Low Water-Activity Series: Part 1 of 4 – Persistent Strains and Source Tracking Principles in Low aw Food Processing Environments

**Moderator:** Joshua Gurtler, USDA-ARS, United States

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Please consider making a contribution

This webinar is being recorded and will be available to IAFP members within one week.
Dr. Kornacki is an industrial forensic food microbiologist. He has assisted and continues to assist many companies during environmental and product contamination concerns including FDA and USDA recalls, and has made well over 850 troubleshooting related plant visits across a vast assortment of food processing industries in his career. He is an active member of IAFP and several PDGs including the Low Moisture Foods PDG. He received the IAFP Sanitarian award (2010), its Wisconsin chapter’s (WAFP) Laboratorian of the Year award (2010) and is past Chairman of IAFP’s Food & Hygiene PDG from 2011 to 2013. He became an IAFP Fellow in 2017 and has published on a wide variety of food microbiology topics.

Joshua Gurtler is a Research Scientist at the USDA/ARS in Wyndmoor, PA, where he has worked for 14 years. Dr. Gurtler’s current work involves interventions for the inactivation of foodborne pathogens in fresh produce, soil, water, compost, and dried foods. Joshua has published numerous peer-reviewed scientific manuscripts, 9 book chapters, is an editor of three books and author of one patent and one patent that is pending. He has served as a member or chair on several IAFP committees. Dr. Gurtler is a co-scientific editor for the Journal of Food Protection. He has delivered numerous scientific research presentations, including invited presentations, in the U.S., Canada, China and Korea. He resides with his wife and three children in Phoenixville, PA.
IAFP Low Water Activity Webinar Series

Persistent Strains and Source Tracking Principles in Low $a_w$ Food Processing Environments

By:

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March 29, 2021
“Bacteria, by any reasonable criterion, were in the beginning, are now, and ever shall be, the most successful organisms on earth.”

Stephen Jay Gould,

*Full House: The spread of excellence from Plato to Darwin*, 1996.
## Estimated Annual Foodborne Disease From Selected bacterial pathogens in United States*

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>No. Total Illnesses*</th>
<th>% Total Illnesses*</th>
<th>% Hospitalized</th>
<th>% Deaths</th>
<th>No. Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> (non-typhoidal)</td>
<td>1.0 M</td>
<td>11</td>
<td>27.2</td>
<td>0.5</td>
<td>378</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>970,000</td>
<td>10</td>
<td>0.6</td>
<td>&lt;0.1</td>
<td>9</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>241,000</td>
<td>2.6</td>
<td>6.4</td>
<td>&lt;0.1</td>
<td>6</td>
</tr>
<tr>
<td><em>STECS</em></td>
<td>176,000</td>
<td>1.9</td>
<td>59.0</td>
<td>0.8</td>
<td>20</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>98,000</td>
<td>1.0</td>
<td>34.4</td>
<td>2.0</td>
<td>29</td>
</tr>
<tr>
<td>B. cereus</td>
<td>63,400</td>
<td>0.7</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ETEC</td>
<td>18,000</td>
<td>0.20</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>1600</td>
<td>&lt;0.02</td>
<td>94</td>
<td>15.9</td>
<td>255</td>
</tr>
</tbody>
</table>

Persistent strains are those specific molecular subtypes which are repeatedly isolated “in the same factory during an extended period of time; typically months or years.”

“Hence, the persistent strains may cause repeated product contamination”

Synonymous Terms

- Persistent strains
- Endemic strains
- "Systemic" strains
- House bugs/pets
- Recurrent strains


Strain Persistence

How common is this phenomenon? - VERY!

The *Salmonella* experience –

“*Salmonella* can persist for long periods of time in the dry state and in low-moisture products”¹

2463 different serotypes²

32 years active troubleshooting

>850 facility visits (about 2/3 Salmonella related)

One serotype for years, even decades in many dry food processing facilities visited in which a Salmonella strain or strains have previously been isolated

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Transient vs. Persistent

- **Transient strain**
  - Enters the facility but does not stay
    - Programs eliminate organism
    - Unable to adapt and replicate

