Part 2: What Could Be In Municipal Water?

Monday, April 30 2018, 11:00 a.m. Central Time U.S.

Part 1 gave the basics of EPA rules and what they might mean. But what could be in the water you get? Learn what municipal water indicators indicate and whether they predict the presence of microbes that may impact the safety of your product.

Hear from Dr. Shay Fout, recently retired from the EPA about what indicators do and do not indicate, from leading researcher Arizona State University’s Dr. Paul Westerhoff about De facto reuse, how wet weather and variability can impact food safety and the latest on heat resistant microbes from University of Alberta Professor Norman Neumann and what they could mean to food processors.

Speakers

- G. Shay Fout, U.S. EPA, National Exposure Research Laboratory, Retired
- Paul Westerhoff, Vice Dean for Research and Innovation – Ira A. Fulton Schools of Engineering Arizona State University
- Norman Neumann, Professor School of Public Health University of Alberta

Moderator

Elisabetta Lambertini, PhD, Principal Investigator, Research Scientist Food Safety and Environmental Health Risk Center for Health and Environmental Modeling RTI International

Sponsored by IAFP’s Water Safety and Quality PDG & Atlantium Technologies
Does Water Matter?
Part 2: What could be in Municipal Water?

What Do Indicators Really Indicate?

G. Shay Fout
U.S. EPA, National Exposure Research Laboratory, ret.

Sponsored by IAFP's Water Safety and Quality PDG & Atlantium Technologies
Virus and Indicator Relationships

Outline
• Indicators
• Virus occurrence studies
• Virus and indicator relationships
• Conclusions

Adenovirus

E. coli
Indicators are microbial agents that indicate whether a pathogen (or just fecal pollution) is present

Perfect Indicators
- Must be present in higher concentration than pathogens
- Must always be present when pathogens are present
- Must always be absent when pathogens are absent

There are no perfect indicators for virus occurrence
- Bacterial indicators are always present in human stool while pathogens are only present when people are infected and then normally only for short periods
- Bacterial and bacteriophage indicators are excreted from animal as well as human sources, but most viral pathogens of concern are human-specific
- In general bacterial indicators die off faster than virus, so while their concentrations are higher than those of viral pathogens close to the source of contamination, the difference in concentrations decreases with time and distance
Why are indicators less valuable for groundwater? It depends on the hydrogeology of the aquifer.
## Virus and Indicator Relationships

### Virus Occurrence Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Wells</th>
<th>Samples</th>
<th>Study Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA/AWWARF (US)</td>
<td>30</td>
<td>333</td>
<td>9/92-12/94</td>
</tr>
<tr>
<td>USGS (MO)</td>
<td>182</td>
<td>322</td>
<td>5/97-7/98</td>
</tr>
<tr>
<td>USGS/EPA (MI)</td>
<td>38</td>
<td>169</td>
<td>6/99-7/01</td>
</tr>
<tr>
<td>USGS (PA)</td>
<td>60</td>
<td>60</td>
<td>9/00-2/01</td>
</tr>
<tr>
<td>AWWSC (US)</td>
<td>20</td>
<td>235</td>
<td>3/01-5/02</td>
</tr>
<tr>
<td>UT Knoxville (TN)</td>
<td>4</td>
<td>6</td>
<td>3/04-8/04</td>
</tr>
<tr>
<td>Armand-Frappier (Canada)</td>
<td>36</td>
<td>243</td>
<td>3/04-12/12</td>
</tr>
<tr>
<td>Univ. Rome (Italy)</td>
<td>8</td>
<td>14</td>
<td>6/05-12/05</td>
</tr>
<tr>
<td>Univ. Tokyo (Japan)</td>
<td>46</td>
<td>46</td>
<td>11/05-1/06</td>
</tr>
<tr>
<td>Marshfield Clinic (WI)</td>
<td>36</td>
<td>391</td>
<td>4/06-11/07</td>
</tr>
<tr>
<td>NIER (Korea)</td>
<td>220</td>
<td>383</td>
<td>7/07-12/08</td>
</tr>
<tr>
<td>Iowa DNR (IA)</td>
<td>66</td>
<td>71</td>
<td>3/13-6/13</td>
</tr>
<tr>
<td>EPA (US)</td>
<td>823</td>
<td>1055</td>
<td>7/13-12/15</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>1569</strong></td>
<td><strong>3328</strong></td>
<td></td>
</tr>
</tbody>
</table>

