CURRENT ISSUES RELATED TO MEATBORNE PATHOGENIC BACTERIA

John N. Sofos

John.Sofos@colostate.edu
Food Safety Problems

FRANKFURTER AND LUNCHEON MEAT LISTERIOSIS OUTBREAK, USA 1998-1999:
21 deaths (6 stillbirths or abortions) and 99 cases

POULTRY RTE PRODUCTS LISTERIOSIS OUTBREAK, USA 2002:
50 sick, 7 deaths, 3 miscarriages in 8 NE states
# Foodborne Illness in the United States

- **Illnesses per year**: 75,814,924
- **Hospitalizations**: 323,854
- **Deaths**: 5,209
- **Costs**: $8.4 Billion

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Illnesses</th>
<th>Deaths (%)</th>
<th>Fatality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em></td>
<td>2,453,926</td>
<td>124 (2.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1,413,322</td>
<td>585 (11.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>Escherichia coli O157:H7</em></td>
<td>73,480</td>
<td>61 (1.1)</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2,518</td>
<td>504 (9.7)</td>
<td>20</td>
</tr>
</tbody>
</table>

Mead et al. (1999)  
CDC (1999)
### U.S.A. NATIONAL HEALTH OBJECTIVES FOR 2010

#### Incidence of Foodborne Illnesses (per 100,000 population)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incidence 1996</th>
<th>Incidence 2002</th>
<th>Target 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>23.5</td>
<td>13.37</td>
<td>12.3</td>
</tr>
<tr>
<td>Salmonella</td>
<td>14.5</td>
<td>16.1</td>
<td>6.8</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>0.5</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>2.7</td>
<td>1.73</td>
<td>1</td>
</tr>
</tbody>
</table>

**SITES/POPULATION** 5/14.2M  9/37.4M  

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*CDC, FoodNet-Final Report, 1998 / CDC Preliminary FoodNet Data, 2002*
# FSIS Product Recalls, 2000-2003

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>Listeria monocytogenes</th>
<th>Salmonella</th>
<th>E. coli O157:H7</th>
<th>Total/%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FRESH PRODUCTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEEF</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>84/24.7</td>
</tr>
<tr>
<td>PORK</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>4/1.2</td>
</tr>
<tr>
<td>POULTRY</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>8/2.4</td>
</tr>
<tr>
<td><strong>READY-TO-EAT PRODUCTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEEF</td>
<td>26</td>
<td>2</td>
<td>-</td>
<td>28/8.3</td>
</tr>
<tr>
<td>PORK</td>
<td>67</td>
<td>7</td>
<td>-</td>
<td>74/21.8</td>
</tr>
<tr>
<td>POULTRY</td>
<td>26</td>
<td>2</td>
<td>-</td>
<td>28/8.3</td>
</tr>
<tr>
<td><strong>TOTAL/%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128/37.8</td>
<td>12/3.5</td>
<td>86/25.4</td>
<td>339/100*</td>
<td></td>
</tr>
</tbody>
</table>

*INCLUDING RECALLS NOT ASSOCIATED WITH BACTERIA 113/33.3

Adapted from USDA-FSIS Recall Information Center database: [http://www.fsis.usda.gov/OA/recalls/rec_pr2.htm](http://www.fsis.usda.gov/OA/recalls/rec_pr2.htm)
MEAT PRODUCT RECALLS

Major USA Meat Recalls:

- Hot dogs/packaged meats; *Listeria monocytogenes*; 35M Lbs; December 1998
- Various products; *Listeria monocytogenes*; 35M Lbs; January, 1999
- Fresh and frozen RTE poultry products; *Listeria monocytogenes*; 27.4M Lbs; October, 2002
- Ground beef; *Escherichia coli* O157:H7; 25M Lbs; August, 1997
- Beef trim and ground beef; *Escherichia coli* O157:H7; 19M Lbs; July, 2002
MICROBIAL COMMUNITY

SOURCES OF MICROBIAL CONTAMINATION

- Food
- People
- Facilities
- Animals
- Equipment
- Pests
- Environment (Feces, Soil, Water, Dust)
FSIS MEAT SAFETY PROGRAMS

