii) peracetic acid and hydrogen peroxide or iii) didecyl dimethylammonium chloride.

Quantification of viable cultivable (VC), viable (VC and viable but non-cultivable (VBNC)) and total (dead and viable) populations were performed by plate count agar, by qPCR coupled with propidium monoazide treatment, and by qPCR, respectively.

**Results:** Microscopic observations showed that biofilms grown on the surfaces containing silver ions with a density and architecture close to these carried out without silver ions. Quantification data confirmed that the VC, viable, and total populations were in similar amounts on the surfaces containing or not containing silver ions. Even if biocides have an effect on the VC population, some VBNC cells remain on the surfaces containing or not containing silver ions.

**Significance:** Under culture conditions tested, we didn't observe the effect of surfaces containing silver ions to prevent the formation of monospecies and mixed *Lm* biofilms.

## P2-10\* Investigating the Impact of Natural Anti-Microbials on *Escherichia coli* O157 in Processed Meat Products for Enhanced Safety and Shelf Life

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**Introduction:** Shiga toxin-producing *Escherichia coli* can cause serious illness in humans, and ruminant animals and meat are recognised transmission sources. Practical strategies to control this pathogen are thus needed by the meat processing industry.

**Purpose:** This study investigated the impact of five natural antimicrobial agents (carvacrol, thyme essential oil, cranberry extract, chitosan from shrimp and chitosan from mushroom *Agaricus bisporus*) on survival of *E. coli* O157 (3-strain cocktail) in beef burgers.

**Methods:** The antimicrobial agents were added at different concentration levels (0.05%, 0.1%, 0.2% carvacrol; 0.13%, 0.25%, 0.5% thyme essential oil; 2.5%, 3.75% cranberry extract; 0.63%, 1.25%, 2.5% chitosan from both sources). The concentrations selected were based on previous studies to determine the Minimum Inhibitory Concentration, whilst the potential impact on organoleptic properties of the burgers was also taken into account. The antimicrobial agents were added to the beef burgers (80% VL) which were inoculated with approximately 3.5 log CFU/g *E. coli* O157. The beef burgers were then vacuum-packed and stored at 3°C for 16 days. *E. coli* O157 was enumerated on selective agar (CT-SMAC), with colonies confirmed using latex agglutination test.

**Results:** The addition of carvacrol and thyme essential oil at highest concentration used yielded small reductions in *E. coli* O157 after 16 days of storage. Beef burgers containing 3.75% of cranberry extract had *E. coli* O157 counts approximately 0.7 log ( $P < 0.05$ ) lower after 16 days at 3°C compared to control samples, while the addition of chitosan to beef burgers yielded the highest bactericidal activity against *E. coli* O157 during cold storage. Beef burgers containing 2.5% of either chitosan resulted in significant ( $P < 0.05$ ) bactericidal effects, with approximately 2 log difference compared with the control samples after 16 days at 3°C.

**Significance:** These results show that some natural antimicrobials, such as chitosan have potential use as natural preservatives in processed meats.

## P2-11 Controlling Salmonellosis in Poultry Industry through the Use of Aptamers: Identification of Protein Targets

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**Introduction:** Salmonellosis causes a global economic loss of more than €3 billion/year and still is the second leading cause of foodborne zoonosis. Regulatory restrictions on the use of antibiotics and increasing consumer demand for antibiotic residue-free poultry products have directed research in this area towards the development of alternative products to antibiotics. Aptamers, single-stranded oligo-

nucleotides, have been applied as antimicrobial agents with promising neutralizing properties to combat pathogenic microorganisms. The antimicrobial activity of these molecules comes from their ability to bind to target molecules with high affinity and specificity.

**Purpose:** The present work aimed at identifying and characterizing proteins crucial for the invasion of *Salmonella* spp. in intestinal epithelial cells of the host, using *in silico* methodologies. Such proteins will be used as targets for aptamers selection.

**Methods:** Proteins crucial for the *Salmonella* spp. adhesion and invasion process of host cells were selected after a search on Virulence Factor Data Base (VGDB). The selection process was performed applying the following criteria: (1) *Salmonella* spp. virulence factors, (2) extracellular localization, (3) number of genomes and (4) function. Using UniProt, the selected proteins were evaluated for the existing conserved domains, their presence on proteins families, and homology with other proteins. Protein quaternary structure similarity was also evaluated in Pymol.

**Results:** *Salmonella* spp. has a multitude of virulence factors encoded in pathogenicity islands. Such factors help to overcome the intestinal barrier and escape cellular defense and host immunity system. The FimH adhesion mediator protein, located at the top of type 1 fimbriae, was selected since it is responsible for the first contact with host cells. The SipA, secreted by the SPI 1-type 3 (T3SS) secretion system, was also selected. It is capable of destabilizing the host cytoskeleton of membrane-associated actin.

**Significance:** FimH and SipA proteins were selected as viable candidates to be used as targets for aptamers selection.

**Purpose:** To study the antimicrobial activity against *L. monocytogenes* and *Salmonella* spp. of encapsulated OEO in two different inclusion complexes ( $\beta$ -CDs and liposomes), compared to free OEO in Kefir milk and Katiki Domokou cream cheese.

**Methods:** The encapsulation of OEO in  $\beta$ -CDs was performed with the co-precipitation method in two different ratios of OEO: $\beta$ -CDs (1:99 and 8:92). The thin-film hydration method was used for the incorporation of OEO into liposomes at a concentration of 0.88% v/v. The samples were inoculated with pathogens (6 log CFU/g) and incubated at 7°C for 17 days. Yeasts, lactic acid bacteria, pH and  $a_w$  were also measured.

**Results:** At the end of storage time in Kefir, free OEO caused about six-log reduction of *L. monocytogenes*, OEO encapsulated in 8:92  $\beta$ -CDs and liposomes caused 5.1 and 3.4 log reduction, respectively. Ratio 1:99 could not be applied to kefir because changed its physico-chemical characteristics. *Salmonella* spp. was much more sensitive as it was below detection limit after 1st day of incubation at all treatments. In Katiki cheese, when free, 8:92  $\beta$ -CDs, 1:99  $\beta$ -CDs and liposomes were applied, the population of *L. monocytogenes* reduced at  $4.6 \pm 0.9$ ,  $3.9 \pm 0.2$ ,  $5.6 \pm 0.3$  and  $5.3 \pm 0.0$  log CFU/g, respectively, after 17 days. When free and 8:92  $\beta$ -CDs OEO were applied, *Salmonella* spp. dropped below quantification limit by the end of storage time. Conversely, the population of *Salmonella* was  $1.0 \pm 0.8$  and  $1.3 \pm 1.3$  log CFU/g in the presence of 1:99  $\beta$ -CDs and liposomes, respectively. Free and encapsulated OEO reduced lactic acid bacteria and yeasts in kefir while had no effect against the microbiota of katiki cheese. The addition of liposomes increased the pH of Kefir.

**Significance:** Different inclusion complexes had different antimicrobial activity, which may also be affected by food matrix and microorganisms.

## P2-12 Sustainable and Safe Poultry Burgers

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**Introduction:** Currently, the search for new alternatives that allow reuse and recycling of waste are focused on agricultural by-products with beneficial effects on food safety.

**Purpose:** To evaluate the anti-listerial effect of a white grape pomace seasoning carrying out a challenge test on poultry burgers stored in vacuum and refrigeration for 26 days.

**Methods:** The challenge test was performed across five batches of burgers: control, control in vacuum, control with seasoning (3%), inoculated with a cocktail of four *Listeria monocytogenes* strains (4 log CFU/g), and burgers with seasoning (3%) and 4 CFU/g of *L. monocytogenes*. During the stored period, six sampling points were performed to study the evolution of total mesophilic aerobic microorganisms (TMA), psychrophilic aerobes (PS), and *L. monocytogenes* using the ISO methodology. Moreover, physicochemical parameters, such as pH and water activity ( $a_w$ ) were also analyzed in the uninoculated batches.

**Results:** The addition of the seasoning significantly modified the pH of the burgers; however this was not observed with the  $a_w$ . The anti-listerial effect was observed as bacteriostatic, delaying the growth of the pathogen during the storage period. Although the number of *L. monocytogenes* was not reduced from the inoculated level, its counts in the presence of the seasoning were significantly lower than those in the control batch. Despite these promising results, the seasoning was not able to improve the shelf life of the product as TMA and PS evolved the same in all the batches.

**Significance:** The anti-listerial property of a promising seasoning coming from wine by-products was proofed in this study opening the gate of using these subproducts to enhance food safety and sustainability.

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## P2-13 B-Cyclodextrins and Liposomes as Encapsulating Vectors of Oregano (*Origanum vulgare* L.) Essential Oil: Evaluation of Their Antimicrobial Activity Against *Listeria monocytogenes* and *Salmonella* spp. in 'Kefir' and 'Katiki' Cheese

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**Introduction:** Encapsulation of oregano essential oil (OEO) protects it from oxidation and volatilization and is considered an alternative antimicrobial application.

## P2-14 Do Materials Containing Silver Improve Antimicrobial Efficacy Towards *Vibrio parahaemolyticus* Biofilm?

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**Introduction:** *Vibrio parahaemolyticus* is one of the main foodborne pathogens known to cause gastroenteritis infections due to consumption of shellfish or seafood products. Biofilms can develop on surfaces of processing environments and be transferred to food. Reducing *V. parahaemolyticus*-related surface contamination is thus crucial to guaranteeing food safety.

**Purpose:** This study aimed to evaluate the gain in antimicrobial efficacy of silver-containing materials applied alone and with sanitizer products to eradicate *Vibrio* biofilms.

**Methods:** One day-old biofilms of *Vibrio parahaemolyticus* were produced on five different materials (flooring resin, paint, plastic film, plastic crate, doors with straps) with or without silver and in presence of shrimp juice as soiling or water (control). Biofilms were treated or not with 3 commercial sanitizer products (quaternary ammonium compound (QAC), sodium hypochlorite (SH), or peracetic acid (PA)). Viable cultivable (VC) and viable but not cultivable (VBNC) *Vibrio* were quantified.

**Results:** Without complementary sanitizing treatments, silver didn't result in better prevention of biofilm formation compared with materials without silver. VC *Vibrio* were around  $10^4$  CFU/cm<sup>2</sup> (water) and  $10^6$  CFU/cm<sup>2</sup> (shrimp juice).

Treatments with QAC and PA based sanitizers were very effective as a VC *Vibrio* reduction of at least 4 log CFU/cm<sup>2</sup> was obtained whether or not the material contained silver. A large amount of VBNC was also detected. Treatment with SH was the most effective by destroying all VC and VBNC *Vibrio*. Only for one material with shrimp juice, silver showed a better efficacy by reducing VC from  $10^4$  to  $10^2$  CFU/cm<sup>2</sup> with QAC and from  $10^2$  CFU/cm<sup>2</sup> to below the detection limit ( $10^{1.4}$  CFU/cm<sup>2</sup>) with SH.

**Significance:** Findings from this study provided information about the different performance of silver-containing materials on *Vibrio* biofilm formation.

## P2-15 *Escherichia coli* in Blue Light: Gold Standard to Decipher the Fate and Lifestyle Decisions of *E. coli* and Increased Safety of Leafy Vegetables

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**Introduction:** Blue light treatment (400-500 nm) has gained attention as a non-chemical method to combat microorganisms in the environment, on food and in medicine. A systematic literature review and meta-analysis<sup>1</sup> of the interaction between blue light and *E. coli* revealed theoretical and methodological pitfalls.

**Purpose:** We therefore created a standardised approach (gold standard) with a clear distinction between killing and different degrees of microbial growth inhibition that provides a basis for intervention.

**Methods:** Based on the findings of a recent systematic review and meta-analysis, we specifically depicting four basic areas of improvement; (i) definition of the apparatus conditions, including scattering interception and of light beams and treatment time, (ii) definition of the genetic conditions of the bacteria as well as nutritional and environmental conditions during bacterial propagation and blue light treatment, (iii) implementation based on experimental setup and the physiological response of the bacteria, (iv) development of scripts for calculation and normalisation.

**Results:** The protocol is currently subject to validation with pathogenic *E. coli* strains in the near future.

**Significance:** The gold standard is a contribution to mitigate safety hazards caused by pathogenic *E. coli* in leafy vegetables from processing to plate.

<sup>1</sup>Lawrence, C.D., Waechter, S., and Alsanus, B.W. (2022). Blue light inhibits *E. coli*, but decisive parameters remain hidden in the dark: Systematic review and meta-analysis. *Frontiers in Microbiology* 13, 867865. doi: 10.3389/fmicb.2022.867865.

## P2-16 Highly Sulfonated, Alginate/Polyacrylamide Hydrogel Beads for Efficient Pectinase Separation and Recovery

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**Introduction:** Pectinases is a heterogeneous group of enzymes widely used for clarification of fruit, vegetables juices. The use of free pectinase is inappropriate due to the difficulty of separating the free form from the reaction environment and recycling use.

**Purpose:** in this study we presented a scalable method for creating highly sulfonated hydrogel beads (SSAHB) for selective adsorption of pectinase from degradation broth. The obtained ion-exchange anionic beads exhibit a unique cellular structure endowing them with outstanding under compressive fatigue resistance.

**Methods:** The sulfonated hydrogel beads were prepared using an in situ formed network of polyacrylamide and natural polysaccharide alginate through an emulsion polymerization. Sulfonation process was obtained by simple nucleophilic substitution. The pectinase adsorption was through non-covalent interactions.

**Results:** The pectinase separation based on electrostatic attraction and ion exchange mechanisms which enhance pectinase adsorption capacity (250 mg.g<sup>-1</sup>) within 30 minutes and recovery (98% of adsorbed amount). Moreover, dynamic breakthrough capacity of the SSAHB could reach up to 230 mg.g<sup>-1</sup>, which was almost six times higher than that of the commercial Sartorius Sartobind membrane. Furthermore, SSAHB could directly extract pectinase from degradation broth of orange juice solely driven by gravity with excellent regenerability (upon 10 cycles).

**Significance:** This work may provide a new avenue to design and develop next-generation high-performance separation media for recovery of free enzymes used in food industry for several times of reusing cycles significant change in its activity.

## P2-17\* Using Cold Atmospheric Plasma (CAP) for Bacterial Decontamination of Bioaerosols

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**Introduction:** Aerosol droplets are able to carry and transfer bacteria and other microorganisms. If this happens in a food production environment, e.g., when producing liquid food products or aerosols originating from (contaminated) processing water, foodborne and other pathogens could be spread and cross-contamination on the food could occur. Additionally, this could impact the health of operators at the production site. On the other hand, aerosols originating from those operators (e.g. spread during exhaling) might contaminate the food products as well. It is thus of utmost importance to ensure that, among other things, aerosol droplets are free of hazardous microorganisms.

**Purpose:** One possible method of decontamination of those bioaerosols is the use of cold atmospheric plasma (CAP) treatment. During the current study, the CAP decontamination method was evaluated for aerosols using an in-house design set-up (Rotating Dielectric Barrier Discharge (RDBD) pin-to-plane plasma source) which was also compared with a commercial air purifier based on CAP (Jonix cube).

**Methods:** The device was placed in a treatment chamber, and a bioaerosol containing *Staphylococcus epidermidis* cells in phosphate buffered saline (PBS) was introduced and treated for several minutes. Sampling inside the chamber was done before and after treatment by collecting aerosol for two minutes using an impinger with 20 mL PBS.

**Results:** Both devices resulted in complete inactivation of *S. epidermidis* in the bioaerosol. When introducing a duty cycle of 60% to the RDBD, ozone levels decreased significantly. This poses some health benefits, as less of the harmful ozone is released into the environment. Nevertheless, this also impacted the efficacy of the treatment, as lower inactivation levels were observed.

**Significance:** Decontamination potential of both devices is sufficient and ozone production is comparable. However, release of this ozone to the environment could be lowered for the RDBD when applying activated carbon filters.

## P2-18 Development of a Clean, Nutritious, and Safe Ready-to-Drink, High Pressure-Processed Almond Milk Product

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**Introduction:** The rising occurrence of health, environmental, and ethical concerns are leading toward a growing consumer demand for dairy alternatives. In addition, consumer demand for clean and safe food, without compromising the nutritional and sensory qualities, has also become a recent trend. Non-thermal technologies such as high-pressure processing (HPP) have been successfully used to comply with these trends.

**Purpose:** The process parameters for two ready-to-drink almond milk product prototypes were standardised, with a focus on the formulation, shelf life, nutrition and microbial safety as well as to determine the effectiveness of HPP on *Listeria innocua* introduced into almond milk to validate the process at 4°C and 20°C.

**Methods:** Two prototypes were both processed in 350 mL PET plastic bottles by the Hiperbaric 55 HPP unit at 600 MPa for 3 min. Chemical and microbiological analyses were performed by a laboratory which is accredited in accordance with the recognised International Standard ISO/IEC 17025:2017. The ISO 11290-2/A1:2005 horizontal method for the detection and enumeration of *Listeria monocytogenes* was used for the challenge-lethality and storage tests at refrigerated (4°C) and 20°C. For the challenge study, *L. innocua* ATCC 33090 was used in a separate trial as a nonpathogenic surrogate for *L. monocytogenes*.