### References:
- Fout et al., Virus occurrence in small groundwater public systems located in karstic regions of the U.S. *In preparation*
## Virus and Indicator Relationships

### Indicator- and Virus-Positive Wells

<table>
<thead>
<tr>
<th>Indicator/Virus</th>
<th>%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>21</td>
<td>1558</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>6</td>
<td>1558</td>
</tr>
<tr>
<td>Enterococci</td>
<td>8</td>
<td>1241</td>
</tr>
<tr>
<td>Aerobic spores</td>
<td>39</td>
<td>838</td>
</tr>
<tr>
<td>Anaerobic spores</td>
<td>13</td>
<td>50</td>
</tr>
<tr>
<td>F-specific coliphage</td>
<td>8</td>
<td>1446</td>
</tr>
<tr>
<td>Somatic coliphage</td>
<td>5</td>
<td>1446</td>
</tr>
<tr>
<td>Culturable virus</td>
<td>3</td>
<td>1174</td>
</tr>
<tr>
<td>PCR-virus</td>
<td>6</td>
<td>1419</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>6</td>
<td>1234</td>
</tr>
<tr>
<td>Norovirus</td>
<td>8</td>
<td>1250</td>
</tr>
</tbody>
</table>
Virus and Indicator Relationships

Borchardt et al. 2012
- Virus exposure – AGI model: mean concentration GI norovirus, all ages
- 22% of the AGI in the study communities was from virus-contaminated tap water
- For children < 5 yrs, in the spring of 2006, the fraction of AGI from drinking water was 63%!

Borchardt et al. 2012
## Spearman Rank Order Correlation for Wells (Rho value)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Culturable virus</th>
<th>PCR-virus</th>
<th>Enterovirus</th>
<th>Norovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Aerobic spores</td>
<td>0.1</td>
<td>0.0**</td>
<td>-0.0**</td>
<td>0.0**</td>
</tr>
<tr>
<td>Anaerobic spores</td>
<td>0.1**</td>
<td>0.1**</td>
<td>-0.0**</td>
<td>-0.0**</td>
</tr>
<tr>
<td>F-specific coliphage</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Somatic coliphage</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Any indicator</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1*</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Unmarked values are significant at $P < 0.001$; * $P = 0.01$ to 0.05; ** $P > 0.05$
Virus and Indicator Relationships

Virus-Indicator Relationships

Sensitivity = the percentage of virus-positive wells the indicator correctly identified as virus-positive

Specificity = the percentage of virus-negative wells the indicator correctly identified as virus-negative

Positive predictive value (PPV) = the percentage of indicator-positive wells that were virus-positive

Negative predictive value (NPV) = the percentage of indicator-negative wells that were virus-negative

Risk Ratio = the increase in odds of finding a virus-positive well when an indicator is present versus when it is absent = PPV-(1-NPV)
## Virus and Indicator Relationships