- **Persistent strains**
  - Enters the facility and stays
    - Programs fail to eliminate organism
    - Establishes niche(s)
  - Surface chemistry (e.g., serotype) and/or genetic discriminatory testing
<table>
<thead>
<tr>
<th>Food</th>
<th>Years</th>
<th>Country</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>4</td>
<td>Switzerland</td>
<td>4b</td>
</tr>
<tr>
<td>Cheese, blue veined</td>
<td>7</td>
<td>Sweden</td>
<td>3b</td>
</tr>
<tr>
<td>Fish smoked</td>
<td>1.2 (14 months)</td>
<td>Finland</td>
<td>1/2a and 4b</td>
</tr>
<tr>
<td>Ice cream</td>
<td>7</td>
<td>Finland</td>
<td>1/2</td>
</tr>
<tr>
<td>Meat, sliced luncheon</td>
<td>4</td>
<td>Norway</td>
<td>Not determined</td>
</tr>
<tr>
<td>Mussels, smoked</td>
<td>3</td>
<td>New Zealand</td>
<td>1/2</td>
</tr>
<tr>
<td>Pâté</td>
<td>2*</td>
<td>UK</td>
<td>4b(x), 4b</td>
</tr>
<tr>
<td>Poultry, cooked</td>
<td>1</td>
<td>Ireland</td>
<td>1/2</td>
</tr>
<tr>
<td>Poultry, cooked deli products</td>
<td>12</td>
<td>United States</td>
<td>4b</td>
</tr>
<tr>
<td>Salmon, cold smoked</td>
<td>4</td>
<td>Denmark</td>
<td>ND</td>
</tr>
<tr>
<td>Trout, smoked/salmon, gravad</td>
<td>&gt;4</td>
<td>Sweden</td>
<td>1/2a</td>
</tr>
</tbody>
</table>


**Product from one plant was source of an outbreak from 1987 to mid 1989.
Other Examples of Persistence

Same *Salmonella* serotype across 10 years in cereal plant environment

Same *Listeria* across 12 years: RTE Meat

Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment

BMS Genomics. 2008. 9:539
Biofilms

“Growth on surfaces offers numerous advantages to microorganisms and therefore biofilms are the predominant growth form of microorganisms in natural environments.”

“Failure to clean and effectively sanitize a microbial growth niche site will likely lead to development of a biofilm at that site that may be many orders of magnitude more resistant to destruction by sanitizers.”

“…biofilms and polysaccharide capsules … facilitate persistence of pathogens in the processing environment…”

1 Joseph Frank, Professor, Food Science Department and Center for Food Safety, University of Georgia (personal communication).


Native Biofilms – Some Examples

Ripening Shelves During Cheese Manufacture


Steel Surface in Industrial Water System


Acridine Orange Stained Biofilm-Catch Pan Below Condensor Unit– RTE Meat Plant

3Slide Provided Courtesy of Dr. Amy Wong, Food Research Institute, Madison, WI July, 2008
Biofilms: Advantages to the Organism

Access to nutrients under low nutrient conditions that predominate in nature

Protection from environmental or host stress

Ease of genetic exchange

Sanitizer and Heat Resistance: “What Doesn’t Kill me, makes me stronger”

**Biofilm sanitizer resistance:** Widely reported as up to 1000x greater

**Listeria monocytogenes**

- Planktonic cells vs biofilms:

  Pasteurization: (wet heat): 71.7°F (161°F) 15 seconds: 3.6 to 5.2 log$_{10}$ reduction of *Listeria monocytogenes* ¹

  *Listeria monocytogenes* biofilm: 40 minutes at 70°C (158°F)² (wet heat): (>160X more heat resistant in a biofilm)

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Evicting and Preventing Development of Persistent strains

1. *Find them or the conditions that favor microbial growth*

2. *Eliminate the conditions that favor their development (Do’s and Don’ts)*

3. Eliminate the organisms
Principal Source of Microbial Contamination in Processed Foods: Processing Environment

“... cross contamination ...was mentioned as the most important factor relating to the presence of pathogens in prepared foods”

Environmental contamination is the principle source of contamination of processed foods

It is from the post-processing (post-CCPm) environment

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### Examples of Outbreaks Attributed to Environmental Contamination