**Results:** HPP treatment and storage at 4°C achieved a 12-day shelf life for both prototypes. These were both deemed nutritious in claiming a source of energy, free of cholesterol, high in vitamin B3 and high in vitamin E. Results were within microbiological specification; therefore, safe for consumption. HPP treatment also achieved a five-log reduction of *L. innocua* at the end of shelf-life stored at 4°C.

**Significance:** This study found that HPP technology can produce a clean, nutritious and safe almond milk product. It also highlights the importance of HPP-treated almond milk on the elimination of *Listeria innocua*.



## P2-19\* Combined Effect of High Hydrostatic Pressure (HHP) and Chitosan on *Listeria monocytogenes* LO28

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**Introduction:** During the last decades, High Hydrostatic Pressure (HHP) has been studied and used, as a novel non-thermal technology, against various spoilage and pathogenic microorganisms such as *Listeria monocytogenes*. Meanwhile, chitosan has been broadly investigated for its antimicrobial activity extending the shelf life and improving the quality of food products. Various studies have shown the separate effect of HHP and chitosan in the reduction of *L. monocytogenes* but only few the combined effect.

**Purpose:** By using the most effective combination of high pressure and chitosan, this research aims to identify the strategies of the decontamination efficiency optimizing the HHP technology.

**Methods:** This study focused on the effect of HHP (200 and 300 MPa) alone or in combination with chitosan (0.02 to 0.2%) in 20 ± 2°C and 35 ± 2°C against *L. monocytogenes* LO28 strain. LO28 strain was grown in rich medium to stationary phase. Centrifugation and resuspension in ACES buffer (pH 6) were carried out before the chitosan application and the HHP treatment. Each treatment was performed in three independent biological replicates.

**Results:** The combined effect of HHP and chitosan was always higher in 35°C than in 20°C by 0.2 to 0.6 and 1 to 2 log CFU/mL at 200 and 300 MPa, respectively. The effect of HHP without chitosan was significantly lower than the combined effect. In 300 MPa, the combined effect reduced the LO28 cells >5 log CFU/mL in both temperatures. Statistically significant synergistic effect was observed in 300 MPa at 35°C.

**Significance:** HHP at 300 MPa in 35°C combined with chitosan was the most effective treatment reducing *L. monocytogenes* below the detection limit. Synergistic effect was observed in 200 MPa – 35°C and 300 MPa (both temperatures). Finally, higher reduction against LO28 can be achieved by combining HHP and chitosan than by the individual hurdles separately.

## P2-20 Bacterial Inactivation Using Cold Atmospheric Pressure Plasma (CAPP) as a Non-Thermal Processing Treatment

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**Introduction:** Cold atmospheric pressure plasma (CAPP) is a novel non-thermal processing technology that employs reactive gases to inactivate contaminating microorganisms on foods.

**Purpose:** The aim of this work was to (i) evaluate the effect of CAPP on the inactivation of selected bacteria and (ii) develop inactivation kinetic models under different CAPP conditions.

**Methods:** CAPP was created by a jet plasma device (KINPen® IND model) operating with argon as carrier gas at a flow rate of 4.0 L/min. The microorganisms assayed were *Pseudomonas fragi*, *P. fluorescens*, *Bacillus subtilis*, *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, *Brochothrix thermosphacta* and *Enterobacter sakazakii*. The microorganisms were spread on petri dishes containing TSA or MRS agar (for *L. plantarum* and *L. mesenteroides*) to obtain an initial population of ca. 8.0 to 9.0 log CFU/mL. The plates were subjected to CAPP treatment for 3, 6, 9, 12, or 15 min. After treatment the plates were incubated at 25°C for 48 h for *P. fragi*, *P. fluorescens*, *B. thermosphacta* and *E. sakazakii* and 30°C for 48 h for *L. mesenteroides* and *B. subtilis*; the surviving bacterial populations were enumerated. Finally, the models of Weibull and Geeraerd were fitted to the data to determine the inactivation kinetic parameters.

**Results:** After 15 min treatment with CAPP the lowest and highest population decrease was observed for *L. plantarum* (1.20 log CFU/mL) and *E. sakazakii* (3.36 log CFU/mL), respectively. The population of *L. mesenteroides* exhibited a decrease of 3.13 log CFU/mL, whereas the reduction in *Pseudomonas* counts were 2.19 and 2.82 log CFU/mL for *P. fragi* and *P. fluorescens*, respectively. Finally, the decrease in the population of *B. thermosphacta* and *B. subtilis* was 2.21 and 1.54 log CFU/mL, respectively. Both Weibull and Geeraerd models described adequately microbial inactivation as inferred by the low values of RMSE (< 0.43 log CFU/mL).

**Significance:** CAPP presented potential as a non-thermal treatment to reduce bacterial counts.

## P2-21 Plasma-Treated Water: Industrial Application on Minimally-Processed Leafy Greens

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**Introduction:** Minimally processed, RTE produce such as leafy greens may contain a very high microbial load, and despite a good safety record overall, has been associated with major human pathogen associated outbreaks. The need for sustainable, non-thermal interventions and effective antimicrobial agents at post-harvest stages to assure microbial safety remains a challenge.

**Purpose:** Sanitation steps based on non-thermal plasma (NTP) and therefore plasma treated water (PTW) opens up innovative food processing possibilities through application at different points and modes of delivery along the food chain. This talk describes the implementation of PTW in an industrial washing process for mixed lettuce.

**Methods:** Different PTW washing applications were compared to the industrial standard of portable (ice) water washing in a commercial washing line based on food safety (proliferation, metabolic activity and membrane integrity), food quality (color, chlorophyll content), and toxicological investigations.

**Results:** The PTW application increased the reduction of microbial load on the lettuce itself and within the wash water compared to portable water use. Up to 2 log reduction could be achieved. The reduced ability of proliferation went along with reduced membrane integrity. The metabolic activity was less influenced. On the other hand, the food quality and toxicological results were comparable between portable water and PTW application.

**Significance:** The industrial scalability and applicability of PTW for minimally processed leafy greens was demonstrated. With regard to promising antimicrobial effects on the lettuce itself and the process water, the later reuse to reduce water consumption and meanwhile provide minimization of water-mediated cross-contamination seems to be possible.

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## P2-22 Exposure to Mycotoxins through the Consumption of Rice in Lebanon and United Arab Emirates: A Comparative Study

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**Introduction:** Rice is one of the world's most staple food products. Being cultivated in hot and humid areas, fungi can contaminate rice and produce mycotoxins including the hepatotoxic aflatoxin B1 (AFB1) and nephrotoxic Ochratoxin A (OTA).

**Purpose:** Our study evaluated AFB1 and OTA levels in rice marketed in Lebanon and United Arab Emirates (UAE), and determined the exposure to this toxin from rice consumption.

**Methods:** All rice brands available in the market (105 and 128 in Lebanon and UAE, respectively) were collected twice. Enzyme-linked immunosorbent assay was used to measure AFB1 and OTA. A comprehensive food frequency questionnaire was completed in both countries to determine patterns of rice consumption and, subsequently, the exposure levels to mycotoxins from rice consumption.

**Results:** In Lebanon, AFB1 was detected in all rice samples (100%). The average concentration was 0.5±0.3µg/kg. Contamination range was 0.06-2.08µg/kg. Only 1% of the samples had an AFB1 level above the European Union limit (2µg/kg). Exposure was calculated as 0.1 to 2ng/kg of body weight per day. In UAE, AFB1 was detected in 48 out of 128 rice samples (38%). The average contamination among positive samples was 1.66±0.89 µg/kg, ranging from 1 to 4.69µg/kg. The calculated mean daily exposure level of the Emirati population from consuming rice was 4.83 ng/kg. On the other hand, OTA in 56 (53%) samples in Lebanon and 73 (58%) samples in UAE were above the limit of quantification (0.8 µg/kg). Average concentration of the positive samples was 1.29±0.32 and 1.40±0.42µg/kg in Lebanon and UAE, respectively. Exposure was calculated as 1.27 ng/kg body weight/day in Lebanon and 1.42 ng/kg body weight/day in UAE.

**Significance:** Our study was the first in Lebanon and UAE that assessed the estimated daily exposure to mycotoxins from consuming rice. Measures on the governmental, retail markets, and household levels must take place to reduce the exposure to mycotoxins.

## P2-23 FunShield4Med – Shielding Food Safety and Security by Enabling the Foresight of Fungal Spoilage and Mycotoxin Threats in the Mediterranean Region Under Climate Change Conditions

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**Introduction:** FunShield4Med is a new Coordination and Support Action, funded by Horizon Europe programme under Twinning call. The Consortium consists of the University of Parma from Italy, University of Lleida from Spain, National Kapodistrian University of Athens from Greece, and Cranfield University from UK, all representing advanced partners, and the Hellenic Agricultural Organization (ELGO) – DIMITRA, representing the widening country participant and coordinator of the project.

**Purpose:** FunShield4Med's overall aim is the reinforcement of research and innovation capacity of the Institute of Technology of Agricultural Products of ELGO – DIMITRA. This will be achieved by transferring key knowledge and state of the art expertise from the advanced participants on food safety relating toxigenic fungi & mycotoxins within climate change, taking into consideration a special provision for upgrading as well on project management and administrative skills of the institute's personnel. FunShield4Med has been structured to bridge the gap between Greece and advanced EU member states in securing consumers from imported goods contaminated with mycotoxins.

**Methods:** To address the challenges and support its scientific objectives, FunShield4Med's overall methodology foresees a joint research project on mycotoxins surveillance, and a series of dissemination/exploitation/communication measures which briefly include: five seminars, four workshops, two summer schools, 12 short-term staff exchanges for training, short-term experts visits, site-visits via open days, organization of international conferences, publications to peer-reviewed journals, and participation in conferences, scientific round tables and meetings with stakeholders.

**Results:** The results will level up research and scientific cooperation of participants, help securing consumers from mycotoxins threats, develop educational/training activities and material, and level-up administrative and financial staff skills.

**Significance:** Finally, FunShield4Med will raise public and scientific awareness on food safety and mycotoxins threats under climate change.

## P2-24 Results of a Multi-Year, Inter-Laboratory Proficiency Testing Program for Zearalenone in Corn

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**Introduction:** Proficiency testing programs are important to measure the reliability of results from HPLC/MS determination of mycotoxins in ground corn.

**Purpose:** This research provides results from a double-blind study of accuracy, precision, and reproducibility for zearalenone quantitated in paired ground wheat and corn samples determined by HPLC/MS over several years.

**Methods:** Double-blind ground corn samples with known zearalenone levels were analyzed by each laboratory. Neogen's sample preparation used five-gram samples extracted in 70% methanol/water + 125ng of <sup>13</sup>C zearalenone. Filtered and centrifuged samples were diluted in PBS and passed over immunoaffinity columns prior to HPLC/MS analysis. Samples from the same lot were analyzed once per year for five years. Statistical analysis including ANOVA was completed using Minitab 18 (Minitab, LLC).

**Results:** The inter-laboratory mean for four laboratories was 94.2 ± 16.4 ppb zearalenone (CV= 17%, N=12) for a ground corn check sample containing nominal 90.5 ppb zearalenone; 218.4 ± 22.4 ppb (CV = 10.3%, N=12) for a 273.0 ppb sample and 1105.4 ± 149.6 (CV = 13.5%, N=12) for a 1004.6 ppb sample. Over five years of testing, six laboratories participated. For the 90.5 ppb sample, standard deviations (SD) were < 9 ppb for results from samples of the same lot for all laboratories except one lab that had a statistical outlying result and another lab that had 16.7ppb SD for three samples in one year. For the 273.0 ppb sample, SDs were <32 ppb for all labs and one lab that had statistical outliers from previous year results. For the 1004.6 ppb sample, standard deviations were < 115 ppb for all labs. Accuracy, precision and repeatability of Neogen's multi-year HPLC/MS results were acceptable since all results were within USDA FGIS specifications.

**Significance:** The comparison of multi-year results provided information about the quality of the ground corn check samples and long-term laboratory proficiency.

## P2-25 Growth Kinetics Do Not Improve Differentiation of Persistent and Sporadic *Listeria monocytogenes*

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**Introduction:** *Listeria monocytogenes* is a ubiquitous, facultative anaerobic, Gram-positive foodborne pathogen. This aetiological agent can cause severe illness in vulnerable populations. Furthermore, and contrasting many of its innocuous neighbours, this pathogen is known for persisting in various environments, including food processing environments (FPEs). Interestingly enough, only certain strains are routinely isolated from these FPEs while others are sporadically isolated. One possible explanation for this persistence has been attributed to the formation of cell subpopulations capable of withstanding adverse conditions, which may encompass high salinity, low temperature, or low pH.

**Purpose:** To evaluate if persistent and sporadic strains manifest significant differences in their growth.

**Methods:** We set out an experiment to evaluate if persistent *Listeria monocytogenes* isolates collected from cheese factories possessed better fitness regarding their growth kinetics, focusing on two growth parameters ( $\mu_{max}$  and lag phase) when faced with frequent stresses found in these FPEs compared with transient strains. To do so, a two-level three condition full factorial design was implemented, with 18 *L. monocytogenes* strains being grown in culture media with varying combinations of pH (7.0 and 6.0 adjusted with lactic acid), NaCl (2.5 and 8.0%), and temperature (11 to 30°C).

**Results:** From the 18 tested strains, we did not observe statistically meaningful differences between the persistent and transient *L. monocytogenes* groups. Regarding the three tested, low pH and low temperature were the most impactful variables in the growth kinetics of our isolates.

**Significance:** Although a better understanding of the growth kinetics of *Listeria monocytogenes* is crucial for controlling and preventing its spread in FPEs, given our data, persistence does not appear to be linked with the growth fitness of specific strains of this pathogen.

## P2-26 Method Comparison and Interlaboratory Study for the ISO 16140-6:2019 Validation of a Commercially Available Real-Time PCR-Test, for the Confirmation and Typing of *Salmonella* spp.

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**Introduction:** Standard protocols for detection of *Salmonella* are time-consuming, taking several days to generate a final positive or negative test result (ISO 6579-1 and ISO 6579-3). This study reports a quick and simple multiplex real-time PCR test to confirm and type *Salmonella* spp. within 2.5 hours from colony.

**Purpose:** Method comparison and interlaboratory study for the ISO 16140-6:2019 validation of Check & Trace Salmonella 2.0, for the confirmation and typing of *Salmonella* spp.

**Methods:** ISO 16140-6 was used as reference. For the method comparison, study strains were inoculated on Nutrient Agar (NA) and Xylose Lysine Deoxycholate agar (XLD) and tested on both the Biorad CFX96 and Biorad CFX Opus real-time machine. Amount of tested samples was according to ISO 16140-6 for both the inclusivity and exclusivity study for confirmation of *Salmonella* spp. and serotyping of 59 serovars (five strains per serovar). For inclusivity,



313 *Salmonella* spp. strains were used and for exclusivity, 175 non-target strains. For the interlaboratory study a total of 30 strains were tested by 15 operators in 13 different laboratories.

**Results:** For confirmation, no deviating results were found. For typing, a few deviations were found, but were all within the acceptability limits described in ISO 16140-6 (AL: deviations  $\leq 3$  for both inclusivity and exclusivity). For the interlaboratory study a few deviations were found within the acceptability limits (AL: deviations  $\leq 3$  for both inclusivity and exclusivity)

The presented test is a validated alternative method for ISO 6579-1 and ISO 6579-3 for the confirmation and typing of *Salmonella* spp.

**Significance:** Food producers or laboratories testing for *Salmonella* are able to use the test as a validated alternative method for ISO 6579-1 and ISO 6579-3 for the confirmation and typing of *Salmonella* spp.

## P2-27 Development of an Impedance-Based Method for the Rapid Evaluation of Raw Sheep Milk Microbiological Quality

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**Introduction:** Microbiological evaluation of raw milk is based on the enumeration of different microbial parameters (hygienic indicators) on selective substrates. The time needed to obtain results is a drawback. There is a requirement for microbial testing methods that will minimize this disadvantage and rapidly determine the microbial count of a raw milk sample.

**Purpose:** The objective of this work was to evaluate and validate the BioTrac 4250 Microbiological Impedance Analyzer (SY-LAB) as a means of determining the microbial count of raw sheep milk as compared to analysis of the methods of Standard Plate Count (SPC), *Enterobacteriaceae* (ENT), and Coliform Counts (CC).

**Methods:** The raw sheep milk samples (n=120) were collected from the bulk tank of a commercial dairy sheep farm (Lacaune) over a period of one year. Four samples were collected at each visit. SPC, ENT, CC were analyzed on the respective 3M Petrifilm and impedance media.

**Results:** The three developed models (one for each microbial category) and their predictability were successfully validated (values distribution  $>4$  logs, correlation coefficient  $>90\%$ , and dispersion  $<0.5$  logs, Bland-Altman graph, two-sided t-test) against the reference method.