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms (TC)</td>
<td>64</td>
<td>88</td>
<td>15</td>
<td>98.7</td>
<td>11</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>36</td>
<td>96</td>
<td>24</td>
<td>98.0</td>
<td>12</td>
</tr>
<tr>
<td>Enterococci</td>
<td>47</td>
<td>92</td>
<td>15</td>
<td>98.3</td>
<td>9</td>
</tr>
<tr>
<td>Aerobic spores</td>
<td>67</td>
<td>61</td>
<td>2</td>
<td>99.4</td>
<td>3</td>
</tr>
<tr>
<td>Anaerobic spores</td>
<td>40</td>
<td>68</td>
<td>25</td>
<td>81.3</td>
<td>1**</td>
</tr>
<tr>
<td>F-specific coliphage</td>
<td>38</td>
<td>95</td>
<td>21</td>
<td>97.9</td>
<td>10</td>
</tr>
<tr>
<td>Somatic coliphage</td>
<td>39</td>
<td>97</td>
<td>31</td>
<td>98.0</td>
<td>16</td>
</tr>
<tr>
<td>TC or aerobic spores</td>
<td>75</td>
<td>59</td>
<td>2</td>
<td>99.6</td>
<td>4*</td>
</tr>
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Unmarked values are significant at $P < 0.01$; * $P = 0.01$ to 0.05; ** $P > 0.05$
<table>
<thead>
<tr>
<th>Indicator</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms (TC)</td>
<td>48</td>
<td>85</td>
<td>35</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>20</td>
<td>96</td>
<td>49</td>
<td>88</td>
<td>4</td>
</tr>
<tr>
<td>Enterococci</td>
<td>30</td>
<td>94</td>
<td>29</td>
<td>93</td>
<td>5</td>
</tr>
<tr>
<td>Aerobic spores</td>
<td>41</td>
<td>62</td>
<td>3</td>
<td>97</td>
<td>1**</td>
</tr>
<tr>
<td>Anaerobic spores</td>
<td>28</td>
<td>80</td>
<td>85</td>
<td>22</td>
<td>1**</td>
</tr>
<tr>
<td>F-specific coliphage</td>
<td>24</td>
<td>95</td>
<td>43</td>
<td>89</td>
<td>4</td>
</tr>
<tr>
<td>Somatic coliphage</td>
<td>22</td>
<td>97</td>
<td>51</td>
<td>89</td>
<td>5</td>
</tr>
<tr>
<td>TC + aerobic spores</td>
<td>48</td>
<td>59</td>
<td>3</td>
<td>98</td>
<td>1**</td>
</tr>
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</table>

Unmarked values are significant at $P < 0.01$; * $P = 0.01$ to 0.05; ** $P >0.05$
## Virus and Indicator Relationships

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Wells with spores</th>
<th>Wells without spores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk Ratio</td>
<td>P-value</td>
</tr>
<tr>
<td>Total coliforms (TC)</td>
<td>0.0</td>
<td>0.69</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.0</td>
<td>0.92</td>
</tr>
<tr>
<td>F-specific coliphage</td>
<td>0.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Somatic coliphage</td>
<td>0.0</td>
<td>0.99</td>
</tr>
</tbody>
</table>

ND – value could not be determined
## Virus and Indicator Relationships

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliform Rule (TCR)</td>
<td>All U.S. wells with &gt;2 health-related TCR violations plus all international wells with &gt;2 likely violations</td>
</tr>
<tr>
<td>Hydrogeology</td>
<td>All wells located in karst, fractured bedrock, or gravel/cobble settings</td>
</tr>
<tr>
<td>U.S. Groundwater Rule indicators (GWR)</td>
<td>All wells with total coliforms and any of the three GWR-triggered indicators (E. coli, enterococci, or coliphage)</td>
</tr>
</tbody>
</table>
## Virus and Indicator Relationships

<table>
<thead>
<tr>
<th>Category</th>
<th>Culturable Virus</th>
<th>PCR-Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR</td>
<td>1.3 (672)</td>
<td>1.2 (148)</td>
</tr>
<tr>
<td>Hydrogeology</td>
<td>1.5 (131)</td>
<td>0.9 (65)</td>
</tr>
<tr>
<td>GWR</td>
<td>3.9 (59)</td>
<td>1.6 (118)</td>
</tr>
</tbody>
</table>
# Virus and Indicator Relationships