<table>
<thead>
<tr>
<th>Product</th>
<th>Pathogen</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice Cream</td>
<td>S. Enteritidis</td>
<td>Pasteurized ice cream mix in tanker truck previously used for transporting raw liquid eggs</td>
<td>Hennessy <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Infant formulae</td>
<td>S. Eealing</td>
<td>Contamination from the processing environment, insulation material of the drying tower</td>
<td>Rowe <em>et al.</em> (1987)</td>
</tr>
<tr>
<td>Soft cheese</td>
<td>S. Berta</td>
<td>Cheese ripening in buckets previously used for chicken carcasses</td>
<td>Ellis <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>Cooked sliced ham</td>
<td>S. Typhimurium</td>
<td>Cooked ham placed into containers previously used for curing raw pork</td>
<td>Llewellyn <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>Chocolate</td>
<td>S. Napoli</td>
<td>Possibly contaminated water used in double-walled pipes, tanks,</td>
<td>Gill <em>et al.</em> (1983)</td>
</tr>
<tr>
<td>Chocolate</td>
<td>S. Eastbourne</td>
<td>Contamination from the processing environment</td>
<td>Craven <em>et al.</em> (1975)</td>
</tr>
<tr>
<td>Butter</td>
<td>S. Eastbourne</td>
<td>Contamination from the processing environment</td>
<td>Lyytikainen <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>Hot dogs</td>
<td><em>L. monocytogenes</em></td>
<td>Contamination from the processing environment</td>
<td>Anonymous (1999)</td>
</tr>
<tr>
<td>Canned salmon</td>
<td><em>C. botulinum</em></td>
<td>Contamination from the processing environment, cooling water</td>
<td>Anonymous (1984); Stersky <em>et al.</em> (1980)</td>
</tr>
<tr>
<td>Lasagna</td>
<td>S. aureus</td>
<td>Growth of <em>S. aureus</em> in the processing equipment, improper cleaning</td>
<td>Woolaway <em>et al.</em> (1986); Aureli <em>et al.</em> (1987)</td>
</tr>
</tbody>
</table>
Examples of Outbreaks Attributed to Environmental Contamination

<table>
<thead>
<tr>
<th>Product</th>
<th>Pathogen</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different foods</td>
<td><em>E. coli</em> O157:H7</td>
<td>Contaminated meat grinder and equipment at retail level</td>
<td>Banatvala <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Chocolate milk</td>
<td><em>Y. enterocolitica</em></td>
<td>Probably during manual mixing of pasteurization milk and chocolate or contaminated chocolate syrup</td>
<td>Black <em>et al.</em> (1978)</td>
</tr>
<tr>
<td>Canned meat</td>
<td><em>S. Typhi</em></td>
<td>Use of non-potable water for can cooling</td>
<td>Ash <em>et al.</em> (1964); Stersky <em>et al.</em> (1980)</td>
</tr>
<tr>
<td>Crabmeat</td>
<td><em>S. aureus</em></td>
<td>Contamination during manual picking of cooked meat</td>
<td>Bryan (1980)</td>
</tr>
<tr>
<td>Canned mushrooms</td>
<td><em>S. aureus</em></td>
<td>Possible growth of <em>S. aureus</em> in the brine bath before canning</td>
<td>Hardt-English <em>et al.</em> (1990)</td>
</tr>
<tr>
<td>Flavored Yogurt</td>
<td><em>E. Coli</em> O157:H7</td>
<td>Pump previously used for raw milk</td>
<td>Morgan <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>Pastry</td>
<td><em>S. Enteritidis</em></td>
<td>Equipment previously used for raw eggs or insufficiently cleaned piping and nozzles used for cream</td>
<td>Evans <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Yeasts</td>
<td><em>S. München</em></td>
<td>Contamination from the processing environment</td>
<td>Joseph <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td><em>S. Typhimurium</em></td>
<td>Possibly cross-connection between raw and pasteurized milk</td>
<td>Lecos (1986)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td><em>E. coli</em> O157:H7</td>
<td>Contamination from pipes and rubber seals of the bottling</td>
<td>Upton &amp; Coia (1994)</td>
</tr>
<tr>
<td>Mexican type cheese</td>
<td><em>L. monocytogenes</em></td>
<td>Contamination from the processing environment</td>
<td>Linnan <em>et al.</em> (1988)</td>
</tr>
</tbody>
</table>

Microorganisms Associated with Foodborne Illness
2007-2012 World-Wide and Recalls

Salmonella (94% of US low water activity food recalls and 53% of outbreaks world-wide)

Shiga-Toxin Producing Escherichia coli (STEC)
Bacillus cereus
C. perfringens
C. botulinum
Staphylococcus aureus
Cronobacter spp. (formerly E. sakazakii)
Listeria monocytogenes (recall only)