Zero Tolerance for Visible Carcass Contamination

“Adulterant”: *E. coli* O157:H7 in Ground Beef

Pathogen Reduction/HACCP Rule:

- Sanitation Stand. Operating Procedures/SSOP
- Hazard Analysis Critical Control Point/HACCP
- Microbiological Criteria:
  - Microbiological Performance Criteria: *E. coli*
  - Pathogen Reduction Standard: *Salmonella*

Directives/Guidances for raw and RTE products
ESCHERICHIA COLI O157:H7 “ADULTERANT” IN GROUND BEEF AND OTHER NON-INTACT BEEF PRODUCTS
### USA *E. coli* O157:H7 Ground Beef Testing

<table>
<thead>
<tr>
<th>Year</th>
<th>Tested (#)</th>
<th>Positive (#)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>891</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1995</td>
<td>5407</td>
<td>3</td>
<td>0.06</td>
</tr>
<tr>
<td>1996</td>
<td>5703</td>
<td>4</td>
<td>0.07</td>
</tr>
<tr>
<td>1997 (25/325g)*</td>
<td>6065</td>
<td>4</td>
<td>0.07</td>
</tr>
<tr>
<td>1998</td>
<td>8080</td>
<td>14</td>
<td>0.17</td>
</tr>
<tr>
<td>1999 (IMS)*</td>
<td>7786</td>
<td>32</td>
<td>0.41</td>
</tr>
<tr>
<td>2000</td>
<td>6374</td>
<td>55</td>
<td>0.86</td>
</tr>
<tr>
<td>2001</td>
<td>7009</td>
<td>59</td>
<td>0.84</td>
</tr>
<tr>
<td>2002</td>
<td>6708</td>
<td>55</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Sample size increased from 25 to 325g  
*Method modified to include immunomagnetic separation
BACTERIAL PATHOGEN TESTING

Number of samples needed to detect known or expected levels of contamination with probabilities of 0.95*

Expected level of contamination = 0.1%
\[ n = -\ln \left( \frac{0.05}{0.001} \right) = 2,996 \text{ samples} \]

Expected level of contamination = 1.0%
\[ n = -\ln \left( \frac{0.05}{0.01} \right) = 300 \text{ samples} \]

Expected level of contamination = 7.5%
\[ n = -\ln \left( \frac{0.05}{0.075} \right) = 40 \text{ samples} \]

*Assumes random incidence; which it's not
QUESTION:
How about those positive tests that lead to removal of product from commerce? Does n’t that save lives?

ANSWER:
Good point. There is value to that. However, do not use testing as the sole means of assuring product safety.

Most importantly, do not consider lots of product with negative testing results as pathogen free, and do not let others believe so.
FSIS Enteric Pathogen (*Escherichia coli* O157:H7 and *Salmonella*) Slaughter Guidance

- Reasonably likely to occur in beef production
- Slaughter/processing responsible for risk control
- Effective sanitation needed
- HACCP plan reassessment
- Validation of decontamination CCP
- No product mixing
- Recordkeeping needed
- FSIS inspection to verify validation of CCP
- FSIS to enforce increased O157:H7 testing
FSIS Enteric Pathogen (*Escherichia coli* O157:H7 and *Salmonella*) Grinder/Supplier Guidance

- Specify purchase requirements raw materials
- Processing and recordkeeping controls for safety and traceability
- Effectively recall plan for distributed products
- Grinding operators responsible until product use
Pre-harvest Pathogen Control

- Scientific information is limited
- Numerous complicating variables
- Unknown reservoirs
- Inadequate detection methodology
- Ubiquitous presence of some pathogens
- Lack of interventions
- Economic issues

Promising:
- Vaccines
- Diet modifications
- Feed additives
- Probiotics
- Management practices
Carcass Decontamination Interventions

- Animal Cleaning
- Knife-trimming
- Steam-Vacuuming
- Pre-Evisceration Washing
- Thermal Pasteurization
- Chemical/organic Acid Rinsing
- Sequential Hurdles
Decontamination with Multiple Interventions

