THE ROLE OF EXTRAINTESTINAL FOODBORNE PATHOGENS IN HUMAN ILLNESS: OR HOW WGS TECHNOLOGY IS CHANGING OUR VIEWS ON FOODBORNE ILLNESS

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  James R. Johnson, MD (Veterans Administration, MN)
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  Amee Manges (Canada)
WHAT DO YOU NORMALLY THINK ABOUT WHEN IN COMES TO BACTERIAL FOODBORNE ILLNESS?

- Salmonella
- Escherichia coli O157:H7, etc
- Campylobacter
- Listeria monocytogenes
- Yersinia enterocolitica
- Staphylococcus aureus

- Clostridium perfringens
- Clostridium botulinum
- Bacillus cereus

- Diarrhea, cramps, nausea, vomiting >>>>Sepsis, HUS, etc.
FORGET ABOUT DIARRHEA

LETS TALK ABOUT URINARY TRACT INFECTIONS!

Images from pixabay.com and wikipedia
URINARY TRACT INFECTIONS

• ca. 6 - 8 million cases in the US annually

• ca. 250,000 cases pyleonephritis (kidney infections)

• ca, 23,000 deaths

• 80- 85% caused by *E. coli* (*Multi-Drug-Resistant*)

• 5 - 10% caused by *Staphylococcus saprophyticus*

• Some by *Klebsiella pneumoniae* and other....

• Primarily affect women and girls

• Account for ca.1 percent  of medical office visits (sporadic)

• 50 % of women will have a UTI in their lifetime

• 25% will have a recurrent infection

URINARY TRACT INFECTIONS

• Chance of UTI increases with onset of puberty (women) due to sexual activity.

• Self infection process due to transfer of feces from the anus to the vagina and urethra (4-5 cm distance).

• Isolates from UTI, bladder, kidney infections are a typically genetic match the E. coli or S. saprophyticus in the individual’s fecal microflora.

• Increased chance of UTI due to catheterization (men and women).

• Underlying health conditions

• Can be a sexually transmitted disease.

Conclusion: Its all about contaminated feces going where its shouldn’t go.

Question: How do these bacteria get into the GI tract?

COMMUNITY BASED URINARY TRACT INFECTION OUTBREAKS: SIMILAR TO FOODBORNE ILLNESS OUTBREAKS

Outbreaks of Urinary Tract Infections can occur with a community within the same time period:

USA
Canada
Denmark
United Kingdom
Brazil

Pattern noted as early as 1988 that community-based UTI outbreaks resembled foodborne illness outbreaks.

Tools did not exist to establish a link between food and UTI outbreaks

Vincent et al., 2010; Canada; Pitout et al., 2005; Johnson et al., 2002; Manges et al., 2001; Oleson et al., 1994; Phillips et al., 1988.
LET’S TALK ABOUT E. coli
Escherichia coli Types

- Commensal (harmless background microflora)
- Intestinal Pathogenic *E. coli* (iPEC)
  - STEC
  - EHEC
  - VTEC
  - Adulterants in Foods
  - Regulated by FSIS and FDA
- Extraintestinal Pathogenic *E. coli*
  - Uropathogenic *E.coli* (UPEC)
  - Neonatal meningococcal *E. coli* (NMEC)
  - Avian pathogenic *E. coli* (APEC)
  - Not regulated
- Hybrids (Carry both iPEC and ExPEC Virulence Factors)
O-H ANTIGENS

iPEC: O26, O103, O111, O121, O45, and O145. PCR: stx, eae, ehx

UPEC: O1, O2, O4, O7, O16, O18, O25, and O75. PCR: papA/C, sfa/foc, afa/dra, kpsMII, IutA, (UPSA)

NMEC: O1, O7, O12, O18, and O83

Overlap
## Estimated Number of Illnesses and Deaths: E. coli

<table>
<thead>
<tr>
<th></th>
<th>Illnesses</th>
<th>Hospitalizations</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrheal E. coli (STEC, etc)</td>
<td>ca. 306,000</td>
<td>ca. 3700</td>
<td>ca. 31</td>
</tr>
<tr>
<td>Uropathogenic</td>
<td>6 - 8 million</td>
<td>ca. 100,000</td>
<td>ca. 23,000</td>
</tr>
<tr>
<td>Meningococcal</td>
<td></td>
<td></td>
<td>ca. 500</td>
</tr>
</tbody>
</table>

Scallan et al. (2011)
Nordstom et al. (2013)
**COMMONALITIES BETWEEN iPEC AND ExPEC**

<table>
<thead>
<tr>
<th></th>
<th>iPEC</th>
<th>ExPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat and Poultry</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Produce</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Seafood</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Soil</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Groundwater</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Foodborne</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Animal to Animal</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Animal to Human</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Human to Human</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
PATH TO ILLNESS (UPEC)

Reduce Risk

Predictive Microbiology

Interventions

Genotype

Detection Technologies

Incidence & Prevalence

The Urinary Tract

Kidney

Ureter

Bladder

Urethra
## Incidence and Prevalence of ExPEC in Foods

*Escherichia coli* and ExPEC in 1648 Retail Food Samples (Johnson et al., 2005).