**Significance:** BioTrac 4250 offers a definite time advantage when examining raw sheep milk samples contributing to faster detection of microbiological quality problems allowing better control of raw sheep milk quality and safety.

**Acknowledgments:** We acknowledge support of this work by the project "Research Infrastructure "MilkQuality" in Agri-food: Control of mastitis in small dairy ruminants and improvement of the quality of raw milk and dairy products by applying advanced molecular and statistical methods" (MIS 5045647) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

## P2-28 Application of a Molecular Biological Method for Detection of *Clostridium perfringens*

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**Introduction:** *Clostridium perfringens* (*C. perfringens*) is a food-poisoning bacteria that contaminates meats and produces toxin. To date, the official method of Korea is a culture method which is time-consuming.

**Purpose:** For this reason, this study aims to apply the molecular biological method that is rapid and accurate, to detect *C. perfringens*.

**Methods:** the specificity and selectivity of the primer of target gene (*cpa*) were verified and confirmed by using a PCR assay. We diluted and inoculated *C. perfringens* at several concentrations ( $10^{-1}$  to  $10^1$  CFU) in five different food types and incubated in 37 degrees for 24 h according to the Korean Food Code.

**Results:** The result of the specificity and selectivity of Conventional or Real-Time PCR assays to detect *cpa* were confirmed to be 100%. The limit of detection of the PCR assay on various foods tested showed the same or respectively improved compared to the culture method. The p-value showing no significant difference was 0.9244 between collaborators. This result showed that the Conventional or Real-Time PCR could be applied for rapid and accurate detection of *C. perfringens* in various foods.

**Significance:** Our results show that it is possible to apply the PCR assay to detect *C. perfringens*.

## P2-29 Evaluation of a PCR Workflow for the Detection of *Salmonella* from Pooled Chocolate Ingredients

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**Introduction:** In recent years there have been several *Salmonella* outbreaks linked to nuts, dry fruit, and chocolate, which have led to hospitalisations. A reliable detection method is valuable to ensure that chocolate and low water activity chocolate ingredients are safe to eat; pooling methods offer high throughput testing for matrices with a low incidence of *Salmonella* contamination.

**Purpose:** To evaluate the Thermo Fisher Scientific™ SureTect™ *Salmonella* species PCR workflow for the detection of *Salmonella* from chocolate ingredients after a post-enrichment pooling step.

**Methods:** A total of 36 spiked samples were tested, comprising whole almonds, chopped hazelnuts, hazelnut paste and raisins. Two-hundred-and-fifty gram samples were artificially contaminated using seeding and heat injury methods. Per data point, ten samples were pooled (one part contaminated with nine parts non-contaminated) and tested using the PCR workflow and ISO 6579-1 reference method.

**Results:** The PCR assay was able to successfully detect *Salmonella* from all of the low water activity matrices, following pooling, after a 20 h enrichment. The results were in complete agreement with the ISO reference method.

**Significance:** The PCR workflow is a reliable method for the detection of *Salmonella* from pooled chocolate ingredients including nuts and dried fruit.

## P2-30\* Development of Two PCR-Based Techniques for the Detection of Viable but Not Culturable Cells on Surfaces to Assess the Impact of a Desiccation Stress on the Viability of *Cronobacter* spp. in Milk Powder Manufacturing Plants

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**Introduction:** *Cronobacter* spp. is an opportunistic foodborne pathogen responsible for life-threatening infections such as meningitis and necrotizing enterocolitis in neonates, but also for various complications in elderly and immunocompromised people.

**Purpose:** Monitoring in dairy industries shows that *Cronobacter* can persist in production environments and may enter into a dormant state known as Viable But Non-Cultivable (VBNC) state, making it undetectable by conventional enumeration methods. The aim of this study is to develop a method to assess the viability of *Cronobacter* cells after implementation of stress encountered in industries.

**Methods:** We developed two detection systems specific to the genus *Cronobacter* (qPCR and ddPCR) in combination with a PMA™ (Propidium Monoazide) treatment and agar enumeration to assess the viability of detected cells. qPCR coupled with PMA™ was applied to 36 stainless steel surfaces contaminated with 107 *C. sakazakii* (CIP57.33) to detect VBNC cells. Bacteria were dehydrated in milk at 58°C and 20% relative humidity during 48h to mimic environmental conditions encountered by the bacteria in productions sites.

**Results:** ddPCR was shown to be more sensitive than qPCR. Surprisingly, qPCR was more efficient when PMAxx treatment was applied. The combination of PMA™ with ddPCR consistently produced a background noise that made absolute VBNC quantification uncertain. But, it was demonstrated that ddPCR offered significant advantages, with 100% detection probability for the simple detection of *Cronobacter* traces (20 cg/mix). Application of the PMA™-qPCR approach on *Cronobacter* submitted to the desiccation stress demonstrated that a significant part of viable bacteria, i.e. 103 cells, persisted in the VBNC state on stainless steel surfaces after 48 hours.

**Significance:** The two different PCR-based approaches have great potential to detect and discriminate between physiological states of *Cronobacter* cells on surfaces. However, the combination of qPCR with PMA™ seems to be the most reliable method to study the viability of *Cronobacter* cells.



## P2-31 Which QC Strains to Use for Minimizing Risk of False Positives Due to Cross-Contamination in a Lab?

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**Introduction:** The use of collection strains, including pathogenic ones, is necessary in labs for various applications including daily or regular quality control of methods and verification of methods (e.g., according to ISO 16140-3). However, the use of these strains, which often requires cultures and inoculums preparation, can conduct to cross-contamination of routine samples.

**Purpose:** When using strains from culture collections, labs increase the risk of contaminating their analysis samples. The impact of this cross-contamination, which can lead to false positive results, can be enormous if the laboratory is unable to confirm that the positive samples are really from a natural contaminant. This confirmation can be cumbersome, expensive and may not succeed. However, laboratories do not want to risk cross-contaminations using pathogens for quality control purposes and are looking for alternatives which would be acceptable.

**Methods:** In a new approach, ISO/TC34/SC9 considered that the use of strains easily distinguishable from natural strains was possible to limit the risk of false results. A better definition and conditions of use of these strains has been discussed. This presentation is intended to clarify this definition and conditions of use of these strains, and thus propose alternatives to laboratories. These strains can be genetically modified (Green Fluorescence Protein marker GFP) to show characteristics that are never found in common natural strains. They have the advantage of being easily distinguishable, for example, by reading the fluorescence of isolated colonies.

**Results:** The presence of these particular strains in a food during its analysis proves cross-contamination in the laboratory and therefore a false positive result. By a quick and simple confirmation, the laboratory will be able to demonstrate whether or not it is this particular strain or a natural contamination.

**Significance:** The use of these easily distinguishable strains makes it possible to then confirm positive results on routine samples. Their use thus contributes to limiting the costs of waste or re-controls and to maintaining confidence in laboratory results.

## P2-32 Evaluation of the Neogen Soleris® Enterobacteriaceae Vials for the Rapid Detection of Enterobacteriaceae in a Broad Range of Foods

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**Introduction:** Advances in microbiological analysis has enabled the rapid detection of hygiene indicator organisms including the *Enterobacteriaceae*. Neogens Soleris® system and Soleris Enterobacteriaceae vials reduces time to results from 3 days for confirmed results using culture-based methods to 18h.

**Purpose:** This study evaluated the automated system to detect *Enterobacteriaceae* in broad range of foods at  $\geq 10$  CFU/g. A semi-quantitative analysis following the dilute to specification protocol enables the detection of *Enterobacteriaceae* at defined thresholds.

**Methods:** The performance of Soleris® *Enterobacteriaceae* S2-EBAC9 vials was compared to the direct plating reference method ISO 21528-2:2017 following the validation procedure ISO 16140-2 (2016). A single plate of the reference agar was used with the presence of one or more colonies being equivalent to the detection of *Enterobacteriaceae* at  $>10$  CFU/ml. This approach enabled the plate count to be used as a qualitative rather than a quantitative result.

**Results:** Study results demonstrated that the vials were 100% specific and 100% selective for *Enterobacteriaceae*. Data also indicated that the vials were more sensitive than the reference method in the sensitivity study. The calculated LOD<sub>50</sub> of the ready to use vials ranged from 6.9 to 8.3 CFU/g across the five categories tested in the RLOD study. In addition, no significant differences were seen between results obtained by reference method and alternative method in the interlaboratory trial.

**Significance:** Neogen Soleris® *Enterobacteriaceae* S2-EBAC9 vials offers a novel approach for rapid *Enterobacteriaceae* detection in food products with equivalent performance to ISO 21528-2:2017.

## P2-33 How to Implement the Use of Genetically Modified Microorganisms for Routine Quality Control of Methods in Labs

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**Introduction:** According to ISO/IEC 17025, testing laboratories must ensure they operate competently and are able to generate valid results. Implementation of quality control or verification of methods requires the use of strains. Genetically modified microorganisms (GMM) (i.e. GFP tagged organisms) offer a less risky approach by minimizing the risk of cross-contamination events securing laboratory results.

**Purpose:** Strains need to be rightly qualified prior to routine use in the lab. To qualify and demonstrate the suitability of GMM, BIOBALL® LUMINATE 2.0 range is evaluated on pathogenic detection methods.

**Methods:** Five pathogenic strains of BIOBALL® LUMINATE 2.0 (*Cronobacter sakazakii*, *Salmonella* Typhimurium, *Listeria innocua*, *Listeria monocytogenes* & *Escherichia coli* O157:H7) are evaluated using the most sensitive bioMérieux methods (VIDAS, GENE-UP,...) Relevant matrices are selected, and artificial contamination of a negative sample is carried out at the (pre-)enrichment step. An analysis is then performed according to the recommended protocol. Each BIOBALL® LUMINATE 2.0, containing 100 CFU, is diluted in the recommended rehydration fluid to achieve a target level of between 15 to 21 CFU on average (QC of methods) and to achieve the target level recommended in ISO 16140-3 (verification of method). In the context of this work, we also evaluate two GFP confirmation methods (fluorescence on agar plates & rapid PCR VERIFLOW®) to check their applicability to discriminate a natural contamination from a potential cross-contamination of a positive routine sample.

**Results:** The results obtained show that all five strains are correctly detected with the corresponding detection methods according to Quality Control protocol & Method Verification protocol (ISO 16140-3). Positive samples are also correctly confirmed using the two GFP confirmation methods.

**Significance:** With these supporting data, food testing labs can replace their in-house collection strains with these ready to use GMM to reduce risk of false positives and without any more qualification when testing bioMérieux methods.

## P2-34 Bacillus cereus Isolation and Identification: From Traditional Microbiology Methods to Molecular Methods

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**Introduction:** *Bacillus cereus* is a spore-forming, facultative anaerobic bacterium, well known for its ability to cause food poisoning and spoilage of milk and dairy products. The current method for the isolation and identification of *B. cereus* includes cultivation in mannitol egg yolk polymyxin (MYP) agar.

**Purpose:** This study aims to improve the *B. cereus* isolation and identification method, by comparing the recommended method with cultivation in different media and 16S rRNA method.

**Methods:** Isolates from pasteurised milk (n=131) were obtained from a Greek dairy industry. The isolates were plated on MYP (Oxoid) and on the chromogenic Brilliance™ *Bacillus Cereus* Agar (Oxoid) and the plates were incubated at 30 °C for 24h. At the same time, DNA was extracted by using the ZymoBIOMICS DNA Miniprep kit (Cat. No. D4300, Zymo Research Corp.). The amplification of the 16S rRNA gene was performed by the Polymerase Chain Reaction (PCR) method using the genomic DNA as a template and the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1518R (5'-AAGGAGGTGATCCANCCRCCA-3'). The amplicons were sequenced using the BigDye™ Terminator kit (4337455, Applied Biosystems™, Thermo Fisher Scientific Inc.) on a 3500 Genetic Analyzer (4405673, Applied Biosystems™, Thermo Fisher Scientific Inc.). The resulting sequences were manually corrected for ambiguities. Forward and reverse sequences were joined, and the final sequences were aligned against the NCBI's nt database using the BLASTn algorithm.

**Results:** The obtained results indicated that the chromogenic Brilliance agar performed better in the identification of *B. cereus* group from food isolates compared to the recommended MYP agar.

Moreover, the most accurate identification was achieved through the 16S rRNA methods which revealed that a high number of isolates belonged to the *B. cereus* group.

**Significance:** This study provides a comparison of the available methods for the identification of *B. cereus* from food isolates and highlights the advantage of molecular techniques.

## P2-35 Rapid Alternative Isolation and Confirmation Methods for EHEC and *Salmonella* after Assurance® GDS PCR Analysis

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**Introduction:** Isolation and confirmation of target microorganisms using reference methods are labor-intensive and long in duration. Use of rapid alternative isolation and confirmation methods are advantageous for testing sites after presumptive positive results from PCR testing, herein Assurance® GDS. Rapid alternative isolation and confirmation methods for *E. coli* O157:H7 (EHEC) and *Salmonella* were validated for Assurance® GDS for *E. coli* O157:H7 Tq assay (by MicroVal) and Assurance® GDS for *Salmonella* Tq assay (by MicroVal and AFNOR).

**Purpose:** The purpose of this study was to demonstrate rapid isolation and confirmation of EHEC and *Salmonella* by inoculated food studies and inclusivity and exclusivity studies on chromogenic agars.

**Methods:** For EHEC, 77 inoculated food samples (71 at ≤5 CFU/sample) were enriched in mEHEC® broth. For *Salmonella*, 79 inoculated food samples (65 at ≤5 CFU/sample) were enriched in mEHEC®, and an additional 115 contaminated samples (92 at ≤5 CFU/sample, 23 naturally contaminated) were enriched in Buffered Peptone Water (BPW). Samples were incubated for the minimum enrichment time stated in the Directions for Use (DFU). Samples enriched in mEHEC® were isolated onto three different chromogenic agars by direct streak. Samples enriched in BPW were isolated onto three different chromogenic agars by plating immunomagnetic separation (IMS) bead-captured *Salmonella*. Inclusivity and exclusivity strains were plated on chromogenic agars to verify specificity. For both organisms, target colonies were confirmed from plates by latex agglutination. All samples were also confirmed by ISO standards confirmation methods.

**Results:** For both EHEC and *Salmonella*, all contaminated samples were successfully confirmed using the alternative methods. In addition, all inclusivity strains were accurately detected, and all exclusivity strains were accurately excluded.

**Significance:** Fast isolation and confirmation of target pathogenic organisms is critical for the rapid release of food products. In these studies, the rapid alternative microbiological confirmation methods for EHEC and *Salmonella* utilizing chromogenic agars were selective and specific.

## P2-36 Detection of Shiga-Toxin Producing *Escherichia coli* (STEC) in Large Test Portions of Meat and Vegetables by Immunomagnetic Separation and Real Time PCR

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**Introduction:** Recent multistate outbreaks of Top 6 Shiga-toxin producing *Escherichia coli* (STEC) in beef are prompting regulators and the industry to move toward Top STEC testing.

**Purpose:** The aim of this study is to evaluate the performance of the Assurance® GDS method combining immunomagnetic separation (IMS) and real-time PCR for the analysis of large test portions (up to 375 g) of vegetables and meat, comparing performance with the reference method ISO/TS-13136:2012 and the validated 25 g sample size.

**Methods:** A total of 120 samples of meat and vegetables, including 20 uninoculated and 100 samples spiked with stressed cells at positive and fractional levels of different STEC strains, were analyzed using both methods. The alternative method includes enrichment in proprietary broth (1/5) at 41.5°C for 10 h followed by primary screening of *eae*, *stx* genes and O157:H7 markers, secondary screening for serogroup identification, and cultural confirmation on two selective agars by direct plating and using IMS beads. For the reference method and sample size, enrichment was performed in Buffered Peptone Water (1/10) at 41.5°C for 18 h.

**Results:** Results showed that 70% and 62% of the samples spiked were detected and confirmed by the alternative method and the reference method, respectively. The RLOD<sub>50</sub> ratios (=LOD<sub>50</sub> alternative/LOD<sub>50</sub> reference) ranged between 0.35 and 0.96 for the four food items tested. The performance of the methods and sample sizes were considered equivalent (RLOD<sub>50</sub> < 2.5 according to ISO 16140-2:2020). The IMS step allowed STEC isolation and cultural confirmation for 100% of the positive samples compared with 94% when direct isolation on agar plates were performed.

**Significance:** The alternative method using IMS+PCR technology allowed rapid STEC detection (10 h) of larger sample sizes (375 g) from beef and vegetables with equivalent performance to the reference method. The IMS step also contributed to improve the cultural confirmation of STEC positive samples.

## P2-37 Detection of *Salmonella* in Large Test Portions of Challenging Pet Food Products by Immunomagnetic Separation and Real Time PCR

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**Introduction:** The detection of *Salmonella* contamination in pet food is one of the keys to contain animal and human outbreaks. In this context, the development of rapid tests allows the industry to stay competitive.