**Combinations of treatments:**
- Warm acid solutions
- Steam and vacuum

**Sequential application of:**
- Animal cleaning
- Chemical dehairing
- Knife-trimming
- Steam-vacuuming
- Pre-evisceration washing
- Final carcass washing
- Chemical and/or thermal
- Carcass chilling
## Reduction of Contamination at Slaughter

**Beef Carcass Decontamination in 8 plants**

### Ranges of Bacterial Populations (log CFU/100cm²) on Cattle Hides and Resulting Carcasses in 8 Plants

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC</th>
<th>ECC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle hides</td>
<td>8.2 – 12.5</td>
<td>5.9 – 7.5</td>
</tr>
<tr>
<td>Dehided carcasses</td>
<td>6.1 – 9.1</td>
<td>2.6 – 5.3</td>
</tr>
<tr>
<td>Washed carcasses</td>
<td>3.8 – 7.1</td>
<td>1.0 – 3.0</td>
</tr>
<tr>
<td>Chilled carcasses</td>
<td>2.3 – 5.3</td>
<td>&lt;0.9 – 0.9</td>
</tr>
</tbody>
</table>

Bacon et al. (2000)
Contamination Concerns During Carcass Chilling

- Contamination reduction
- Microbial growth
- Additional contamination
- Chilling rate
- Chilling uniformity
- Sanitation and hygiene
- Inadequate knowledge
Contamination Concerns During Carcass Fabrication

- New and additional contamination
- Cross-contamination
- Spreading/redistribution
- Microbial growth
- Sanitation and hygiene
- Room temperature
- Time duration
- Potential decontamination
  - Application technology issues
  - Labeling issues
Mean TPC On Carcasses, Belts & Subprimals As Fabrication Time Progressed

- Carcass
- Belt
- Subprimals

Bacon et al. (2002)

Fabrication Contamination

Time Of Day

Log CFU/100 cm²
Pathogen Incidence in Raw Beef From 6 Plants

CONTAMINATION SPREADING

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Packaging Plant</th>
<th>Retail Store</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass</td>
<td>7.9%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Subprimals</td>
<td>25.7%</td>
<td>19.1%</td>
</tr>
<tr>
<td>Subprimals</td>
<td>0%</td>
<td>0.9%</td>
</tr>
<tr>
<td>Retail Cuts (0h)</td>
<td>15.6%</td>
<td>0.9%</td>
</tr>
<tr>
<td>Retail Cuts (48h)</td>
<td>7.9%</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

Kain et al. (1996)
Growth/survival of *Salmonella* Typhimurium on fresh beef treated with hot water (HW) (75°C) or 2% lactic acid (LA; 55°C)
Behavior of *Listeria monocytogenes* in meat washings

(Samelis et al., 2001)
**Listeria monocytogenes** attached cells vs. **Listeria monocytogenes** suspended cells

**TSAYE+Rif agar/Attached cells**

<table>
<thead>
<tr>
<th>Sanitizer Exposure Time (sec)</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors (log CFU/cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td></td>
<td></td>
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<tr>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>3</td>
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<td></td>
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<tr>
<td>4</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TSAYE+Rif agar/Suspended Cells**

<table>
<thead>
<tr>
<th>Sanitizer Exposure Time (sec)</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors (log CFU/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**BIOFILMS**

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**Listeria monocytogenes** suspended cells vs. **BIOFILMS**
Nonacid-habituated *E. coli* O157:H7 on beef carcass tissue exposed to spray-chilling
Acid-habituated *E. coli* O157:H7 on beef carcass tissue exposed to spray-chilling

**Survival (log CFU/cm²)**

- Acidified sodium chlorite (0.12%)
- Lactic acid (2%)
- Nontreated control
- Water
- Sodium hypochlorite (0.005%)
- Ammonium hydroxide (0.05%)

**Time (h)**

0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5 0 1 2 3 4 5
Reduction of Contamination at Slaughter

QUESTIONS/CONCERNS/ISSUES

- Variability:
  - Plant
  - Animal lot
  - Intervention
  - Animal type
  - Season
  - Anatomical site
  - Plant site
  - Sampling method

- Spreading of contamination and cross-contamination
- Extent of carcass and product area contaminated
- Prevalence vs population reductions by decontamination
- Transfer of contamination from carcasses to meat
Reduction of Contamination at Slaughter

QUESTIONS/CONCERNS/ISSUES

Meat decontamination interventions:
- Instantaneous or short intensity interventions
- Intensity inadequate for complete inactivation
- Penetration/Biofilms/Removal/Inactivation/Injury
- Alteration of metabolic activity of survivors?
- Selection/Adaptation/Cross-protection/Virulence?