## Risk Ratios for wells in Susceptibility categories (*P*-value)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Category</th>
<th>Culturable Virus</th>
<th>PCR-Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total coliforms</strong></td>
<td>All</td>
<td>4.5 (0.04)</td>
<td>1.3 (0.04)</td>
</tr>
<tr>
<td></td>
<td>Hydrogeology</td>
<td>3.8 (0.02)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Enterococci</strong></td>
<td>All</td>
<td>4.5 (0.002)</td>
<td>1.0 (0.91)</td>
</tr>
<tr>
<td></td>
<td>Hydrogeology</td>
<td>5.8 (0.01)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TCR</td>
<td>5.1 (0.02)</td>
<td>4.9 (0.01)</td>
</tr>
<tr>
<td><strong>F-Specific coliphage</strong></td>
<td>All</td>
<td>7.7 (0.04)</td>
<td>1.2 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Hydrogeology</td>
<td>8.4 (0.005)</td>
<td>2.2 (0.02)</td>
</tr>
<tr>
<td><strong>Somatic coliphage</strong></td>
<td>All</td>
<td>9.1 (&lt;0.001)</td>
<td>1.9 (&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>TCR</td>
<td>NS</td>
<td>2.8 (0.04)</td>
</tr>
</tbody>
</table>

Values from first 12 studies adjusted for study design; NS – not significant
Virus and Indicator Relationships

Major conclusions

- Human enteric viruses may be found in groundwaters from wells across a wide range of vulnerability assessments.
- Indicators are not perfect, but still valuable.
- In wells without indicators, viruses are unlikely to be present.
- However, indicators are often present when viruses are absent.
- And viruses may be present in the absence of indicators.
- And viruses in untreated groundwaters used in food processing or restaurants for foods that are not cooked may be a source of foodborne outbreaks.
Virus and Indicator Relationships

Acknowledgements:
Authors of the various studies, especially Mark Borchardt, Burney Kieke, Sandhya Parshionikar, Larry Wymer, Michael Jahne, Yury Shtarkman

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
De Facto Reuse

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Arizona State University

Email: p.Westerhoff@asu.edu

Sponsored by IAFP's Water Safety and Quality PDG & Atlantium Technologies
Outline

• **What is de facto reuse (DRF) & Why use it?**
• How did we linked data sources?
• How much wastewater is in rivers?
• How much DFR occurs at DWTPs serving >10k?
  – Spatial considerations
  – Temporal considerations
• Can we validate DFR predictions?
• Implications
  – DWTPs serving <10k vs >10k populations
  – Implications of DFR on DWTP installed treatment processes
DeFacto Reuse is The unplanned or incidental presence of treated wastewater in a water supply source.

100% - X% = River water
X% = Treated Wastewater
De Facto Reuse = x%

Where is drinking water impacted by WW?
Outline

• What is de facto reuse (DRF) & Why use it?
• How did we linked data sources?
• How much wastewater is in rivers?
• How much DFR occurs at DWTPs serving >10k?
  – Spatial considerations
  – Temporal considerations
• Can we validate DFR predictions?
• Implications
  – DWTPs serving <10k vs >10k populations
  – Implications of DFR on DWTP installed treatment processes
De Facto Reuse Model Development

Base Map: National Atlas of the United States and USGS

Hydrography: USGS National Hydrography Dataset Plus

WWTPs:
- 14,651 data points
- CWNS 2008
- Permit Compliance System used for data mining missing location points

DWTPs:
- 6,330 total active surface water intake points
- 2,056 with population served > 10,000
Outline

• What is de facto reuse (DRF) & Why use it?
• How did we linked data sources?
• **How much wastewater is in rivers?**
• How much DFR occurs at DWTPs serving >10k?
  – Spatial considerations
  – Temporal considerations
• Can we validate DFR predictions?
• Implications
  – DWTPs serving <10k vs >10k populations
  – Implications of DFR on DWTP installed treatment processes
Stream Dilution Factors in Rivers Influence Fish & Discharge Limits

Dilution is the Solution?