**Purpose:** The aim of this study is to evaluate the performance of alternative method Assurance® GDS combining immunomagnetic separation (IMS) and real-time PCR for the analysis of large test portions (up to 375 g) of challenging pet food raw materials and finished products, comparing performance with the reference method ISO 6579-1:2017 and the validated 25 g sample size.

**Methods:** A sensitivity study was performed comparing alternative method with the reference method by spiking low levels of six different challenging stressed strains of *Salmonella* in four different pet food items (24 tests), including liquid fat, acid liquid digest and finished products. Four selected *Salmonella* strains were selected to perform a RLOD<sub>50</sub> study comparing the alternative and the reference method with the same food items, by spiking 30 test portions from each pet food item. For both methods, samples with low pH were analysed using double concentrated BPW and, for high fat samples (>20%), Tween 80 (1g/L) was added.

**Results:** Sensitivity study showed equivalent results between methods. Deviations (negative deviations – positive deviations=2) were below acceptability limits (AL=3). RLOD<sub>50</sub> ratios (=LOD<sub>50</sub> alternative/LOD<sub>50</sub> reference) ranged between 0.793 and 2.272 for the four food items tested. The performance of the methods and sample sizes were considered equivalent (RLOD<sub>50</sub> < 2.5). Double buffered peptone water with extra buffering capacity (MERCK ref. 107228) was required in order to neutralize liquid digest with pH=2.9 and avoid false negative results.

**Significance:** The alternative method using IMS+PCR technology allowed *Salmonella* rapid detection (18 h) of large sample sizes (375 g) from challenging pet food items with equivalent performance to the reference method and sample size.

## P2-38 Development of a SERS-Based Lateral Flow Immunoassay for Detection of Penicillin in Milk Via Direct Writing of Functional Materials

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**Introduction:** Antimicrobials, such as antibiotics, are widely used for treatment of bacterial infections of both human beings and animals. Residual antibiotics have been found in soil, water and food because of their overuse in agriculture, livestock farming, pharmaceutical industries and aquaculture residual. In particular, for dairy farming, antibiotic presence in milk is associated with intolerances and allergies in humans and is known to affect dairy production.

**Purpose:** The objective of this work is the development of a Surface enhanced Raman Scattering (SERS)-based competitive lateral flow immunoassay (LFIA) for detection of residual penicillin G antibiotic in milk.

**Methods:** The test makes use of gold nanoparticles (GNPs) functionalised with antibodies directed against penicillin and rhodamine B isothiocyanate as Raman reporter to obtain a dual readout method, colorimetric and spectroscopic. Intensely red-coloured GNPs give a colouration of the test line that is inversely proportional to the concentration of penicillin in milk. The SERS readout is produced by the Raman reporter, thanks to the close proximity of gold nanoparticles

in the coloured lines. The deposition of test line and control line in the LFIA strips was achieved by direct pen-writing, in particular using a fountain pen.

**Results:** Different concentrations of penicillin G in whole milk (diluted 1:1 with a buffer solution) were tested on the SERS-based LFIA. The device was able to detect 2 µg/l of penicillin antibiotic, which is under the maximum residue limits (MRL) fixed for the specific antibiotic in milk by the European Commission (4 µg/l).

**Significance:** The development of the direct pen writing procedure could allow a versatile and customizable method to deposit reagents for a point-of-care test to be used *in situ* in different situations without trained personnel and expensive equipment. Moreover, the dual detection could increase the sensitivity of the test helping distinguish among low concentrations.

## P2-39 Method Modification of the *Listeria* Precis Detection Methods in Accordance with ISO 16140-2:2016

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**Introduction:** *Listeria* spp. and specifically *Listeria monocytogenes* are major global foodborne pathogens with a severe impact on public health. The ISO 16140-2:2016 validated Thermo Scientific™ Oxoid™ *Listeria* Precis™ detection methods have been extended to offer an improved time-to-result and flexibility for the detection of *Listeria* spp. and *L. monocytogenes*.

**Purpose:** Perform ISO 16140-2:2016 studies to validate additional enrichment broth, selective media, and confirmatory tests.

**Methods:** The method modifications were validated against the ISO 11290-1:2017 reference method in an unpaired study design. The method consisted of enrichment in fully supplemented Thermo Scientific™ Oxoid™ 24 *Listeria* Enrichment Broth for a minimum of 20 h, followed by plating 10µL on Thermo Scientific™ Oxoid™ Brilliance™ *Listeria* Agar (ISO). Presumptive *Listeria* colonies were confirmed using the appropriate tests for the colony characteristic; this included the Thermo Scientific™ PrecisCheck™ lateral flow test.

**Results:** The sensitivity of the method for the detection of *L. monocytogenes* was 90.4% compared to 89.3% for the reference method. For *Listeria* species, the sensitivity was 91.2% and for the reference method it was 87.4%. The combined relative level of detection (RLOD) for *L. monocytogenes* was 0.930 while for *Listeria* species it was 0.848. These studies showed that the modified *Listeria* Precis detection methods were statistically comparable or superior to the ISO 11290-1:2017 reference method.

**Significance:** The two *Listeria* Precis detection methods for *Listeria* species and *L. monocytogenes* provide a simple, fast, accurate and reliable culture-based method for the detection of *Listeria* in a broad range of foods and environmental surfaces. The method modification providing additional media options offers flexibility and enhanced options for the user.

## P2-40 Method Modification Validation of the *Listeria* Precis Enumeration Methods in Accordance with ISO 16140-2:2016

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**Introduction:** *Listeria* spp. and specifically *Listeria monocytogenes* are major global foodborne pathogens with a severe impact on public health. The ISO 16140-2:2016 validated Thermo Scientific™ Oxoid™ *Listeria* Precis™ enumeration methods have been extended to offer a simpler workflow with greater flexibility for the enumeration *Listeria* spp. and *L. monocytogenes*.

**Purpose:** Perform ISO 16140-2:2016 studies to validate additional dilution broth, selective media, and confirmation tests.

**Methods:** The method modifications were validated against the ISO 11290-2:2017 reference method in an unpaired study design. The methods consisted of a dilution in buffered 24 LEB (without selective supplement), followed by plating procedures on the new Oxoid™ Brilliance™ *Listeria* Agar (ISO), including a pour plate procedure. Presumptive *Listeria* colonies were confirmed using the appropriate tests for the colony characteristic, this included the Thermo Scientific™ PrecisCheck™ lateral flow test.

**Results:** The average difference in the relative trueness studies for *L. monocytogenes* were -0.02 log CFU/g with the pour plate protocol and 0.02 log CFU/g with the surface plate protocol. For *Listeria* spp., the average difference was -0.03 log CFU/g with the pour plate protocol and 0.00 log CFU/g with the surface plate protocol. These results suggest a satisfactory performance of the methods against the reference method. This was further demonstrated by the accuracy profile study results.

**Significance:** The two *Listeria* Precis™ enumeration methods for *Listeria* spp. and *L. monocytogenes* provide a simple, fast, accurate and reliable culture-based method for the enumeration of *Listeria* from a broad range of foods and environmental surfaces. The method modifications provide additional flexibility and enhanced options for the end user.

## P2-41 Thermo Scientific Suretect *Salmonella* Species PCR Assay Method ISO 16140-2:2016 Matrix Extensions

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**Introduction:** *Salmonella* is a major global foodborne pathogen with a severe impact on public health. The Thermo Scientific™ SureTect™ *Salmonella* spp. PCR Assay provides an accurate and reliable method for the detection of *Salmonella* from a broad range of foods and environmental surfaces (validated in accordance with ISO 16140-2:2016). The scope and capability of the method has been extended by adding pet food and animal feed matrices and enhancing protocols for meat, vegetables, and powdered infant formula (PIF) categories.

**Purpose:** Perform ISO 16140-2:2016 extension studies to include pet food and animal feed matrices, and enhance the protocol for meats, vegetables and PIF by increasing the sample size and reducing the time to result.

**Methods:** In accordance with ISO 16140-2:2016 a sensitivity study and relative level of detection (RLOD) were conducted for each category (meat 25 g and 375 g, vegetables 375 g, pet food 375 g, animal feed 150 g, and powdered infant formula (PIF) 375 g). Pet food was tested with both a paired and unpaired design, all other categories used an unpaired design.

**Results:** The results of each category for the sensitivity and RLOD studies met the ISO 16140-2:2016 standard requirements meaning that the performance of the SureTect™ *Salmonella* spp. method was statistically equivalent or better than the performance of the reference method.

**Significance:** The SureTect™ *Salmonella* spp. PCR Assay was proven to be an accurate and reliable method for the detection of *Salmonella* from the additional matrices, and the new protocol for meat, vegetables and PIF categories.

## P2-42 Performance Evaluation of Ready-to-Use Culture Media, Easy Plate AC for Enumeration of Aerobic Plate Count in a Broad Range of Foods, Environmental Samples and Pet Foods

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**Introduction:** The Ready-to-use (RTU) media Easy Plate AC (Kikkoman Biochemifa Company) provides many benefits including reduced time-to-result, simplicity of use and is more sustainable compared to conventional plate count agar. The speed of analysis of the device gives a saving of 24 h over the reference method with results available in 48 h.

**Purpose:** To evaluate the performance of the RTU device for the enumeration of aerobic count as detailed in the ISO 16140-2 validation protocol.

**Methods:** The repeatability, accuracy and relative trueness of the RTU device was evaluated during the study across 5 food categories; dairy products, fishery products, produce and fruits, meat and poultry, and multicomponent foods in addition to environmental samples and pet foods. Analysis of the samples was performed according to the manufacturer's instructions and ISO 4833-1 (2013).



**Results:** Study results revealed good agreement between the RTU device and the reference method in the 105 samples analyzed in the relative trueness study. During the accuracy profile it was demonstrated that all seven categories passed the 0.5 log acceptability limits or the recalculated limits. Additional studies showed that the RTU device prevented the spread of members of the *Bacillus* spp. seen on Plate Count Agar.

**Significance:** Data indicates that the RTU device provides equivalent results to the ISO reference method 4833-1 (2013) for a broad range of foods, environmental samples and pet foods. In addition to the reduced time-to-result compared to PCA, the media allows better colony counting with samples containing *Bacillus* spp.

## P2-43 Performance Evaluation of Ready-to-Use Culture Media, Easy Plate SA for Enumeration of *Staphylococcus aureus* in a Broad Range of Foods

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**Introduction:** Ready-to-use (RTU) media offers many advantages to the end user including shorter time-to-result and ease of use as well as being more sustainable. Easy Plate SA (Kikkoman Biochemifa Company) reduces the time of enumeration of *Staphylococcus aureus* to 24 h, a savings of 24 h compared to the standard ISO 6888-1 protocol.

**Purpose:** To compare the performance of the RTU device and ISO method 6888-1(2021) for the enumeration of *S. aureus* in a broad range of foods following the ISO 16140-2 validation protocol.

**Methods:** This study evaluated the specificity, selectivity, repeatability, accuracy and relative trueness of the RTU device as required by ISO 16140-2. During the study, 225 food samples were analyzed across five food categories: dairy products, fishery products, produce and fruits, meat and poultry and multicomponent foods. All samples were tested according to the manufacturer's instructions and ISO 6888-1.

**Results:** Results from the study revealed the RTU device gave comparable results to the reference method across the 75 analyzed in the relative trueness with no evidence of bias between the methods. In the accuracy profile study, all five categories tested satisfied the 0.5 log acceptability limit or the recalculated acceptability limits. Data also indicated that the RTU device was selective and specific, inhibiting the growth of nine non-target organisms that grew on the reference agar.

**Significance:** The RTU device gives equivalent results to the ISO reference method 6888-1(2021) for a broad range of foods. This RTU device is a rapid convenient alternative for the enumeration of *S. aureus* enabling results in 24h.

## P2-44 *Salmonella* Quantification in Beef Lymph Nodes Using Different Methodologies

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**Introduction:** Because several published studies have demonstrated that *Salmonella* is harbored in the lymph nodes (LNs) of cattle and may be a direct source of contamination in non-intact meat products, the beef industry and regulatory agencies can benefit from the development of rapid pathogen quantification methodologies that provide sensible and accurate results that can lead to risk-based actionable decisions on hazard mitigation.

**Purpose:** To assess the performance of the newly developed methodology known as BAX<sup>®</sup>-System-SalQuant<sup>®</sup> with existing and published methodologies for quantification of *Salmonella* in LNs that include a 3M<sup>™</sup>-EB-Petrifilm<sup>™</sup> + XLD-replica-plate method and a modified Most Probable Number (MPN) technique.

**Methods:** Beef LNs (n=26) collected from a commercial beef processing facility and confirmed to be negative for *Salmonella* presence were trimmed, weighed, surface sterilized, pulverized, spiked with 1.00–5.00 logCFU/LN using *Salmonella* Typhimurium (ATCC-14028), and then homogenized with 80ml of BAX-MP (LN homogenate [LNH]). From each LNH, samples were enumerated by 1) a direct-plating method on 3M<sup>™</sup>-EB-Petrifilm<sup>™</sup> + XLD-replica-plate, 2) a 3x3 MPN in BPW, and 3) the BAX<sup>®</sup>-System-SalQuant<sup>®</sup> methodology for beef LNs. All data were analyzed using R statistical software to evaluate the relationship between enumeration technologies.

**Results:** The slope for both alternative enumeration methods, when compared with MPN, was 1.187 ( $P < 0.001$ ) and 1.092 ( $P < 0.001$ ) with an adjusted-r-square of 0.84 and 0.83 for 3M<sup>™</sup>-EB-Petrifilm<sup>™</sup> + XLD-replica-plate and BAX<sup>®</sup>-System-SalQuant<sup>®</sup> methodologies, respectively. The intercept value was -1.064 for 3M<sup>™</sup>-EB-Petrifilm<sup>™</sup> + XLD-replica-plate ( $P = 0.009$ ) and -0.251 for BAX<sup>®</sup>-System-SalQuant<sup>®</sup> methodologies ( $P = 0.489$ ) when compared against the MPN method.

**Significance:** This validation study provides evidence for the availability of a rapid and feasible quantification methodology that provides a tool for the meat industry when conducting contamination risk assessments for processing decision making and adds support to the use of PCR-based quantification methodologies with advantages over published methodologies with lower limits of quantification (10 CFU/LN).

## P2-45 Performance Equivalency and Stability Analysis of Handling Improvements of the Thermo Scientific Suretect Workflow

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**Introduction:** Real-time PCR detection of foodborne pathogens from food and environmental surfaces is a widely used principle in the food safety industry. The Thermo Scientific<sup>™</sup> SureTect<sup>™</sup> workflow is a real-time PCR detection method for a number of foodborne pathogens. The importance of time-to-result and efficiency in process is a critical factor in food pathogen testing, considering this, a number of handling improvements were identified and analysed for equivalency and stability.

**Purpose:** Analyse improvements to ensure equivalency in performance whilst improving efficiency. Improvements include blue dye indicator moving from one reagent to another providing a visual indicator whilst pipetting, addition of a pierceable lysis seal, rigid lysis/PCR plates with color coding and orientation markers. Improved capping, de-capping and cutting tools are also available but were not analysed due to no impact on performance/stability.

**Methods:** Where applicable, improvements were analysed for stability, performance equivalence and enzymatic activity between the original and new formats. Studies were designed in accordance with the manufacturing site quality system (ISO 13485:2016 certified) with added enhancements where required. A representative range of assays and matrices were included. Acceptance criteria was based on the current variation between SureTect Assays (Ct value  $\pm 1.5$  and dRn at  $\pm 50\%$ ).

**Results:** Where applicable, improvements were analysed for stability, performance equivalence and enzymatic activity between the original and new formats. Studies were designed in accordance with the manufacturing site quality system (ISO 13485:2016 certified) with added enhancements where required. A representative range of assays and matrices were included. Acceptance criteria was based on the current variation between SureTect Assays (Ct value  $\pm 1.5$  and dRn at  $\pm 50\%$ ).

**Significance:** The SureTect workflow improvements offer increased efficiency and a reduced handling step with no impact to performance. New capping, de-capping and cutting tools have also been added.

## P2-46 Growth of *Bacillus cereus* in Fresh Spinach Canederli during Shelf Life

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**Introduction:** As a soil bacterium, *Bacillus cereus* can spread into many types of foods such as plants, eggs, meat, fish and milk.

**Purpose:** The objective of this study was to evaluate the growth of *B. cereus* during the shelf life of spinach Canederli traditional dumpling in northeast region of Italy.

**Methods:** Two batches of Canederli dough were tested. Each batch (10 kg) was separately inoculated with 1% v/v of *B. cereus* cocktail (mesophilic NCTC 11143, mesophilic wild *B. cereus* 186523, psychrotrophic *B. weihenstephanensis* DSMZ 11821) (contaminated units) or 1% v/v of physiological solution (control units). For each unit, three Canederli were prepared and packed in protective atmosphere in plastic trays (450 g each). Food controls (not handled units)

were also used. All the units were stored for 45 days (8°C for seven days and 12°C for 38 days). Two replicate units were tested during the shelf life. On contaminated units, the *B. cereus* enumeration was performed according to ISO 7932:2004. On control units and food controls, total bacteria count (TBC) (ISO 4833:2013), yeasts and moulds count (Y&M) (ISO 21527-1:2008) and pH were analyzed during the shelf life.