Potential changes in plant and meat microbial ecology:
- Inhibition of growth of normal gram-negative flora?
- Acid washings may select for yeasts or lactics
- Lactics more dominant in acetic acid washings
- Water, steam or highly diluted acid washing runoff fluids: establishment of gram-negative bacteria?
- Changes in spoilage patterns
Reduction of Contamination at Slaughter

QUESTIONS/CONCERNS/ISSUES

Red Water spray-washing runoff fluids from meat:
- May support pathogen growth
- High levels of background flora may:
  - Suppress pathogen growth
  - Reduce pathogen acid resistance
  - *E. coli* O157:H7 sensitized to acid when exposed to neutral pH, nutrient deficient water meat washings

Residual undiluted organic acid meat spray-washings:
- Potential residual pathogen inhibition or inactivation
- Survival of low numbers of adapted cells
Reduction of Contamination at Slaughter

QUESTIONS/CONCERNS/ISSUES

Residual diluted organic acid/water meat spray-washings

- Pathogen survival (*E. coli* O157:H7 survives for days)
- Longer survival of acid-adapted pathogens
- May lead to acid-adaptation
- Acid: potential residual antimicrobial effect on meat
- Acid: residual effects may become sublethal

Residual acetic acid runoff fluids:

- Less effective against *E. coli* O157:H7
- Effective against natural flora
MEAT PROCESSING INDUSTRY PATHOGEN CONTROL EFFORTS

Should Carcass Decontamination be Discontinued?
Decontamination interventions are useful:
- Reduce carcass contamination (1-3 logs)
- Reduce pathogen prevalence
- Assist plants meet regulatory/industry criteria

However, they should be:
- Evaluated for potential unpredictable risks
- Optimized for maximum benefits with no risks

Consider potential long term effects of interacting sublethal interventions on the microbial ecology of plants and raw and ready-to-eat products
Reduction of Contamination at Slaughter

SUMMARY

- Select/apply treatments in the right intensity and sequence in order to maximize activity and minimize resistance development
- Potentially beneficial to alternate, or use simultaneously, various decontamination treatments to avoid establishment of acid adapted pathogen niches
- Validation of technologies needed
- Minimize decontamination variations
- Research new technologies and beyond slaughter
- Not ready-to-eat until further processed/cooked
- Useful in reducing probability of illness when product intentionally or unintentionally undercooked
FSIS Directive (7111.1; 1999) Proposed Performance Standards for RTE Products

**RTE Meat Products:**
- 6.5 log *Salmonella* reduction

**RTE Poultry Products:**
- 7.0 log *Salmonella* reduction

**All RTE meat products during cooling:**
- No more than 1.0 log growth of *Clostridium perfringens*
- No growth of *Clostridium botulinum*

**Fermented RTE Meat Products with Beef:**
- 5.0 log *E. coli* O157:H7 reduction
FSIS Directive (10,240.3, 12/9/2002) for Microbial Sampling of RTE Products

- Intensified verification testing program
- Increased FSIS record verification checks
- Increased testing of food contact surfaces, plant environments, and final products
- Plants with environmental testing program that do not share data with FSIS subject to intensified testing
- Plants sharing data from environmental testing program with FSIS: subject to targeted testing
CONTROL OF CONTAMINATION BY FURTHER PROCESSING

- Avoidance of additional contamination
- Inhibition of growth
- Destruction of contamination
- Prevention of post-processing contamination
- Proper storage
- Proper distribution
- Proper serving
Acid-adapted E. coli O157:H7
TSAP