Dilution Factor = \( \frac{Q_{\text{River}}}{Q_{\text{Wastewater}}} \)

Dilution Factor
- Greater than 100
- 10 to 100
- 2 to 10
- Less than 2

Regional Hydrologic Unit Code (HUC)
Figure 3. a. Dilution factors under low flow conditions (Q95) with median MEC. b. Dilution factors under low flow conditions (Q95) with 90th percentile MEC.  Red lines represent the dilution factors required for (1) 17α-ethinylestradiol, (2) 17β-estradiol, and (3) estrone (labeled from top to bottom) to fall below hazard quotients given a 10-fold safety factor. (. Top and bottom of box= 75th and 25th percentiles, respectively; top and bottom of whisker= 90th and 10th percentiles, respectively; line across inside of box= median (50th percentile). Diamonds represent the average of values within between the 10th and 90th percentiles.

Key Findings

- Wastewater discharges make up >50% of instream flow for over 900 receiving streams.
- Dilution factors amongst receiving streams 25th, 50th, and 75th percentile are 8, 43, and 287 respectively (N=14,651).
- Roughly 400 of 1049 reaches are impacted by a HQ value < 10 fold safety factor for all three contaminants under low flow conditions.
- Up to a four-magnitude difference between DF’s based upon stream orders in the same USGS hydrologic region.
Outline

• What is de facto reuse (DRF) & Why use it?
• How did we linked data sources?
• How much wastewater is in rivers?
• **How much DFR occurs at DWTPs serving >10k?**
  – Spatial considerations
  – Temporal considerations
  – Communities with <10,000 people
• Can we validate DFR predictions?
• Implications
  – DWTPs serving <10k vs >10k populations
  – Implications of DFR on DWTP installed treatment processes
Low Magnitude of De Facto Reuse

Legend
- DWTPs Impacted by AVGDFR
  - Less than 1%
  - 1 to 5%
  - 5 to 10%
  - 10 to 15%
  - Greater than 15%
- States (National)
High Occurrence Frequency of De Facto Reuse

National Occurrence: Average Flow Conditions
Influence of Droughts & Floods

Strahler Stream Order
Historic Streamflow Percentile

Stream Order & Streamflow Variations

% Defacto Reuse

Historic Streamflow Percentile

Droughts
Floods

(uri River) Missouri River
De Facto Reuse under Varying Streamflow Conditions

**Impacts of Seasonal Streamflow on De Facto Reuse**

Strahler Stream Order = 3

*Red line represents DFR under Average Streamflow Conditions*

Strahler Stream Order = 6

Impact of Monthly Streamflow Variation on DFR
2011 NRC Report
Suggested:

DWTPs with ≥ 5% DFR received higher levels of CECs than planned reuse schemes
UCMR3 Min Reporting Level (MRL)

Legend: top and bottom of box = 75th and 25th percentiles respectively; top and bottom of whisker = 90th and 10th percentiles respectively; line across inside of box = median (50th percentile).
Comparison of Model Predicted “HITS” vs Observed in UCMR3 for Steroid
Disinfection Impacts

Chloramination is practiced at WTPs serving water to >50% of the US Population
Chloramines react with Wastewater Organics to form DBPs (Nitrosodimethylamine – NDMA)
Wastewater effluents contain antibacterial resistant organics & little is known about chlorine resistance
Predictions of NDMA precursors from wastewater at DWTPs
Summary of key points

• Big data & GIS allows us unprecedented opportunities to understand spatial and temporal impacts of wastewater on our water supplies

• There is a high frequency, but low magnitude, of de facto reuse

• Communities on smaller streams are more susceptible to wastewater impacts

• Next we hope to include industrial and agricultural discharges

• WTPs with de facto reuse have lower treatment goals than planned reuse projects (e.g., RO)
Supporting references & Acknowledgements

Resistant Microbes and VBNC - What Might They Mean to Food Processors?

Norman Neumann
Professor
School of Public Health
University of Alberta

Sponsored by IAFP's Water Safety and Quality PDG & Atlantium Technologies
I hope to convince you that:

• Stress resistance is common in bacteria.