**Results:** During the shelf life, the TBC reached a concentration up to 8.7 log CFU/g in both batches, while heterogeneous growing of Y&M was observed among the units. A significant acidification was observed both in control units and in food controls, with a pH drop from 6.3±0.1 to 5.2±0.1 and to 4.1±0.1 in batch 1 and in batch 2 respectively. *B. cereus* grew up to 7.5 log CFU/g in batch 1, but not in batch 2 where the final concentration was below to 2 log CFU/g.

**Significance:** Canederli are permissive for *B. cereus* growth. *Bacillus* poisoning can be prevented by proper food handling, including hygienic preparation, reducing refrigeration storage and by adequate cooking.

## P2-47 Modeling the *Cronobacter sakzaii* Inactivation in Milk Infant Formula during Microwave Heating Processing

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**Introduction:** The contamination of milk infant formula (IMF) can occur at any point in the food chain, and one of the main microorganisms associated with this contamination includes *Cronobacter* spp. with an increasing resistance in low moisture foods.

**Purpose:** This study aimed to evaluate the inactivation of dry-adapted *Cronobacter* during the domestic microwave heating of IMF and in addition to determining a predictive model to describe this behavior.

**Methods:** A central composite design was implemented to study the effect on *Cronobacter* inactivation during the microwave heating of 3 factors: power (P), treatment time (t) and milk volume (V), considering five levels for each. IMF with the *Cronobacter* type strain ATCC<sup>®</sup> 29544<sup>™</sup> (freeze-dried inoculum maintained for 14 days at room temperature) was treated at different microwave conditions (at 100 to 1000 W; for 20 to 70 sec; in 30 to 180 mL). The *Cronobacter* enumeration was performed by plate count method on selective Chromogenic *Cronobacter* Isolation agar (CCI). The reduction was evaluated as the difference between counts after the treatments (N, log CFU/mL) and the initial inoculum level (N<sub>0</sub>, log CFU/mL). Logarithmic reductions (I) were assessed as the mean of two independent experiments.

**Results:** The results showed high *Cronobacter* inactivation (I) ranged from 5.74 to 4.61 log CFU/mL in *Cronobacter* counts after exposure to microwave heating at 750W (60 s) in 60 mL and 500W (45 s) in 30 mL, respectively. A second-order polynomial regression model was obtained using Microsoft Excel regression (solver) analysis module. The goodness of fit of the models was evaluated using the adjusted determination coefficients (R<sup>2</sup> adj = 0.93). The obtained model equation was:  $I = -7.54 + 8.94 \cdot V + 0.0052 \cdot P + 5.5289 \cdot t - 4.32 \cdot (V \cdot V) + 0.0041 \cdot (V \cdot P) - 0.000009 \cdot (P \cdot P) - 0.0064 \cdot (P \cdot t)$  (SE 0.63).

**Significance:** The results of this study may help food safety agency to select domestic heating conditions for IMF able to prevent *Cronobacter* contamination and to ensure the safety of infant formulas.

## P2-48 Challenge Tests to Study Inactivation Potential and Kinetic Parameters (ISO 20976-2:2022)

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**Introduction:** It is the responsibility of Food Business Operators to control microbiological hazards in foods and to manage microbial risks according to the general principles of the Codex Alimentarius on food hygiene. Challenge test is one of the recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and quality of food, food production processes, food storage conditions and food preparation recommendations for consumers.

**Purpose:** In agreement with already available and valuable guidance documents that exist worldwide, the aim of the ISO20976 standard series is to provide general requirements and guidelines for conducting challenge tests on food and feed products with a distinction made between studies targeting growth (ISO20976-1:2019) or inactivation (ISO20976-2:2022) in a specific micro-organism/food combination.

**Methods:** Within the frame of the International Organization for Standardization (ISO), members from all over the world collaborate to create internationally recognized documents providing requirements, specifications, guidelines or characteristics that can be used consistently to ensure that products, processes and services are fit for purpose. ISO/TC34/SC09/WG19 is an ISO working group operating in the field of microbiological analysis of the food chain. It comprises experts from the food industry, food technology institute, food testing laboratory, research center and regulatory bodies.

**Results:** The ISO 20976-2:2022 Standard for conducting challenge tests to study inactivation potential and kinetic parameters was published in November 2022 and provides recommendations and guidelines on several topics such as the number of batches to be tested, the selection of strains for the challenge test, the inoculation procedure and the rules for interpretation. WG19 is currently working on an additional standard for the determination and use of cardinal values in predictive microbiology (project ISO 23691).

**Significance:** General and consensus documents on best practise for conducting challenge tests will ensure harmonisation of practices to facilitate data interpretation and trade between stakeholders from different countries.

## P2-49 Updated Online Risk Assessment Tools to Help Producers of Fresh Produce and Smoked Fish

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**Introduction:** Certain food products can be linked to higher risks of microbiological contamination. As the Scottish regulator for food safety, Food Standards Scotland aims to support food businesses to follow best practice.

**Purpose:** To reduce risk to consumers and support food businesses, we redesigned and overhauled our online microbiological risk assessment tools for businesses producing fresh produce (primary production of fruit and vegetables), and smoked fish (secondary processing). The online platform includes multiple choice risk assessment tools, resources, a glossary, and a downloadable report page, which provides advice, and can be used as an informal audit resource to demonstrate the actions they are taking to support safe production.

**Methods:** In close collaboration with technical experts, industry stakeholders, and web developers, we created new online multiple choice risk assessment tools. This free to use and anonymous platform allows food businesses to risk assess their practices, and offers advice and recommendations supported by relevant academic literature, guidance, and legislation. After the update an engagement strategy was used to promote the revised platform. This included emailing known stakeholders, presenting to relevant groups, and engaging with interested individuals at events such as the Royal Highland Show.

**Results:** After releasing both tools early in 2022 the hits per month on the platform has increased significantly. Analytic data also indicates longer access time per user.



**Significance:** Using this tool allows businesses (particularly small to medium enterprises) to receive targeted advice on how to achieve best practice to reduce microbiological risk in the products they make. Additionally we now have a template which we can use to create new risk assessment tools for businesses where a need is identified.

## P2-50 The Risk to Vulnerable Consumers from *Listeria monocytogenes* in Ready-to-Eat Smoked Fish: A Qualitative Risk Assessment

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**Introduction:** *Listeria monocytogenes* infections can lead to invasive listeriosis in vulnerable groups, and the case fatality rate in these groups can reach 30%. Ready-to-eat foods pose the greatest risk as there is no heating step at consumption to eliminate any pathogens present.

**Purpose:** Following a recent outbreak of *L. monocytogenes* in smoked fish in the UK, risk managers requested a risk assessment to ensure advice to vulnerable consumers regarding the consumption of smoked fish was appropriate.

**Methods:** A qualitative risk assessment was performed, following Codex Alimentarius guidelines. The production pathway identified points where *L. monocytogenes* could contaminate smoked fish and any existing critical control points. Following this, the risk from cold-smoked fish and hot-smoked fish were considered separately as hot-smoked fish undergo a heat treatment sufficient to control *L. monocytogenes*. Additional evidence presented included published surveys of *L. monocytogenes* in smoked fish products, outbreaks attributed to *L. monocytogenes* in smoked fish, and data on food safety incidents linked to smoked fish products.

**Results:** Based on the evidence, the assessment concluded that the likelihood of invasive listeriosis occurring in vulnerable groups from hot-smoked fish was very low (very rare but cannot be excluded) with medium uncertainty. For cold-smoked fish, the likelihood was low (rare, but does occur) with medium uncertainty. The severity of infection with *L. monocytogenes* in vulnerable groups was high (severe illness) with low uncertainty. The uncertainties associated with the likelihood of infection were mainly due to difficulties in estimating the infective dose in vulnerable groups, variations in consumer practices and differences in smoked fish production processes affecting *L. monocytogenes* contamination.

**Significance:** This risk assessment is supporting work by FSA and FSS risk managers and communicators, which includes consumer research and consultation with stakeholders representing the interests of vulnerable groups, to refine advice targeted to these consumers.

## P2-51 Effects of Temperature, Water Activity, and Strain Variability on the Growth and Growth Boundaries of *Escherichia coli*

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**Introduction:** Due to the low infective dose of pathogenic *Escherichia coli* and its ability to survive in meat and dairy products, there is a need for quantitative tools for assessing the risk associated with *E. coli*.

**Purpose:** Develop a predictive model for the effects of temperature,  $a_w$  and strain variability on the growth and growth boundaries of *E. coli*.

**Methods:** The probabilistic model is based on a cardinal-type model with an interaction term for improved accuracy. To account for strain variability, distributions (derived from literature data) were used for model parameters instead of single values. The growth rates and growth boundaries were predicted by means of Monte Carlo simulations and model outputs compared literature data in broth or in milk (1782 records). Growth curves were also generated for O157:H7 and O26:H111 strains in milk and mascarpone.

**Results:** Based on data for 22 strains, the means of distributions for the minimum, optimum, and maximum growth temperatures are 5.0, 40.2 and 47.8 respectively. The mean for  $a_{wmin}$  was set to 0.953. The lower limit of the predicted growth boundary closely agrees with the

experimental transition between no growth and growth conditions. The  $\mu_{max}$ -values of pathogenic and non-pathogenic *E. coli* in broth and dairy products also fall within limits of prediction. Phenotypic responses of commensal and Shiga toxin-producing *E. coli* (STEC) strains were found to overlap. However, specific serotype behaviour was also observed: for instance, the O26:H111 strain exhibited extended lag time (15-fold increase) at  $a_w$  0.97 compared to the O157:H7 strain.

**Significance:** This probabilistic model can improve risk assessment of *E. coli* in near neutral pH products and serve as a basis for incorporating other environmental factors (pH, organic acids) for broader applicability.

## P2-52 Predicting the Growth of *Bacillus cereus*: An Improved Model Based on Phylogenetic Affiliation

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**Introduction:** Sym'Previus ([www.symprevius.eu](http://www.symprevius.eu)) is a decision support software for prediction of microbiological data. It integrates models for predicting bacterial growth, growth limits and thermal inactivation for a number of pathogenic microorganisms, including *Bacillus cereus*. This microorganism is a common foodborne pathogen, and its broad diversity is difficult to assess and as a source of difficulty for risk assessment. The phylogenetic structure of the *B. cereus* group was resolved by Guinebretiere et al. (2008, 2010) resulting in seven different phylogenetic groups that exhibit high differences in their response to temperature and their ability to cause food poisoning.

**Purpose:** To integrate this classification system into the Sym'Previus models for *B. cereus*.

**Methods:** For each phylogenetic group (from Group II to Group VII), models were developed based on the Gamma concept (multiplicative effects of environmental factors). The (intra-group) strain variability is incorporated in the model by using probability distributions for these strain-dependent parameters (e.g., cardinal temperatures for growth). Several pH terms were also assessed for improved performance at pH levels below 5.5.

**Results:** In comparison with the original Sym'Previus *B. cereus* growth model, this new model integrates growth data on 33 additional strains of *Bacillus cereus sensu lato*. The classical cardinal pH model was not found to be the best performing model and an alternative pH term was suggested taking into account an increasing effect of the pH at values close to the pH growth limits. The growth parameters based on clustering the *B. cereus* strains into phylogenetic groups improved the model performance compared to the classical approach.

**Significance:** The affiliation to phylogenetic groups enables improving the model predictions by taking into account the biodiversity encountered. It is expected that combining group specific heat inactivation models with the developed growth models will allow improved exposure assessment of *B. cereus* in foods.



## P2-53 Improving the Growth Prediction of the Growth of Pathogenic Microorganisms as a Function of pH

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**Introduction:** To simulate the behaviour of pathogenic microorganisms in low acid foods, accurate predictive models for the effects of pH on their growth are needed.

**Purpose:** The objective of this study is to improve predictions of bacterial growth rate and description of biological variability between layers in fermented or non-fermented foods (e.g., sauces, non-fermented deli products, etc.) at pH below 5.5.

**Methods:** The datasets for model development include historical data used for developing the Sym'Previous growth rate models (www.symprevious.eu) and additional data generated in independent scientific projects. The evaluation will be based on the following criteria: goodness of fit, description of the biological variability between layers and the biological significance of the estimated parameters (correspondence between the estimated and experimental minimum growth values). Several pH models from literature were evaluated and the best performing model selected for further study validation on independent data from literature.

**Results:** Depending on the microorganisms, the evolution of the maximum growth rate  $\mu_{max}$  as a function of pH has various forms (e.g., linear as a function of pH, proton concentration). The cardinal pH model did not appear to be the most suitable for describing some of these changes, in particular when a plateau zone is visible, for instance, for *L. monocytogenes*. In this case, the  $pH_{min}$  values estimated with the cardinal model for strains of the same microorganism show a wide dispersion that is not consistent with the range of pH limits observed experimentally. Across the range of microorganisms tested, the performance of the pH term based on Aryani et al. (2015), is at least as good or better than the cardinal model.

**Significance:** These results support the use of the Aryani term to describe the effect of pH on the bacterial growth and its incorporation, within the Sym'Previous framework, into more complex growth rate models.

## P2-54 Development and Validation of a Predictive Model of *Bacillus sporothermodurans* Growth for Estimating the Impact of Climate Change on Non-Refrigerated Food Products

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**Introduction:** Thermophilic and thermo-tolerant spore-forming bacteria are related to spoilage of a wide range of non-refrigerated food products. Due to the fact that the minimum temperature for growth of thermophilic bacilli is quite high, these products are currently considered as microbiologically stable. However, microbiological stability is based on the current distribution and storage conditions. Therefore, an environmental disturbance, such as temperature increase (which is expected due to climate change), may affect their stability.

**Purpose:** The aim of this study was to assess the impact of climate change (e.g., temperature increase) on the spoilage of non-refrigerated food products and re-evaluate their microbiological stability.

**Methods:** In this model, cardinal temperature values, along with the maximum specific growth rate of *Bacillus sporothermodurans* DSM 10559 were estimated, by studying the temperature range between 19 and 48°C. The model was validated in an evaporated milk and a pea beverage, under static and non-isothermal conditions.

**Results:** In the present study the cardinal temperature values, along with the maximum specific growth rate of *Bacillus sporothermodurans* DSM 10559 strain were estimated. In addition, the obtained results indicated that the developed models can significantly predict *B. sporothermodurans* growth in both tested matrices.

**Significance:** This study is of a great importance since the developed model will allow for the assessment of the effect of climate change on the microbiological stability of non-refrigerated food products, under different temperature scenarios.

## P2-55 How Processing Practices and Conditions Affect *Salmonella* Levels in Fresh-Cut Lettuce? Evaluation Using Predictive Microbiology Models

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**Introduction:** The production of RTE vegetables is an emerging segment in Argentina. The processing practices and conditions greatly affect the microbial responses in these products, which can be contaminated with pathogens such as *Salmonella* during all the steps from pre-harvest to consumption. When processing and distribution conditions are well known, simulations using predictive models are useful for validating control measures to prevent the exposure of consumers to microbial hazards.

**Purpose:** To apply predictive models to evaluate *Salmonella* levels in fresh-cut lettuce (FCL) over production and distribution, based on information gathered in the Argentinian fresh-produce industry.

**Methods:** Seven Argentinian FCL companies were surveyed regarding practices and conditions employed during production and storage (e.g., steps, temperatures, times, and disinfectant concentrations). A cluster analysis of the data was performed to identify homogeneous groups among the participating companies. The data collected were used as inputs of predictive microbiology models to estimate *Salmonella* concentrations after chlorine washing, during storage and distribution, and to rank the different practices according to the final estimated *Salmonella* levels.

**Results:** Six different clusters were identified by evaluating the parameters, methods, and controls applied in each processing step, evidencing great variability among companies. Sodium hypochlorite is the disinfectant applied by all companies, although concentrations and washing times differ among them. Simulations using predictive models indicated that the reductions of *Salmonella* in FCL would vary in the range of 1.70 to 3.0 log CFU/g between companies during chlorine washing. This information could be used to prioritize risk-based sampling programmes by Food Official Control or determine more adequate process parameters for pathogen control. Moreover, *Salmonella* would be able to grow in FCL during storage and distribution, achieving levels of up to 2 log CFU/g.

**Significance:** This study provides a deep knowledge of the FCL processing operations, parameters, and conditions in Argentina, which are essential for performing microbiological risk assessments.

## P2-56\* Effect of Herbal Extracts on the Survival of *S. aureus* in Goat's Raw Milk Cheese

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**Introduction:** Raw milk cheeses have shown moderate prevalence of *Staphylococcus aureus*, imposing a health safety issue for consumers. Considering that lemon balm and spearmint hydroethanolic extract possess antimicrobial capacities, it was hypothesised that these could be used as biopreservatives in cheese.