D-value (h) $r^2$

Control 2.18 0.99
Traditional marinade 2.76 0.97
Modified marinade 2.09 0.96
Acetic acid + Traditional marinade 1.65 0.98
Tween 20 + Acetic A. + Traditional marinade 1.40 0.94

BEEF JERKY
**Acid-adapted E. coli O157:H7**

**TSAP**

- **Control**
- **Traditional marinade**
- **Modified marinade**
- **Acetic acid + Traditional marinade**
- **Tween 20+Acetic A.+Traditional marinade**

**BEEF JERKY**
Escherichia coli O157:H7 (TSAP) on Beef Jerky Slices

BEEF JERKY
Frankfurters with antimicrobials included in the formulation inoculated with *Listeria monocytogenes* (Bedie et al., 2001)
Listeria monocytogenes on **nondipped pork frankfurters** formulated with antimicrobials and stored under vacuum.

![Graph showing the growth of Log CFU/cm² over Days of storage at 10°C for different treatments: Control, SL (1.8%), SD (0.25%), SL (1.8%)+SD (0.25%), SL (1.8%)+SD (0.125%).]
Listeria monocytogenes on 2.5% acetic acid-dipped pork frankfurters formulated with antimicrobials and stored under vacuum.
Listeria monocytogenes inoculated on pork bologna formulated with antimicrobials and stored under vacuum
Bologna slices inoculated with *Listeria monocytogenes* and then immersed (1 min)
Growth/Survival of *Listeria monocytogenes* on pork ham dipped in antimicrobials and stored at 10°C under vacuum
Nondipped frankfurters exposed to synthetic **gastric fluid** (pH 1.0)

**GASTRIC FLUID RESISTANCE**

- Control
- Sodium lactate (SL)
- Sodium diacetate (SD)
- SL + SD (0.25%)
- SL + SD (0.125%)

Day 20

Day 30

Day 40
Lactic acid-dipped frankfurters exposed to synthetic gastric fluid (pH 1.0)

Day 20

Day 30

Day 40

GASTRIC FLUID RESISTANCE

log CFU/cm²

Exposure time (min)

Control
Sodium lactate (SL)
Sodium diacetate (SD)
SL + SD (0.25%)
SL + SD (0.125%)

Day 20
Day 30
Day 40
Microorganisms exposed to stresses during food handling and processing

Sublethal treatments may cause metabolic injury or hardening of the organism

Physical: cold shock; sublethal heat; drying; UV

Chemical stresses: acids; sanitizers

Injury: Altered growth capability

Resistance: hardening/cross-protection/virulence

Viable but non-culturable
Optimization of Pathogen Control

In order to resist antimicrobial stresses bacteria:
- Try to maintain homeostasis
- Repair multiple injuries
- May become energetically exhausted (long lag phase or death; exhaustion)
- May also become exhausted when wrong genes activated (psychosis): environmental signals make them unprepared to cope with a sudden stress
Optimization of Pathogen Control

- Develop processes with treatment combinations or sequences that:
  - Do not allow stress adaptation
  - Lead to death through:
    - Pathogen exhaustion, or
    - Sensitization of “psychotic” survivors to stresses

- Select and apply treatments or interventions at:
  - Sufficient intensity
  - Appropriate time
  - Appropriate sequence with other stresses
Optimization of Pathogen Control

- To jointly inactivate pathogens
- To maximize antimicrobial effects and minimize resistance development of pathogens
- Objective: Apply stress to exhaust cells and increases susceptibility to subsequent stress
- Failure to do so:
  - May lead to resistant survivors
  - Increased virulence
  - Increased food safety risks
Optimization of Pathogen Control

Should we discontinue using multiple, individually sublethal, treatments?

No. Apply at appropriate intensity and sequence to cause exhaustion during food processing.

Exhausted survivors may have insufficient energy reserves to cope with subsequent stresses or the acid stress of the stomach.
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- I. Geornaras
- K. Koutsoumanis
- P. Skandamis

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- S. Albright
- C. Anderson
- L. Ashton
- T. Bacon
- I. Barmpalia
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