• Bacteria evolved adaptive stress mechanisms long before humans came onto the scene!

• Humans have simply ‘facilitated’ the natural selection and evolution of extreme resistance.

• We need a ‘re-awakening’ of our research agenda to ensure better food/water safety practices.
The Urban Water Cycle
‘De facto’ Reuse
Could our water disposal practices be facilitating the emergence of pathogen resistance in the food-water nexus?
"We can look forward with confidence to a considerable degree of freedom from infectious diseases at a time not too far in the future. Indeed...it seems reasonable to anticipate that within some measurable time...all major infections will have disappeared”. (T. Aidan Cockburn [1963] in his book the Evolution and Eradication of Infectious Diseases as quoted by Merrill Singer in the book, Anthropology of Infectious Diseases [Page 157], Left Coast Press, 2015).

It is alleged that a couple of years later the Surgeon General of the U.S., Dr. William Stewart, said “It is time to close the book on infectious diseases”. (Merrill Singer in the book Anthropology of Infectious Diseases [Page 157], Left Coast Press, 2015).
Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century.
What about the evolution of water-treatment resistant microbes?

• Like antibiotic resistance, evolutionary selection for treatment resistance has been going on for a very long time....millions/billions of years!!!
• **Examples**
  - The mammalian immune system uses reactive chlorine (e.g., HOCl), reactive oxygen (H₂O₂, O₂⁻, OH⁻) and reactive nitrogen (peroxynitrite [OONO·], nitric oxide [NO·]) as defenses against microbes.
    - Microbes have evolved a number of strategies to deal with these ‘toxic’ molecules

• Many microbes need to survive in an environment until the next host comes along to infect....
  - solar radiation (polychromatic UV)
  - dessication
  - osmotic pressure
  - temperature
  - predation
  - microbial competition

They already have the tools in the toolbox!!

...microbes have had a long time to think about these ‘disinfection’ problems...and...they have ‘invented’ diverse and remarkable solutions!
AN ENGINEER’s VS. AN EVOLUTIONARY MICROBIOLOGIST’s Perspective on Wastewater Treatment

TREATMENT as a series of Microbial Selection/Evolution Pressures
- Microbial competition
- Predation
- Antibiotics
- Temperature
- Treatment (O₃, UV, Cl₂, H₂O₂)

Raw Sewage $10^6$ E. coli per 100mL

Primary Effluent

Secondary Effluent

Tertiary Effluent

Final Effluent $10$ E. coli per 100mL

[5 log₁₀ reduction]

Are we creating treatment resistant/environmentally-persistent, virulent pathogens?

- Direct Potable Reuse
- Indirect potable reuse
- Reuse (Stormwater)
- Irrigation
- Drinking water

Why did 10 E. coli survive and the other 999,990 die? Was ‘disinfection / microbial reduction’ random?
INACTIVATION OR KILLING OF MICROBES IS NOT RANDOM IN A WASTEWATER TREATMENT PROCESS

We do not choose who lives and who dies from treatment! Nature decides!

The 10 E. coli survived...not because they were lucky...but......
because they had MICROBIAL KEVLAR™
(i.e., they were wearing bullet proof vests)!!!!!!!
Some *E. coli* strains have evolved to live and survive in wastewater!
Stress resistance in naturalised waste water

*E. coli* strains

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The authors recently demonstrated that naturalised strains of *Escherichia coli* exist in municipal waste water, characterised by (*a*) biomarker patterns in intergenic regions distinct from human and animal *E. coli* strains and (*b*) an insertion element (*IS30*) located in the *uspc-flhDC* intergenic region of the genome. Remarkably, these strains are naturally adapted to survival and growth in waste water and differentially survive the treatment process. The authors sought to explore the adaptive mechanisms used by these strains for survival. A serial stress experiment (nutrient deprivation and osmotic stress followed by chlorine treatment) was performed and survival was measured using culture. Waste water strains were shown to be approximately 100 times more resistant to chlorine treatment than a wild-type human faecal strain. Naturalised waste water strains were also more robust at producing biofilms – an adaptive strategy for surviving environmental stressors. Since biofilm formation has been linked to increased motility, the authors examined the expression of the flagellar regulator gene, *flhDC*, under serial stress conditions. Chlorine was a potent inducer of *flhDC* expression in waste water strains. The results demonstrate that waste water strains possess adaptive genotypic/phenotypic properties related to their survival in waste water and challenge the understanding of treatment reduction based on *E. coli* as an indicator of treatment performance.
Stress-induced **Chlorine** Resistance in Wastewater Naturalized *E. coli* strains