**Purpose:** To evaluate the antimicrobial effect of lemon balm and spearmint extracts against *S. aureus* in raw milk goat cheeses during maturation.

**Methods:** Lyophilised herbal extracts were produced using ethanol 70% (v/v) in a shaking water bath. Milk was inoculated with *S. aureus* to reach ~5 log CFU/g and 1% (w/w) of each extract was added to the curd, while a non-inoculated control was kept. Cheeses were kept in a chamber at 10°C/98% RH for 15 days. *S. aureus* counts and pH were determined at specific days. For every treatment, a log-decay function with tail in differential form as primary model (with varying D-value), coupled to a secondary model Bigelow equation of D-value as a function of pH (with parameters  $\log D_{ref}$  at pH 7.0 and  $z_{pH}$ ) was adjusted.

**Results:** The dynamic models adequately fitted the survival curves, with RMSE of 0.1172 and 0.0633 for spearmint and lemon balm, respectively, producing significant parameter estimates. The incorporation of extracts influenced  $\log D_{ref}$  (0.621 [SE=0.061] for spearmint; 1.189 [SE=0.200] for lemon balm), compared to the controls (0.932 [SE=0.166]; 0.996 [SE=0.056]). The initial pH drop (until day 4) was affected by the presence of extracts, which was reflected by the higher  $z_{pH}$  values of cheeses with spearmint (3.172 [SE=0.660]) and lemon balm (2.339 [SE=0.835]). Overall, the addition of herbal extracts significantly decreased the time to achieve one log reduction, which in practical terms corresponded to up to 1.36 log CFU/g decrease by the end of maturation.

**Significance:** This work characterised *S. aureus* survival parameters in raw milk goat cheeses and demonstrated that herbal extracts can reduce *S. aureus* burden during cheese maturation.

Characterization of the microbial populations and their dynamics over shelf life, is important to develop targeted preservation strategies to enhance shelf life and reduce food waste.

**Purpose:** To investigate microbial populations and their dynamics over shelf life in fresh minced beef, pork, and poultry and to evaluate the effect of different preservative solutions on these dynamics.

**Methods:** Samples were prepared with and without interventions and analysed in triplicate over 14 days. Samples were stored at 5°C under modified atmosphere. Both culture-dependent and culture-independent (16S rDNA sequencing) analyses were performed. Relative abundance plots were created in R, using OTUs (operational taxonomic units) classified to the genus level. Median values of abundance were calculated for each meat sample per timepoint across the replicates. Any OTU's present at <1% were collapsed into a category termed "Other".

**Results:** Initial microbial diversity was relatively high in beef and poultry but low in pork. The most abundant bacteria differed significantly: Beef - *Carnobacterium*, *Brochothrix* and *Pseudomonas*; Pork - *Photobacterium*; Poultry - *Lactobacillus* and *Lactococcus*. A dramatic decrease in bacterial diversity was observed in beef and poultry samples during storage. Conversely, increased diversity was observed in pork samples. At the end of the storage period, almost all samples, including those with interventions, showed microbial counts above the spoilage level of 6 log CFU/g. All interventions extended shelf life compared to the control. Interestingly, the shift in microbial abundance over shelf life differed depending on the substrate and intervention used.

**Significance:** This work demonstrates the importance of implementing both culture-dependent and independent methods to understand the true microbial populations and their dynamics in order to optimise preservation strategies for fresh meat products.

## P2-57 Comparative Genomic Analysis and Antimicrobial Resistance Profile of Pathogens Isolated from Raw Sheep Milk and Associated with Ovine Mastitis in Greece

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**Introduction:** Staphylococci and streptococci are the main pathogens causing mastitis in ruminants followed by *Escherichia coli*. Use of antibiotics is the most common practice to deal with mastitis-causing bacteria. There is a lack of comprehensive information regarding genetic diversity, complete profile of virulence factors (VFs), and antimicrobial resistance (AMR) genes for these microorganisms associated with ovine mastitis in Greece.

**Purpose:** The objectives of this work was to estimate the prevalence of mastitis-causing bacteria in raw sheep milk, determine genetic diversity and evolution, and identify their VFs and AMR genes.

**Methods:** Raw sheep milk samples were collected from the bulk tank of a commercial dairy sheep farm (Lacaune) over a period of one year. Four samples were collected at each visit and cultured on CHROMagar Mastitis. Suspected colonies were isolated, cleaned, and subjected to whole-genome sequencing, antimicrobial resistance profiling (Sensititre MIC) and screening for enterotoxin production (*S. aureus*). Genetic analysis was performed for all strains.

**Results:** The isolates were divided into clusters and the corresponding sequence types and clonal complexes were identified as well as their VFs and AMR profiles. Pan-, core and accessory genomes, enterotoxin, and AMR profiles were also determined.

**Significance:** Contribution to a better understanding of pathogens epidemiology providing comprehensive profiles of virulence and resistance genes. Contaminated raw sheep milk is particularly alarming for cheese production in artisan dairies.

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## P2-58 Bacterial Population Dynamics in Fresh Minced Meat Using Culture-Dependent and Independent Analysis

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**Introduction:** Fresh minced meat is one of the most widely consumed meat products among consumers. These products are highly perishable with short shelf life, resulting in >20% wasted annually.

## P2-59 Prophage Analysis of *Salmonella enterica* Serovar Adjame

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**Introduction:** Bacteriophages upon integration into the bacterial chromosome are stably transmitted from generation to generation as prophages. The nucleotide composition of prophages is different from the rest of the bacterial chromosome which permits easy identification. Prophages also display properties similar to other viruses which nevertheless make them unique in the context of a bacterial genome, including a high frequency of mutation and recombination.

**Purpose:** We analyzed the prophage composition of *Salmonella enterica* serovar Adjame, a rare serotype.

**Methods:** We applied a genomics based prophage typing (PST) tool which allows related and unrelated isolates of *Salmonella* to be distinguished from one another as distinct strains, using a total population of 38 isolates a fraction of which was recovered from an outbreak of *S. Adjame* in England in 2017 (n=14 isolates) and the results were compared to that of a single nucleotide polymorphism (SNP) analysis.

**Results:** The PST analysis of the *S. Adjame* isolates showed a high degree of strain heterogeneity. We observed small clusters made up of 2 to 6 isolates (n = 27 isolates) and singletons (n = 11 isolates), in stark contrast with the three clusters observed by SNP analysis. In total, we detected 24 prophages of which only four were highly prevalent among the *S. Adjame* strains, namely: Entero\_p88 (36/38 strains), Salmon\_SEN34 (35/38 strains), Burkho\_phiE255 (33/38 strains) and Edward\_GF (28/38 strains). Analysis of the four conserved prophages agreed with strain characterization as determined by SNP analysis.

**Significance:** Despite the marked strain diversity seen with prophage analysis, the distribution of the four most common prophages matched the clustering observed using core genome analysis. The confirmation of multiple strain involvement using conserved prophages and an added layer of greater resolution of the isolates into smaller clusters provides the investigator with a better understanding of the relatedness among members of the populations and the spread of outbreak strains.



## P2-60 Wastewater Based Epidemiology: A Useful Tool in Public Health Preparedness across One Health Sectors

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**Introduction:** Wastewater based epidemiology (WBE) is a non-invasive surveillance method that has become a routine public health monitoring tool for detecting and tracking emerging pathogens and variants at a community level.

**Purpose:** Our objective was to evaluate targeted and non-targeted metagenomic approaches for profiling wastewater as a potential early warning system for the emergence of pathogens and resistant bacteria that may negatively impact food systems.

**Methods:** Raw wastewater sample and 24 h composite were collected daily for 7 months in Maryland, USA beginning in January 2022. For the detection of SARS-CoV2, total nucleic acid was extracted using the Promega Enviro TNA kit. Target enrichment was performed with QIAseq DIRECT or NEBNext VarSkip Short amplicon kit and sequenced on MiSeq or ONT Gridlon respectively. Sequences were analyzed using CFSAN Wastewater Analysis Pipeline. Microeukaryotes were concentrated using Ceres Nanotrap particles. Shotgun metagenomics sequencing was performed using Illumina DNAprep and an Illumina Nextseq2000. Data were analyzed using Kraken2, customized bacterial kmer tool, taxaTarget, CARD DB, AMRfinderplus, and AMRplusplus pipeline.

**Results:** Onset of surges in Sars-CoV-2 Omicron sub-lineages BA.2, BA.2.12 and BA.5 were detected in wastewater about two weeks ahead of clinical data. Shotgun metagenomic analyses of ~300 samples identified multiple antibiotic-resistant *Escherichia coli*, *Salmonella enterica*, *Vibrio* and ESKAPE pathogens. *Cryptosporidium* and *Giardia* were detected in composite samples. Temporal trends were observed in the relative abundance of *E. coli*, which increased in late April. The relative AMR abundance per antimicrobial class, such as Oxazolidinone, was highest in January and declined in the following months.

**Significance:** WBE along with metagenomic sequencing have the potential to make culture-independent pathogen and AMR surveillance a reality. From a clinical perspective, the metagenomic level of resolution of community prevalence of foodborne disease could underpin traditionally monitored clinical foodborne isolates to delimit the scope and extent of foodborne outbreaks in a region or community.

## P2-61 Whole Genome Sequencing of *Escherichia coli* Isolated from Fresh Produce at the Point-of-Sale of South African Formal and Informal Retail Markets

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**Introduction:** Globally, *Escherichia coli* is recognised as a valuable indicator organism for contamination and carriage of antimicrobial resistance (AMR) in fresh produce production systems. In South Africa, limited sequence data for *E. coli* from fresh produce sold in formal and informal markets are available, hindering risk mitigation strategies for food safety.

**Purpose:** This study aimed to characterize 33 extended-spectrum  $\beta$ -lactamase (ESBL)-producing *E. coli* isolates from fresh produce sold in formal and informal markets in South Africa, using whole genome sequencing.

**Methods:** Presumptive ESBL-producing *E. coli* were isolated from fresh produce sold at formal and informal markets through selective enrichment and plating onto chromogenic media and confirmed using matrix-assisted laser-desorption ionisation time-of flight analysis. The selected 33 isolates were subjected to whole-genome-sequencing (WGS) (Illumina MiSeq). Antimicrobial resistance genes, virulence genes, plasmid typing, detection of mobile genetic elements, multilocus sequence typing (MLST) and prediction of pathogenicity of the strains were determined using GalaxyTrakr and the Centre for Genomic Epidemiology (CGE) platform.

**Results:** Antimicrobial resistance genes from at least three different classes were present in all isolates, with *bla*<sub>CTX-M-14</sub> the dominant ESBL gene. Notably, some isolates harboured plasmid associated  $\beta$ -lactamase genes and/or virulence genes and similar WGS-predicted antimicrobial resistance genotypes were found across the isolates from different fresh produce samples. Using PathogenFinder on the CGE platform, all strains were predicted as human pathogens with a probability >90%.

**Significance:** This is the first study to analyze *E. coli* isolated from fresh produce sold in different South African formal and informal markets with WGS. The results show the presence of clinically significant ESBL genetic determinants within environmental strains and contribute to a global database for surveillance and interpreting isolate relationships in outbreak investigation and/or prevention.

## P2-62\* A Novel Gas-Washing Bottle Incubation System Allowing for Modeling *Listeria monocytogenes* Growth Under Well-Controlled Conditions

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**Introduction:** The prediction of microbial growth and survival under various modified atmosphere packaging (MAP) conditions is an important tool for assessing safety and shelf life of food products. *Listeria monocytogenes* has become a serious problem in refrigerated MAP products because this pathogen can grow at low temperatures. Much attention has been paid to predicting *L. monocytogenes* growth under different atmospheres, which requires data collection under constant conditions. However, microbial activity as well as production, consumption, and dissolution of CO<sub>2</sub>/O<sub>2</sub> may occur during storage. Therefore, there is a need for a specific setup to eliminate these possible dynamic changes.

**Purpose:** This work aims to develop and to use a gas-washing bottle incubation system (GBIS) for investigating and modeling *L. monocytogenes* growth at 4°C while maintaining constant well-defined atmospheres during storage.

**Methods:** *L. monocytogenes* inoculated (3.0 log CFU/ml) in brain heart infusion (BHI) was investigated at 4°C for 13 days under different atmospheres (O<sub>2</sub>%/CO<sub>2</sub>%: 20/0, 0/20, 0/40, 0/60). BHI broth was placed in three gas-washing bottles connected in series to test one atmosphere. Each condition was performed twice. Gas flushing was performed once a day to keep headspace atmosphere stable. Sampling was done before each flushing to obtain growth curves for each bottle. Primary nonlinear growth models (Logistic, Gompertz, Baranyi, and Huang) were used to fit these curves.

**Results:** GBIS was found to be successful in investigating the effect of modified atmospheres on *L. monocytogenes* growth, by keeping the desired atmosphere constant. Growth reduced significantly ( $P < 0.05$ ) and  $\mu_{max}$  decreased as CO<sub>2</sub> concentration increased, indicated by primary models and growth curves.

**Significance:** GBIS is promising in fulfilling the need to study bacterial growth in different atmospheric conditions. Predictive microbiology modeling will be employed for designing optimum MAP systems and ensuring food safety.



## P2-63 Confident and Satisfactory Identification of *Pseudomonas* spp. Delivered by a Protocol Combining Multiple Molecular Techniques

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**Introduction:** *Pseudomonas* spp. are psychrotrophic spoilers of concern in the dairy industry. Growth of these bacteria causes discoloration, texture loss, and unpleasant flavors, with fatal implications for the quality and shelf life of products. The heat-resistant enzymes produced by *Pseudomonas* spp. could also remain active following pasteurization and ultra-high temperature (UHT) treatments, causing whole-batch spoilage of liquid milk.

**Purpose:** *Pseudomonas* has become the largest Gram-negative genus in terms of the number of validly published type species, with 300 entries with correct names in the current List of Prokaryotic Names with Standing in Nomenclature. Therefore, a reliable identification method is key for root cause analysis and potential shelf-life assessment.

**Methods:** In this study, different identification methods have been compared; *Pseudomonas* spp. strains were identified at the species level by MALDI-TOF MS, multi-locus sequence analysis (MLSA), and whole genome sequencing (WGS).

**Results:** Seventy *Pseudomonas* spp. strains were analyzed by using MALDI-TOF MS against the commercial library, but the resulting identities were not accurate. Subsequently, 20 *Pseudomonas* spp. isolates were identified with whole genome sequence alignment, as the gold standard. As a faster and more affordable approach, two MLSA schemes have been explored and results cross-compared to WGS identifications.

**Significance:** These results highlight a protocol (selectively applying three molecular techniques) that can deliver confident and satisfactory identification of *Pseudomonas* spp. with a good balance of speed, accuracy and affordability.

## P2-64 Consumers' Knowledge, Attitudes, and Perception Towards the Safety of Street Foods in Guyana, South America

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**Introduction:** Street foods play a significant socioeconomic role for Guyanese in obtaining their daily-recommended nutritional intake. There has been an increase in street-food vending over the last five years. Diarrheal diseases affect about 7.7% of the Guyanese population every year.

**Purpose:** The objectives of this study were to ascertain the extent of consumer food safety knowledge and their attitudes toward street-food vending.

**Methods:** A cross-sectional survey was conducted with the receipt of 398 valid responses. The questionnaire was sectioned into demographics, food safety knowledge, and attitudes towards food safety of street foods using a 5-point Likert scale. Data were analyzed using SPSS by ANOVA and chi-square analysis at 95% confidence level.

**Results:** Most respondents were female (66.3%), of African descent (49.7%), aged 18-30 years (40.7%) and had tertiary level education (56.3%). Majority (79%) believed that there were food safety risks associated with eating street foods, and age of the consumers significantly ( $P < 0.05$ ) influenced the selection of vendors from whom to purchase. Respondents' self-reported food safety knowledge were: 43% stated 'good' knowledge, 36% 'somewhat' knowledgeable, 9% 'very good knowledge', and 12% 'not being knowledgeable'. Those with tertiary and post-graduate level education were more concerned ( $P < 0.05$ ) about the safety of street foods than those with secondary level education and more women ( $P < 0.01$ ) agreed that good hygienic practices were very important in street food preparation.

**Significance:** The study could guide food safety training of consumers and street vendors by the various regulatory bodies and the food industry.

## P2-65\* How Do Pride, Stress, and Emotional Toll Influence Food Safety in Food Service?

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**Introduction:** To cultivate a positive food safety culture in food service establishments, food-handlers should be committed to implementing safe food handling practices. We suggest that positive cognitive drivers, such as pride, and negative cognitive drivers, such as stress and emotional toll, may influence food handler food safety attitudes and behaviour, and may impact commitment to food safety implementation.

**Purpose:** This study aimed to explore stakeholder perceptions of working in food service to understand how cognitive factors such as pride, stress and emotional toll could influence food safety culture.

**Methods:** Interviews conducted with food service management (n=9) and employees (n=4) were analysed using thematic analysis approach. Emerging themes relating to pride, stress and emotional toll were interpreted with considerations to food safety commitment and food safety culture.