7315 cases of bacterial infection
63 deaths

Low $a_w$ Foods Implicated in Outbreaks 2007-2012 World-Wide

Rice (including Imperial, fried, Spanish, rice cereal)
Rice and corn snack
Seeds including Turkish Pine Nuts
Nuts
   Almonds, raw shelled walnuts, in-shell hazelnuts, peanut butter, roasted pistochios

Herbs & Spices
White ground pepper, black pepper in salami
Dry pet food
Dry milk
Dried tofu
Infant formula
Sweets and chocolate

<table>
<thead>
<tr>
<th><strong>Salmonella</strong></th>
<th><strong>E. coli O157:H7</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Black, white and red pepper</td>
<td>Organic cacao nibs</td>
</tr>
<tr>
<td>Curry spice</td>
<td></td>
</tr>
<tr>
<td>Garlic powder</td>
<td>Hazelnut and mixed nuts</td>
</tr>
<tr>
<td>Nuts, hazelnuts (shelled and unshelled, raw kernels), walnuts, pistachio kernel products</td>
<td></td>
</tr>
<tr>
<td>Nutmeg</td>
<td></td>
</tr>
<tr>
<td>Organic celery seed</td>
<td></td>
</tr>
<tr>
<td>Parsley powder</td>
<td></td>
</tr>
<tr>
<td>Peppermint organic tea</td>
<td></td>
</tr>
<tr>
<td>Sesame seeds, pine nuts</td>
<td></td>
</tr>
<tr>
<td>Soybean flour and soy meal</td>
<td></td>
</tr>
<tr>
<td>Spice packages, seasonings, blends, seasoning salt</td>
<td></td>
</tr>
<tr>
<td>spice rub, gravy mix, onion dip mix, soup mix, sauce mix four cheese risotto mix</td>
<td></td>
</tr>
</tbody>
</table>

## U.S. Recalls 2007-2012: Processed Low $a_w$ Products

<table>
<thead>
<tr>
<th>Salmonella</th>
<th><em>L. monocytogenes</em></th>
<th><em>C. botulinum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushed roasted Thai <strong>red pepper</strong></td>
<td>Popcorn with flavors</td>
<td>Chai concentrate</td>
</tr>
<tr>
<td>Dry <strong>pet foods</strong> including dog and cat food, flake fish food</td>
<td>Spreads</td>
<td></td>
</tr>
<tr>
<td><strong>Peanut butter</strong></td>
<td>Salsa</td>
<td></td>
</tr>
<tr>
<td><strong>Snack products</strong> with chili, also corn chips, corn sticks, potato chips, snack mixes (including with cashews), potato crisps, potato chips (including with barbeque sauce), crackers</td>
<td></td>
<td>Italian sausage</td>
</tr>
<tr>
<td><strong>Snack mixes</strong>, including with Cashews, pretzels, mixes with pretzels</td>
<td>Dips</td>
<td></td>
</tr>
<tr>
<td><strong>Egg noodle</strong></td>
<td>Peanut butter</td>
<td></td>
</tr>
<tr>
<td><strong>Pancake, cake, cookie mix, batter mix</strong></td>
<td>Cheese</td>
<td></td>
</tr>
<tr>
<td><strong>Spreads, cheese ball mix</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dry roasted hazlenut kernels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chocolate</strong> covered peanuts, white chocolate baking squares</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oatmeal</strong>, instant variety pack, and with brown sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein powders</strong>, NFDN, dry whey, sweet dairy whey powder, prebiotic powder formual</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Coming Storm in the Spice Industry


So, Where are they?

“Chance favors only the prepared mind”

- Louis Pasteur
It is impractical and likely impossible to maintain a sterile food production facility; but we can control microbial growth and niche creation and disruption.
The risk of post-process contamination is increased if

the product is not biocidally treated in the end-use container

High numbers usually required to inoculate foods at a measurable level

$1 \times 10^6$ cells per ml in a niche; 10,000 #’s of product ~2 cells per 10 gram product
2. Eliminate the conditions that favor their development (Some “Do’s” and “Don’ts”)

Microbial Growth Niches and Biofilm Creation and Disruption
(Overlapping Categories)

Operating practices (e.g., misapplied sanitation)

Maintenance / repair practices

Design / fabrication of factory / equipment

Misapplied Sanitation of a Rotary Valve Used in a Low $a_w$ Bagging Operation
Design of Facility and Location of Cleaning Implements
“Have an equipment maintenance record and monitoring program to check for broken, pitted, rusty, peeling or dirty, equipment that need replacing, repair, cleaning, etc.”
Design of Plant: Flat Roofs and Water Leaks

“Immediately fix leaky roofs, broken and cracked equipment, floors, doors, windows, etc.”