These wastewater strains were originally isolated in the lab by treating raw sewage with a \( \sim 5 \log_{10} \) microbicidal treatment with chlorine (bleach)!

Wastewater strains were \( \sim 100 \) times more resistant to chlorine than some fecal and lab strains, as well as better biofilm producers!
Killing of Chlorine-Resistant Bacteria by Chlorine-Bromine Solutions

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Effects of Starvation on Physiological Activity and Chlorine Disinfection Resistance in Escherichia coli O157:H7

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Characterization and identification of a chlorine-resistant bacterium, *Sphingomonas* TS001, from a model drinking water distribution system

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**HIGHLIGHTS**

"...this strain was very resistant to chlorine, and 4 mg L⁻¹ of chlorine with 240 min retention time provided only approximately 5% viability reduction..."
Tetracycline-resistant *E. coli* showed tolerance to chlorine at high doses.

Chlorination with a high dose shifted tetracycline-resistant *E. coli* to become even more tolerant to tetracycline.
Divergent adaptation of *Escherichia coli* to cyclic ultraviolet light exposures

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**Experimental design**
- SINGLE strain of *E. coli* (PQ30) exposed to increasing UVC .... for 80 generations!
- ~ final irradiating natural selection dose was 640 J/m²

**Findings**
- 5 log₁₀ difference in susceptibility (100,000X more resistant)!
- Vertical heredity potential—parent to progeny
- Does NOT include horizontal gene transfer potential

Fig. 2. Dose-responses to UV light of wild-type *E.coli* PQ30 and UV-resistant derivatives isolated after 80 UV irradiation cycles. See Figure 1 for details.
Additional characteristics of wastewater *E. coli* strains

- Generalized stress response (*rpoS*), universal stress response (*usp*)
- Presence of a Heat Resistant Genomic Island (Locus of Heat Resistance)

Our wastewater *E. coli* also show a resistance phenotype to UV!

- Originally described in *Klebsiella* heat/disinfection treatment tolerant strains in hospitals. Recently found in *E. coli* by Mercer et al., (2015). Can withstand 60°C for 5 minutes.
Heat tolerance of Wastewater Naturalized *E. coli* strains (60°C)

Why would wastewater strains want to be resistant to ‘heat’ when the temperature doesn’t exceed 18°C?!!!!!!

- LHR Encodes 16 proteins believed to be involved in DNA repair, protein turnover, chaperones, etc.
- LHR probably not a good name for this locus....
Other Examples of Heat Resistance

• We have isolated strains of:
  • *E. coli* that can survive temperatures reaching >55°C (maximum temp. of 55°C) for 8 days, and potentially persist in a viable-but-non culturable (VBNC) state for >30 days!
  • *Salmonella* that can survive > 55°C for 13 days and persist in a VBNC state for >30 days!

Extremely Heat Resistant *E. coli* and *Salmonella*...originating from sewage treatment plants (biosolids)!
Are we seeing co-evolutionary selection between virulence and treatment-resistance in *E. coli* as a result of our engineering practices?

WHERE’S THE EVIDENCE?
Virulence and plasmidic resistance determinants of *Escherichia coli* isolated from municipal and hospital wastewater treatment plants

Vera Calhau, Catarina Mendes, Angelina Pena, Nuno Mendonça and Gabriela Jorge Da Silva

**ABSTRACT**

“WWTPs contribute to the dissemination of virulent and resistant bacteria in water ecosystems, constituting an environmental and public health risk.”