**Results:** Expressed comments highlighted three key areas of importance for the food service management and employees – customer focus, teamwork, and personal wellbeing. Respondents talked about stress created by "irregular schedules", pressure from the customers and senior staff, "people not pulling their weight", and lack of management support. Opinions and experiences such as: "nobody realises how hard it is sometimes" and "I do feel like some people treat us like servants" indicate that emotional toll is high among the food service workers. However, feeling pride in one's work: "I love that place! I don't want it to sink!" was indicated to motivate commitment to safeguarding the consumer, building positive teamwork and food safety compliance.

**Significance:** This study determined that positive cognitive drivers, such as pride in one's work, may have a positive impact on commitment; whilst stress and emotional toll may have a negative impact. It is suggested that amplifying positive cognitive drivers through effective communication, recognition and rewards may help cultivate positive food safety culture in food service establishments and counteract the impact of negative cognitive drivers, such as stress or emotional toll.

## P2-66 Survival and Growth of *Salmonella enterica* and *Listeria monocytogenes* in Falafel Paste at Different Storage Temperatures

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**Introduction:** Recently, fried falafel (fried paste of chickpeas with herbs and spices) has been linked to foodborne outbreaks due to the contamination with *Salmonella*, *Escherichia coli* O121 and *Bacillus*.

**Purpose:** The objective of the current study was to investigate the behavior pattern of *Salmonella enterica* and *Listeria monocytogenes* in falafel paste at different storage temperatures.

**Methods:** Falafel paste was divided into 50 g samples which were inoculated with 5.0 to 6.0 log CFU/g of a cocktail culture of five strains of *S. enterica* and *L. monocytogenes* and stored at 4, 10 and 25°C for 14 days. Samples were taken at different time intervals.

**Results:** *S. enterica* survived in falafel paste stored at 4°C till the end of storage period with 1.5 log CFU/g reductions. In contrast, the *S. enterica* numbers increased by 2.1 log CFU/g at 10°C by 14 d and by 3.3 log CFU/g at 25°C by 3 d. However, at 25°C, the numbers were gradually decreased beyond day 3 to reach the undetectable levels by 10 d. *L. monocytogenes* grew at all storage temperature tested and the initial numbers (5.3 log CFU/g) gradually increased to 7.2 log CFU/g at 4°C by 14 d, 9.7 log CFU/g at 10°C by 7 d, and 9.2 log CFU/g at 25°C by 1 d. Similarly, the numbers were gradually decreased at 25°C after 1 d to reach 3.8 log CFU/g by 14 d. The initial pH value of falafel paste was 6.4; and remained constant at 4°C until the end of storage period. However, the pH was reduced to a range of 4.1 to 4.7 at 10 and 25°C.

**Significance:** *S. enterica* and *L. monocytogenes* grew or survived well in falafel paste under different storage temperatures which indicates the necessity of preventing the contamination of falafel paste to reduce the potential the risk associated with foodborne pathogens.

## P2-67 Metagenomic Analysis in Supermarket Brines of Table Olives Disclose Microbial Composition and Support Next-Generation Microbiological Risk Assessment

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**Introduction:** The era of next generation sequence (NGS) could impact food industry by unraveling the microbiome during storage and distribution. Also, it could contribute to the rapid identification of species including their technological potential or their ability to cause diseases or spoilage.

**Purpose:** The aim of this study was to apply metagenomics as an approach to understand the table olive's ecosystem and to assess their safety at retail level.

**Methods:** Samples corresponding to Kalamata, Halkidiki and Konservolia cultivars were collected from supermarkets of Greece. The respective brines were analyzed microbiologically for lactic acid bacteria, yeasts and moulds, coliforms, *Enterobacteriaceae*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*. Shotgun metagenomics utilized to determine microbial communities at species level of the different brines. Isolates of the cultivable community were identified by MALDI-ToF/ToF.

**Results:** The dominant microbial species for cv Kalamata and cv Konservolia, was *Lactiplantibacillus pentosus* followed by *L. plantarum*, while in cv. In Halkidiki, *Debaryomyces hansenii* prevailed. MALDI-ToF/ToF results verified the prevalent microorganisms. From a safety point of view, the culturable approach showed negative results for the hygienic indices. Analysis of the shotgun data with more than one bioinformatics tools corroborated these findings at large. However, there were important variations in the low to very low abundance species including foodborne pathogens like *Clostridium botulinum* and *Bacillus cereus* among the different analysis results.

**Significance:** The findings of the study showed that commercial table olives at retail level could serve as a pool of new starters or adjuncts in olives production. Further it was confirmed that NGS could be employed as a next generation microbiological risk assessment approach, but benchmarking of the bioinformatics tools is required.

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## P2-68\* The Prevalence of Foodborne Pathogens in Retail Delicatessens from South Africa

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**Introduction:** RTE food products from delicatessens have a risk of cross-contamination when they come into contact with a contaminated raw food or contaminated preparation surface.

**Purpose:** The purpose was to determine the prevalence of foodborne pathogens in delicatessens of a popular retail chain in South Africa.

**Methods:** A total of 80 environmental swabs were collected from four delicatessens across the Western Cape, South Africa. Swabs (4 cm<sup>2</sup>) were taken from different points within each facility, including hand contact points, scullery areas, meat slicers, food cooking areas and drains. Each swab collected was tested for *Listeria monocytogenes* (ISO 11290-1/A1:2005), *Salmonella* spp. (ISO 6579:-1:2017) and *Escherichia coli* (ISO 16649-2:2001). Results were confirmed with VITEK<sup>®</sup>2 Compact Automated System.

**Results:** *L. monocytogenes* was detected in 100% of the drains, 75% of the scullery areas and 50% of the food preparation areas throughout the sampling period. No *Salmonella* spp. were detected. *E. coli* results indicated the drain swabs averaged from log 4.95 to 5.88 CFU/cm<sup>2</sup>, the meat slicer swabs averaged from log 4.43 to 5.88 CFU/cm<sup>2</sup> and the display handle swabs averaged from log 5.26 to 5.64 CFU/cm<sup>2</sup>.

**Significance:** These results emphasise the importance of having a food safety system in place and ensuring that training is conducted and that the standards are upheld. Findings of this study contribute to the knowledge and importance of having a food safety plan correctly within RTE delicatessen food processing facilities in South Africa. Limited research is available on the incidence of foodborne pathogens in the retail delicatessen environment where there should be an awareness campaign implemented as consumption of RTE food products is on the rise.

## P2-69 Acceleration of Artificial Intelligence and Machine Learning – Innovative Approaches and Emerging Technologies in Food Systems

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**Introduction:** Food systems are undergoing a transformation with the aim to provide safer, more affordable, and healthier diets for all, produced in a sustainable manner. This transformation needs to be executed in the global context of major challenges facing the food and agriculture sectors, with drivers such as climate change, population growth, urbanization, and natural resources depletion compounding these challenges.

**Purpose:** Application of artificial intelligence (AI) and machine learning (ML) to early identification and evaluation of drivers and trends promote strategic planning and preparedness to take advantage of emerging opportunities and address challenges in food safety. The use of artificial intelligence (AI) and machine learning (ML) to rapidly identify and assess trends and drivers encourages strategic planning and readiness to take advantage of new opportunities and overcome hazards or/and risk in the field of food safety.

**Methods:** There are considerable opportunities for gathering, integrating and analyzing data to predict, assess and manage food safety risks. The potential for machine learning to inform microbial risk assessments is still less developed yet progressing quickly. Machine learning is being employed to harness the wealth of foodborne pathogen genomic sequence data to predict health outcomes and improve hazard characterization of specific pathogens in risk assessment models.

**Results:** Results of this study will show that how quickly food systems are adopting the artificial intelligence and machine learning approaches to innovate and to ensure the safety of foods and how this innovative technology will be helpful for food safety operations to enhance the accuracy and efficiency.

**Significance:** This study will be quite helpful for global food services and retail sector operators, food regulatory authorities, research organizations and food safety auditors to know and to adopt innovative ways of food safety to make the food systems more robust and accurate.

## P2-70 Digital Transformation and Smart Food Safety Solutions – Emerging Trends in the Food Sector

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**Introduction:** The global COVID pandemic that leaves wide-ranging and catastrophic effects at normal ways of living, has placed the food sector under immense pressure with rapid transformation by replacing manual, outdated and analog processes with modern, fully integrated technology-based solutions. This gives rise to digital innovations and technological advancements, making the food sector smarter, safer, and more sustainable.

**Purpose:** The purpose of this study is to depict and illustrate how the integration and incorporation of smarter food safety technologies immediately reduces the inaccuracies, uncertainties, and lead times. as well as enhanced the transparency, authenticity, and traceability. This has turned food safety more automated, customized, and digitalized with real-time monitoring and reporting.

**Methods:** The present study will further emphasize the paradigm shift that global players from manufacturing, retail and service sectors began using robotics, RFID tags for enhanced customer service and brand experience.

**Results:** Adoption of digital and smart food safety technologies have opened the doors of availability of diverse and rich information for the customers and can deliver high-impact results by reducing operational expenses, preventing product loss, minimizing food safety risks, brand protection and to equip the system with greater innovations, improved quality and performance, reduce labour costs, lower energy consumption and above all enhancing the global sustainability development goals.

**Significance:** The study will be quite helpful for global QSR chains, retail markets and food manufacturers that have immediately adopted and transformed to technology-based food safety solutions. They can complete checks in seconds rather than minutes by using high quality IoT sensors, web-connected devices, wireless Bluetooth temperature probes, and digital checklists.

## P2-71 Acceleration of Artificial Intelligence and Machine Learning – Innovative Approaches for Food Safety Compliance in Food Service and the Retail Sector

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**Introduction:** Innovations and new technologies are drastically changing the world and have revolutionized our lives with the advent of various emerging technologies over the last few years. Use of robots, GPS technology and smartphone apps are becoming more effective and efficient at improving food processes. Acceleration in the use of artificial intelligence and machine learning technologies with software applications has an incredible impact on food safety operations, especially in ensuring food safety compliance in food service and the retail sector by giving robust and more accurate results and data analytics.

**Purpose:** The purpose of this study will be to depict adoption of digital innovations and technological advancements, especially artificial intelligence and machine learning in smarter ways to transform and automate the existing food safety operations to make them more robust and efficient.

**Methods:** In the present study, an online survey method was used to assess the adoption and transformation of artificial intelligence and machine learning technologies to automate tasks to enhance accuracy and efficiency at various food services and retail markets.

**Results:** Results of this study show how quickly food service and retail sectors are adopting the artificial intelligence and machine learning approaches to innovate and to ensure the safety of foods. Also, how this innovative technology will be helpful for food safety operations to enhance accuracy and efficiency.

**Significance:** This study will be quite helpful for global food services and retail sector operators, food regulatory authorities, research organizations and food safety auditors to know and to adopt innovative ways of food safety to make food systems more robust and accurate.

## P2-72 Application of Artificial Intelligence (AI) and Machine Learning (ML) in the Food and Beverage Sector

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**Introduction:** Innovations and new technologies are drastically changing the world and have revolutionized our lives with the advent of various emerging technologies over the last few years. AI-based technologies are emerging tremendously and becoming more effective and efficient ways to improve the food processes throughout the food and beverages sector. AI-based cameras, object recognition, facial detection, robotics, virtual reality (VR), augmented reality (AR), drone delivery, automated online ordering and checkouts, shelf life algorithms and simulated based trainings will leave incredible impact and will transform the current technology with innovative and advanced technology with the acceleration of artificial intelligence and machine learning.

**Purpose:** The purpose of this study will be to depict potential adoption of AI tools, benefits of its applications, insights to turn data into actionable information and technological advancement especially artificial intelligence and machine learning in smarter ways to transform and automate the existing food safety operations to make them more robust and efficient.

**Methods:** In the present study survey techniques find the potential information for use and application of emerging technologies such as artificial intelligence and machine learning technologies to automate the tasks to enhance accuracy and efficiency at food and beverages sector.

**Results:** Results of this study will depict that food and beverage sectors are adopting the artificial intelligence and machine learning approaches to innovate food systems and how this innovative technology will be helpful for food operations to enhance the accuracy and efficiency.

**Significance:** This study will be quite helpful for food and beverages sector global food services and retail operators, food regulatory officials' consumers, research organizations and food industry stakeholders to know and to adopt innovative ways of food safety to make the food systems more robust and accurate.

## P2-73\* The Presence of *Listeria monocytogenes* Lineage Type I in South African Ready-to-Eat Factories

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**Introduction:** *Listeria monocytogenes* lineage II isolates are better suited for growth within food factories over the more pathogenic lineage I isolates. To help prevent further outbreaks of listeriosis, an understanding of a factory's sanitiser resistance profile is important. Quaternary ammonium compound (QAC)-based sanitisers have been used extensively in food processing environments (FPEs) as part of sanitation procedures and *L. monocytogenes* resistance towards QACs is well noted.

**Purpose:** The purpose of this study was to identify and assign lineage types to *L. monocytogenes* and determine the effect of historical sanitiser usage on strain diversity in a factory that had never used a QAC (Factory A) and another with extensive QAC usage (Factory B).

**Methods:** *L. monocytogenes* (n=42) from two South African food factories (Factory A (n=21) and Factory B (n=21)) were isolated from the factory environment and identified as *L. monocytogenes* using the *hlyA* housekeeping gene, since this gene encodes for the virulence of this pathogen, using conventional PCR. Following confirmation, their respective lineage groups were assigned through a PCR-RFLP methodology for lineage typing. Tolerance to QACs is often encoded by the *emrC* and *bcrABC* genes. A PCR assay was used to identify the resistance genes.

**Results:** All the isolates (n=42) from both factories were confirmed to be *L. monocytogenes* due to the presence of the *hlyA* housekeeping gene. All *L. monocytogenes* isolates from both factories were confirmed as lineage I and only two isolates from factory B were found to have the *emrC* gene, encoding resistance towards QACs.

**Significance:** This research highlights that the more virulent lineage I isolates are outcompeting the less virulent lineage II isolates within these two factories, despite a notable absence of QAC-resistance genes.

## P2-74 Observation of Hand Hygiene Practices at a Sandwich-Making Factory

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**Introduction:** Improper hand hygiene by food handlers has been highlighted as a major contributing factor in the spread of foodborne illnesses. Cognitive data do not equate to behaviour and have limitations of desirability biases. Consequently, observation data are indicative of actual behaviour. Although a few research studies have observed hand hygiene behaviour of food handlers prior to entering food production areas, research observing the hand hygiene practices of food handlers during production are seldom undertaken.

**Purpose:** To undertake covert observation at sandwich manufacturing business to assess food handlers' hand hygiene behaviour during production.

**Methods:** Footage from a closed-circuit television (16 h) were reviewed to evaluate food handler (n=12) hand hygiene compliance with company protocol during production using an electronic behavioural checklist.

**Results:** A total of 588 occasions that required hand hygiene by employees were observed; of which 32% of occasions hands were not washed in events when handwashing was required. Of 401 attempts to implement hand hygiene practices, only 1% were compliant with the company protocol. Observations indicated that 95% of attempts did not adhere to the recommended handwashing duration (≥20 seconds), and 62% failed to apply sanitizer after drying. Food handlers were significantly more likely ( $P < 0.001$ ) to wash hands when entering (89%) than exiting (8%) the production area.

**Significance:** Observations identified widespread hand hygiene malpractices during production indicating that appropriate interventions are needed to improve hand hygiene compliance among staff. Given that observation data during production in manufacturing settings are lacking, further research studies to assess hand hygiene compliance in the production areas are needed to allow comparison. Future cog-



native research is required in production areas to explore the potential barriers that may influence food handlers to adequately implement hand hygiene practices.

## P2-75 The Inclusion of Alcohol-Based Hand Sanitizers as a Hand Hygiene Intervention Option May be Appropriate in Retail Food Service in Certain Situations – A Systematic Study Evaluating Hand Hygiene Interventions during Meal Preparation

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<sup>1</sup>Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, <sup>2</sup>Department of Agricultural and Human Sciences, North Carolina State University, Raleigh, NC, <sup>3</sup>GOJO Industries, Inc., Akron, OH

**Introduction:** Per the FDA Food Code (FC), alcohol-based hand sanitizers (ABHS) cannot be used in lieu of handwashing (HW) during food preparation. A recent study investigated the impact of hand hygiene interventions (HHI) on microbial cross-contamination to surfaces and hands during food preparation, finding no difference in the performance of ABHS vs HW.

**Purpose:** Identify associations between worker behaviors, HHI compliance, and microbiological contamination of surfaces and hands.

**Methods:** Food handlers (n=85) were assigned to one of five groups: control (no HHI); FC-directed HHI (HW or ABHS); and natural HHI (no direction; with and without access to ABHS). Workers prepared meals using raw meat containing *Escherichia coli* DH5- $\alpha$  (NCSU IRB #21056). All meal preparations were recorded and coded. Afterwards, surface swabs (n=850) and hand rinsates (n=85) were collected and screened for *E. coli* contamination. Associations were determined using one-way ANOVA and Chi-square tests.