Sloped roofs much better
Correlation of % *Listeria* spp. Isolated from Packaging Lines and Floors to RTE Meat


Correlations of % Environmental to % Finished Product Contamination

Smoked fish plant: Correlation of environmental *L. monocytogenes* to finished product (p<0.0001)

Variables Affecting Likely Contamination From the Processing Environment

“The probability of product contamination from the environment is dependent upon a number of variables…”

1. Proximity of microbial growth niches to the product stream (e.g., processing equipment, zone 1 and 2)
2. No. of niches in the food production facility
3. Spatial relationships of niches and product stream
4. Microbial population in niches
5. Degree of niche disruption during operations
6. Exposure of the product stream to the environment (e.g., processing equipment zones 1 and 2)

Exceptions to the Rule: Correlation Vs Causation

Nature 332, 495-495
EMP Can be a Profit Center

Sanitation

How and Why Environmental Monitoring Programs Add to the Bottom Line

Over three decades of active consultation with the food industry, largely on microbiological matters related to investigation, risk assessment, and control, I have seen companies in both the best of times and the worst of times. During hundreds of visits to many food

February 26, 2021
Jeffrey L. Kornacki Ph.D.

Tracking Strains

A variety of molecular techniques can be applied.

Common approaches- Genetic (e.g., Rep PCR\textsuperscript{1}, Ribotyping\textsuperscript{1}, PFGE\textsuperscript{1}, MLST, WGS), and phenotypic (surface chemistry; serotyping, FTIR).

**Microarray-based multi-target sequencing**

**Whole Genome Sequencing**
Environmental Map with Comparison of Indicator Isolates
WGS VS. Other Approaches: An Industrial Perspective Or To Test or Not To Test?

It Depends

Parking Tickets Vs. Parking Permits
PFGE and WGS Called a “Fingerprint”

- “PulseNet compares the DNA fingerprints of bacteria from patients to find clusters of disease that might represent unrecognized outbreaks.” CDC in reference to PFGE

- https://www.cdc.gov/pulsenet/pathogens/pfge.html

- “…PulseNet has transitioned from PFGE to Whole Genome Sequencing (WGS)”.  

  https://www.cdc.gov/pulsenet/pathogens/pfge.html
What is a Fingerprint?

Noun- “An Impression or mark made on a surface by a person’s fingertip, able to be used for identifying from the unique pattern of whorls and lines on the fingertips. - Oxford Dictionaries

“The impression or mark left by the underside of the tips of the fingers or thumbs. The impression is formed by a pattern of ridges on the skin surface. This pattern is unique for each individual and therefore can serve as a means of identification.

http://www.dictionary.com/browse/fingerprint
Bacteria

- Exist as populations not as individuals
- They can be cloned, they are not as complex as humans, but they can mutate

(3 million vs 3 billion base pairs)
If You Don’t Have a Parking Ticket

WGS - Not a good idea for routine environmental samples in any of your Zones

Remember the WGS Draft Sequences

FDA records access

(Plausible scenario or not?)
Correlation Vs Causation: Plausible Scenario and Perceptions

“Nothing So Complicated as Perception” – Anij to Jean Luc Picard - Star Trek Insurrection

- Can two strains that are unrelated geographically be the same strain?
The Evil Identical Twin Who Moved to England and Robbed Banks

Same clone
Same WGS pattern
No causal relationship with the good twin living in America
But what if that twin visited England?
Might he be arrested?
Hypothetical Cases
Case 1: No Parking Ticket

Environmental WGS pattern “A” in manufacturing environment

Popular band coast-to-coast and widely consumed

WGS pattern “A” in Clinical Samples: 5 years ago

Plausible scenario? A slippery slope

Chances of a recall/regulatory action?
Case 2: No Parking Ticket

History of environmental samples with WGS pattern “B” (Persistent strain)

Also, product with WGS pattern “B” which company reprocessed (e.g., repasteurized, re-treated, etc.): No positive product released

No illness

FDA visit and records access

Recall/regulatory action?

Root cause?
Case 3: No Parking Ticket

*Clinical* cases of WGS pattern “C”

*Product* with WGS pattern “C” which was reprocessed and sold

Popular brand

Recall/regulatory action?