*aac(6’)-Ib-cr*. Aminoglycoside resistance and multidrug-resistant phenotypes were also detected. PAI IV, PAI II, and PAI I were detected. With regard to the clinical ST131 clone, it carried *blaCTX-M-15*, *blaTEM-type*, *qnrS* and *aac(6’)-Ib-cr*, IncF and IncP plasmids, and virulence factors PAI IV, PAI II, PAI I, *iutA*, *sfa/foc* and *papAH* were identified in the effluent of a hospital plant. WWTPs contribute to the dissemination of virulent and resistant bacteria in water ecosystems, constituting an environmental and public health risk.

**Keywords** | *Escherichia coli*, phylogeny, plasmidic resistance determinants, virulence factors, WWTP
"Strains surviving UV irradiation were...carrying virulence genes associated with urinary pathogenic E. coli (UPEC) and intestinal pathogenic E. coli (IPEC)."

“Our data suggest that some E. coli strains have a better ability to survive sewage treatment plants utilizing chlorination and UV irradiation for disinfection.”
Our results indicate that certain...UPEC strains can survive the treatment processes of sewage treatment plants.

strains. Of these, 120 (76.4%) strains belonged to seven persistent C-BPTs and were found in all four STPs. Our results indicate that certain clonal groups of *E. coli* with virulence characteristics of uropathogenic strains can survive the treatment processes of STPs. These strains were common to all STPs and constituted the highest proportion of the strains in different treatment tanks of each STP.
Molecular characterization revealed five pathotypes...: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%) and UPEC (41.7%).

“We conclude that municipal wastewater effluents are important reservoirs for dissemination of potentially pathogenic E. coli (and possibly other pathogens).....”
Question #1:
Are we actually ‘creating’ new MICROBIAL MONSTERS for our industry?

Question #2:
If we are creating these problems then what are the solutions?

- More chlorine?
- More ozone?
- More UV?
- More heat?
- More dessication?
- More sanitizers?
- More disinfectants?
- More additives?

Or will this lead to more resistance?
Implications for Food Processors

- Resistant bacteria are part of nature...don’t assume they’re not a problem in your facility.
  - Are you complacent or diligent?

- These principles apply to all microbes, including foodborne pathogens (*Salmonella, Campylobacter, Arcobacter, Listeria*, etc.)
  - Evolutionary principles govern all living organisms (i.e., survival of the fittest)
    - Antibiotic-resistance, vaccines, pesticide resistance (mosquitoes, molluscs), clinical resistance (viruses, bacteria, parasites, worms)

- Don’t rely on a single barrier for food safety. Multi-barrier approach to HACCP programs needed.
The Role of Water Quality in Food Safety: Does Water Matter?

Part 3: Does Water Quality Matter To My Food Company?
Monday, June 4, 2018, Noon, Eastern Daylight Time U.S.

Part 1 gave the basics of EPA rules and how time lags might impact food processors. Part 2 described what could be in the compliant Safe Drinking water you get.

In Part 3, learn what to do about it!
University of Arizona’s Dr. Chuck Gerba explains the basics of Quantitative Microbial Risk Assessment (QMRA) and determining your risk profile, including what information you need to evaluate your risk and where to get it;

Dr. Vince Hill of the CDC explains why we don’t hear much about the nexus between water and food contamination;

Will Daniels, President, Produce Division, IEH Laboratories will advise on Measures you can take if your water isn’t as safe as your business requires.

Speakers

Dr. Chuck Gerba
Professor
University of Arizona

William C. Daniels
President, Produce Division
IEH Laboratories & Consulting Group

Moderator

Phyllis Butler Posy
Chair - Water Quality Safety PDG
Vice President of Strategic and Regulatory Affairs
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Vincent Hill
Chief, Waterborne Disease Prevention Branch – Division of Foodborne, Waterborne and Environmental Diseases, (CDC)

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