**Results:** Compared to treatment groups, the absence of HHI led to significantly higher *E. coli* contamination on surfaces ( $P < 0.001$ ) and hands ( $P < 0.05$ ). Behavior groups, receiving no FC-HHI direction, had statistically lower HHI attempts and successful compliance according to FC-guidelines compared to FC-HHI directed groups ( $P < 0.001$ ), however, surface and hand contamination did not significantly differ between HHI treatments ( $P > 0.05$ ). The *E. coli* concentration on contaminated hands was similar when workers had access to ABHS ( $2.6 \pm 0.7 \log_{10}$  CFU/rinsate) compared to the FC-HHI directed HW group ( $2.6 \pm 1.0 \log_{10}$  CFU/rinsate;  $P = 0.958$ ).

**Significance:** Our research showed that any HHI, including ABHS, reduced microbial contamination on kitchen surfaces and hands of food workers, regardless of the number of successful HHI attempts performed according to FC-guidelines. These results suggest inclusion of ABHS may be a viable option in food service in certain situations, but additional studies are necessary.

## P2-76\* The Effect of Cleaning and Disinfection on *Campylobacter* spp. and *Listeria monocytogenes* in the Slaughterhouse

Madeleine Moazzami<sup>1</sup>, Sofia Boqvist<sup>1</sup>, Emma Bergenkvist<sup>1</sup>, Sara Frosth<sup>1</sup>, Solveig Langsrud<sup>2</sup>, Trond Møretrø<sup>2</sup>, Ivar Vågsholm<sup>1</sup> and Ingrid Hansson<sup>1</sup>  
<sup>1</sup>Swedish University of Agricultural Sciences, Uppsala, Sweden, <sup>2</sup>Nofima, Ås, Norway

**Introduction:** Cleaning and disinfection (C&D) are critical for slaughterhouses for the production of safe food. Deficient C&D have caused outbreaks of *Listeria monocytogenes*, *Campylobacter* spp. and *E. coli* O157:H7.

**Purpose:** To study the survival and elimination of *Campylobacter* spp., *Listeria monocytogenes* and ESBL *E. coli* on surfaces by C&D procedures used in the slaughterhouses.

**Methods:** Twenty-one surfaces at two slaughterhouses in Sweden were sampled before and after C&D by swabbing with sponges and swabs with neutralizer. The total number of samples were 115. Detection of *Campylobacter* spp., ISO 10272-2 (2017), *Listeria monocytogenes*, ISO 11290-1 (2018) and ESBL *E. coli* (using Chrom-Agar Orientation with 1 mg cefotaxime) was performed.

**Results:** *Campylobacter* was identified before C&D at least once from 17 of the surfaces at the broiler slaughterhouse and in 9/53 of the samples at the cattle and swine slaughterhouse. No *Campylobacter* was isolated after C&D. *Listeria monocytogenes* was mainly detected in the drains before C&D in both slaughterhouses. *Campylobacter* spp. and *Listeria monocytogenes* were detected before C&D on food contact surfaces (FCS) in both slaughterhouses and ESBL *E. coli* was not identified in any of the samples.

**Significance:** The removal of pathogens from surfaces by efficient C&D is crucial to avoid cross-contamination of food and the creation of in-house floras in meat establishments.

## P2-77\* *Salmonella* and STEC Mitigation on Raw Beef Fabrication Surfaces Using Ozonated Water as an Antimicrobial Intervention and Pathogen Detection Using a Commercial Platform

Angelica Sanchez, Makenzie Flach, Rodrigo Portillo, Mindy M. Brashears, Markus F. Miller and Marcos X. Sanchez Plata  
Texas Tech University, Lubbock, TX

**Introduction:** Pathogen monitoring on food contact surfaces can help to develop strategies to prevent product contamination.

**Purpose:** To monitor the presence of *Salmonella* spp. and Shiga-toxin producing *E. coli* (STECs) on conveyor belts in the fabrication floor of a beef processing facility with and without a sanitation intervention.

**Methods:** The fabrication lines considered were, chuck line, which is constantly treated with ozonated water (BioSafe™) at ~810 ORP, and trim line that has no antimicrobial intervention throughout the day. MicroTally™ cloth was used to collect the samples by pressing against the running belt for 30 seconds on each side. *Salmonella* prevalence was tested using BAX®-System-*Salmonella*, positive samples were enumerated with BAX®-System-SalQuant™. STEC was tested using BAX®-System-STECS panel 1&2. *Salmonella* quantification results were transformed into log CFU/sample and analyzed using *t*-test, while Chi square analysis was conducted to determine differences in pathogen prevalence by fabrication line with a 0.05 probability threshold.

**Results:** *Salmonella* prevalence showed 5/60 positive results for the trim line and no positives for chuck line ( $P < 0.05$ ). Presumptive positives that were quantifiable had a mean concentration of 3.8 log CFU/sample. For STEC, the positive results were: In the chuck line, 22/68 for O26, 0/68 for O111, 5/68 for O121, 66/68 for O45, 1/68 for O103 and 0/68 for O145. In the trim line, 44/60 for O26, 0/60 for O111, 26/60 for O121, 54/60 for O45, 16/60 for O103 and 0/60 for O145. There were statistical differences between lines for O26, O121, O45 and O103 with a  $P < 0.05$ , but no differences were observed for O111 and 145 ( $P > 0.05$ ).

**Significance:** There is a greater prevalence of pathogens in the trim line as it has no antimicrobial treatment throughout the day. Therefore, an intervention is recommended in the non-treated line to improve hygiene and reduce potential contamination during operating hours. *Salmonella* prevalence and concentration was low in both fabrication lines.

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# EUROPEAN STUDENT TRAVEL SCHOLARSHIP RECIPIENTS



**Cristina Serra-Castelló**  
**University of Wageningen**  
**Wageningen, The Netherlands**

Cristina Serra-Castelló is a recipient of the 2023 IAFP European Student Travel Scholarship. Ms. Serra-Castelló received her Ph.D. in January 2023 in the Food Safety and Functionality Program

of the Institute of Agrifood Research and Technology (IRTA) in Catalonia, Spain. She is currently a postdoctoral researcher in the Food Microbiology Group of the University of Wageningen in the Netherlands. Her research focuses on the assessment of shelf life and safe shelf life of ready-to-eat (RTE) meat products and, recently, of plant-based foods.

Ms. Serra-Castelló is also involved in research activities dealing with the assessment of the efficacy of processing and/or preservation treatments, such as high-pressure processing or the use of bioprotective cultures, to control pathogens in RTE foods. Her research has been constantly developed in the framework of projects funded through public-private partnerships, making the industry needs and concerns the basics of her research. This prompts her to strengthen her commitment in the development of user-friendly tools (apps) integrating predictive microbiology approaches for the food industry.



**Beatriz Nunes Silva**  
**University of Minho**  
**Minho, Portugal**

Beatriz Nunes Silva is a recipient of the 2023 IAFP European Student Travel Scholarship. Ms. Silva is a Ph.D. candidate in the Food Science and Technology Nutrition Program at the University

of Minho in Portugal, and is conducting research at the Polytechnic Institute of Braganca, Portugal. Her research focuses on the application of predictive microbiology to optimize the use of biopreservation strategies to control *S. aureus* grown in traditional raw goat milk cheeses. The biopreservatives investigated include herbal extracts and lactic acid bacteria. She will present this research during the IAFP European Symposium on Food Safety in Aberdeen, Scotland.

Ms. Silva's project also involves the characterization of *S. aureus* heat resistance in raw goat's milk at sub-pasteurisation temperatures, thus evaluating thermization as a strategy to improve the safety of unpasteurised milk cheeses.

## Past European Student Travel Scholarship Recipients

2014 – Erika Georget

2015 – Emily Jackson

2016 – Amanda Demeter

2017 – Christian Hertwig

2018 – Katrien Begyn and Giannis Koukkidis

2019 – Maria Gkerekou and Yifan Zhang

2020 – Alessia Delbrück and Hannah Pye

2021 – None presented

2022 – None presented



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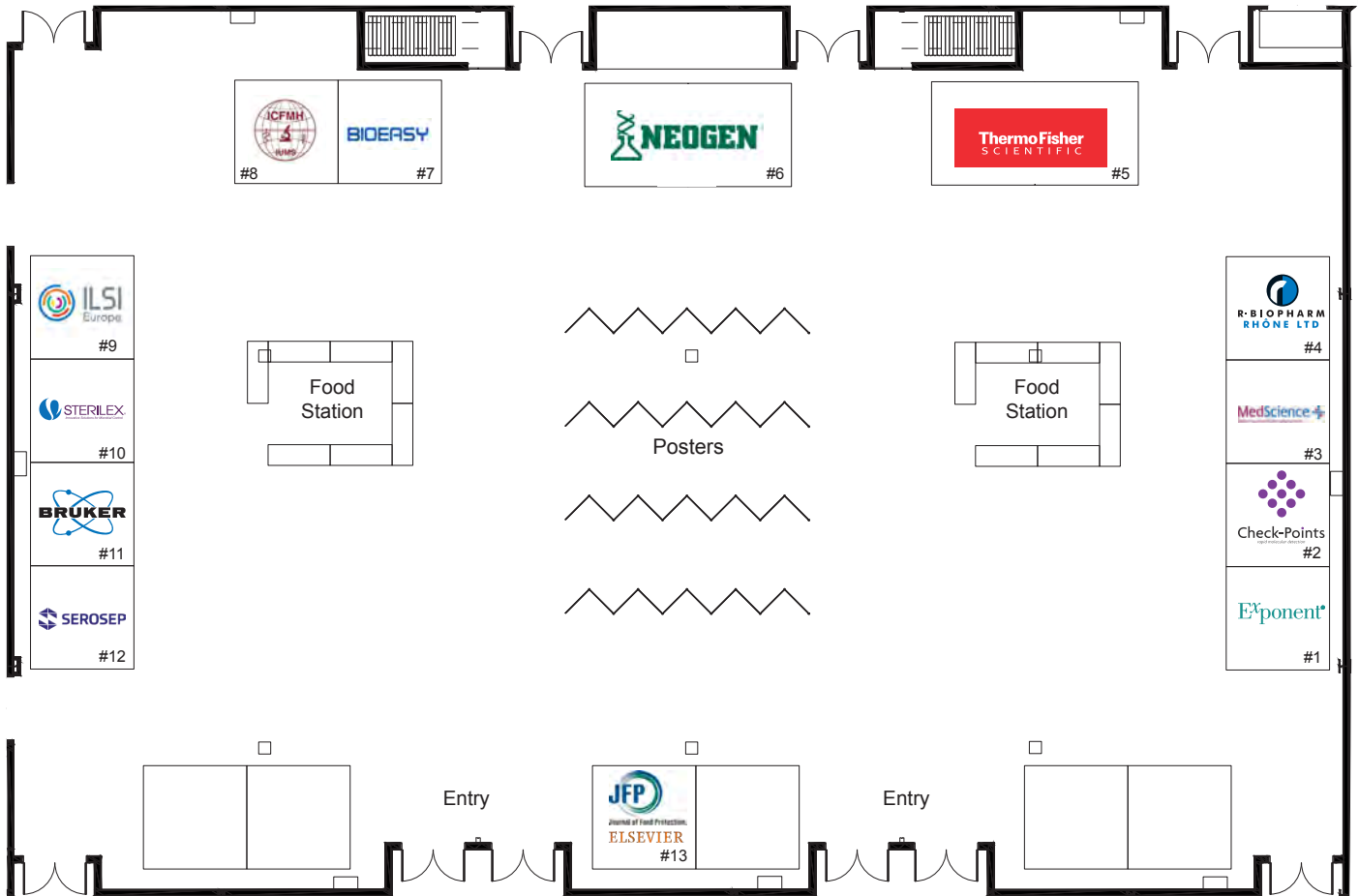
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| 12 | Serosep UK Limited  |
| 13 | The <i>Journal of Food Protection</i> (Elsevier)                                    |

### EXHIBIT HOURS

#### Wednesday, May 3

10.00 – 18.00

#### Thursday, May 4

10.00 – 16.00

### EXHIBIT EVENTS

#### Wednesday, May 3

- 10.00 Networking Coffee Break
- 12.00 Lunch
- 15.00 Networking Coffee Break
- 17.00 Reception

#### Thursday, May 4

- 10.00 Networking Coffee Break
- 12.00 Lunch
- 15.00 Networking Coffee Break

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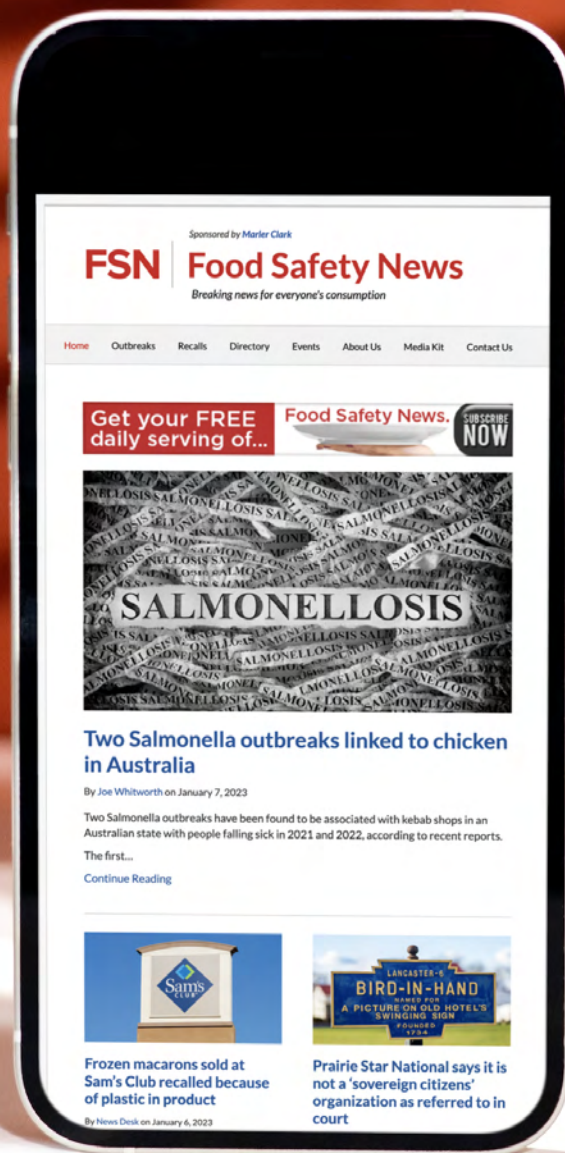


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# PAST EUROPEAN RECIPIENTS

## STUDENT TRAVEL SCHOLARSHIP

2014 – Erika Georget  
2015 – Emily Jackson  
2016 – Amanda Demeter  
2017 – Christian Hertwig

2018 – Katrien Begyn and Giannis Koukkidis  
2019 – Maria Gkerekou and Yifan Zhang  
2020 – Alessia Delbrück and Hannah Pye  
2021 – None presented  
2022 – None presented

## STUDENT AWARD COMPETITION

2009 **Overall:** Peter Rossmannith  
**Posters:** Antje Frohling and Mary Pia Cuervo  
2010 **Technical:** Rocio Morales-Rayas  
**Posters:** Orla Condell and Shane Cooney  
2011 **Technical:** Sriant Wanasen  
**Poster:** Era Taludhar  
2012 **Technical:** Sriant Wanasen  
**Poster:** Sriant Wanasen  
2013 **Technical:** Kai Reineke  
**Poster:** Brenda Magajna  
2014 **Technical:** Sungyul Yoo  
**Posters:** Cristina Rodriguez and Renáta Kugler

2015 **Technical:** Bernhard Merget  
**Poster:** Hend Al Gahmi  
2016 **Posters:** Cristina Rodriguez and Ifigeneia Makariti  
2017 **Technical:** Marcia Boura  
**Poster:** Ifigeneia Makariti  
2018 **Technical:** Lena Fritsch  
**Poster:** Aurelien Maillet  
2019 **Technical:** Krishna S. Gelda  
**Poster:** Beatriz Nunes Silva  
2020 Meeting Cancelled  
2021 **Technical:** Alessia Delbrück  
**Poster:** Xingchen Zhao  
2022 **Technical:** Rosa Heydenreich  
**Poster:** Dimitra Tsourekí

## PAST EUROPEAN LOCATIONS

2005 Prague, Czech Republic  
2006 Barcelona, Spain  
2007 Rome, Italy  
2008 Lisbon, Portugal  
2009 Berlin, Germany  
2010 Dublin, Ireland  
2011 Ede, The Netherlands  
2012 Warsaw, Poland  
2013 Marseille, France

2014 Budapest, Hungary  
2015 Cardiff, Wales  
2016 Athens, Greece  
2017 Brussels, Belgium  
2018 Stockholm, Sweden  
2019 Nantes, France  
2020 Meeting Cancelled  
2021 Virtual Meeting  
2022 Munich, Germany

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