Root cause?
Case 4: Parking Ticket

Product tests positive for WGS pattern “D” AND you have filed an RFR and are in a recall

Do a Root Cause Investigation, best to do this under Attorney Client Privilege (Levitt)

Test post-lethality ingredients: Do WGS
But do it in a Statistically Robust manner

Test the Environment: Do WGS

Why?

"In Order for the Government to Feel Comfortable to Let You Operate they Need To Know that the Root Cause was Found and Eliminated."
Environmental Root Causes

Three Types

1. How did the microorganism get into the product? (Usually a Zone 1-2 investigation)

2. How did the microorganism get into the equipment (if that was the source; usually a Zone 3-4 investigation)

3. How did the microorganism get into the building or the processing area (traffic, drains, HVAC, Air flow)
Root Cause 1: Post-Process Preventive Control added Ingredients

Test Number Needed to Detect One or More Positives per Lot

<table>
<thead>
<tr>
<th>Percent positives</th>
<th>90% confidence</th>
<th>95% confidence</th>
<th>99% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>1</td>
<td>230</td>
<td>299</td>
<td>461</td>
</tr>
<tr>
<td>0.1</td>
<td>2,303</td>
<td>2,996</td>
<td>4,605</td>
</tr>
<tr>
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Adapted: Compendium of Methods for the Microbiological Examination of Foods 3rd ed.

Environmental Post Process-Preventive Control Sites

All Zones

RC 1: Extensive equipment break down and swabbing: Avoid paradigms that prevent problem solving

RC 2: Extensive Zone 3-4 sampling

RC 3: Traffic into the building (birds, trucks and loading docks), roof leaks, backed up drains
Alternatives for Those Without a Parking Ticket

- HQA/HTEB (*Listeria*-like and *Salmonella*-like organisms, respectively)

- And REP PCR or Riboprinting with Unique Restriction Endonucleases or phenotypic Biotyping (e.g., FTIR approach).
Tracking Strains: Parking Tickets Vs. Parking Permits

- *Listeria* and *Listeria*-like organisms with Ribotyping, REP PCR, Biotyping
- HTEB assay with Rep PCR or Biotyping

Multiple subtypes suggest multiple sources and can inform the investigation.

HTEB Subtypes in a Limited Investigation of a Low $a_w$ Food Processing Facility

**REP PCR With Generic E. coli Primer**

1. Citrobacter freundii
2. Escherichia hermannii
3. Citrobacter freundii
4. Citrobacter freundii
5. Enterobacter amnigenus-2
6. Citrobacter freundii
7. Morganella morganii
8. Citrobacter freundii
9. Citrobacter freundii
10. Citrobacter freundii
11. Citrobacter freundii
12. Morganella morganii
13. Morganella morganii
14. Enterobacter amnigenus-2
15. Escherichia hermannii
16. Salmonella arizona
17. Proteus mirabilis

**REP PCR With Enterobacter Primer**

1. Escherichia hermannii
2. Morganella morganii
3. Morganella morganii
4. Citrobacter freundii
5. Morganella morganii
6. Salmonella arizona
7. Enterobacter amnigenus-2
8. Enterobacter amnigenus-2
9. Citrobacter freundii
10. Citrobacter freundii
11. Citrobacter freundii
12. Morganella morganii
13. Proteus mirabilis
14. Morganella morganii
15. Citrobacter freundii
16. Salmonella arizona
17. Citrobacter freundii
Summary: Selected Capabilities of Microorganisms

“Bacteria are smarter than we are because they do not have a brain to worry about”

– R. Behling

Their astounding capabilities makes them difficult to control
Summary

Microbes are highly adaptable and successful creatures

Environmental contamination is likely to be the most significant source of finished product contamination.

Persistent strains in processing plant environments may result from biofilms rendering them more resistant to sanitizers and thermal inactivation.

Investigations of product contamination should be done with robust (not routine) statistical sampling of ingredients that are not subjected to a lethal treatment.

Investigations should include observations and appropriate sampling in areas related to operating practices, maintenance & repair practices, and appropriate sanitary design of the facility and equipment.

Persistent strains can be tracked with various molecular subtyping approaches.

Consider all the consequences to any molecular subtyping approach you choose.
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Low Water Activity Food Safety Series

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Part 2: Microbiological Safety of Nuts and Products       April 15, 11:00 AM (EDT)

Part 3: Microbiological Safety of Dried Spices          May 12, 11:00 AM (EDT)

Part 4: Grain Based Foods and Ingredients               June 9, 11:00 AM (EDT)
This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

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