<table>
<thead>
<tr>
<th>Food Type</th>
<th>No. of samples containing <em>E. coli.</em></th>
<th>Samples containing of antibiotic resistant <em>E. coli.</em></th>
<th>No. of samples containing ExPEC.</th>
<th>No. of samples containing <em>E. coli</em> with UTI O-antigens.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscellaneous</td>
<td>N=121 (9.2%)</td>
<td>N=31 (2.4%)</td>
<td>N=5 (0.38%)</td>
<td>N=12 (0.91%)</td>
</tr>
<tr>
<td>(N=1315)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>N=95 (68.8%)</td>
<td>N=73 (52.9%)</td>
<td>N=18 (13.0%)</td>
<td>N=13 (9.4%)</td>
</tr>
<tr>
<td>(N=138)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>N=180 (92.3%)</td>
<td>N=165 (84.6%)</td>
<td>N=83 (42.6%)</td>
<td>N=28 (14.3%)</td>
</tr>
<tr>
<td>(N=195)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Produce ca. 1% ExPEC with UTI O-antigen.

MEAT CONSUMPTION AND UTI PATTERNS

• Manges et al. (2007) examined the acquisition of UTI due to antimicrobial resistant *E. coli*.

• 99 adult women aged 18 to 45 years old, with history of UTI.

• The dietary habits examined included consumption of chicken and pork, and the method of meat preparation.

• *E. coli* isolates from the women who had UTI were then subjected to PFGE analysis.

• Women infected with multi-drug resistant *E. coli* reported frequent chicken consumption.

• Those infected by ampicillin or cephalosporin resistant isolates frequently consumed more pork.

ISOLATION OF *E. coli* FROM FOOD ANIMALS, RETAIL MEAT AND POULTRY, AND ADULT WOMEN WITH UTI.

- *E. coli* collected from farm animals (pigs, chicken, cattle), retail meat and poultry, and adult women with from the same geographic area during the same time period.

- Multiple countries: Denmark, Spain, US, Canada.

- Isolates compared for PFGE Pattern, MLVA, Virulence Factor Profile, Antibiotic Resistance Profile.

- High degree of relatedness and identity between animals>food>human.

- Food isolates caused UTI in animal model systems.

- New approach uses whole genome sequencing and single nucleotide polymorphism analysis.

(Muller et al., 2016; Davis et al., 2015; Mulata et al., 2014; Bergeron et al., 2014; Danzeison et al. 2013; Mora et al., 2013; Bergeron et al., 2012; Cortez et al., 2010; Vincent et al. 2010; Jakobsen et al. 2010a, 2010b; Johnson et al., 2006; Burman et al., 2003)
NOW: WHOLE GENOME SEQUENCING
>95% OF BACTERIAL GENOME
ASSEMBLE FRAGMENTS BY COMPUTER
PRODUCE CHROMOSOME MAP
PERFORM SNP AND PHYLOGENETIC ANALYSIS


Images from scienceprofile.com, Wikipedia, and Pacific Biosystems
HOW DO WE KILL THEM IN FOODS?

• Start with poultry meat.

• High pressure and irradiation for ground chicken.

• UV-C for chicken meat purge on food contact surfaces and chicken breast surfaces

• Multi-isolate cocktails (ATCC)

• 10⁸-10⁹ CFU/g, cm², or ml.

• UV-C intensity 2 mW/cm²/s, 5 °C
• Cs-137 gamma ca. 0.07 kGy/min, 5 and -20 °C.
• HPP from 200 to 500 MPa/time/5°C

• Recovered on E.coli petri films (ca. 24 h.)

• Calculate $D_{10s}$, reciprocal of slope for log reductions.

• Experiments conducted independently 3 times.
FOOD IRRADIATION

- Process of exposing food to a ionizing radiation field
- Sources: Cobalt-60, electron beam, x-rays
- Does not make the food radioactive
- Safe and effective
- Target is nucleic acids and proteins.
- Used to control insects (phytosanitary)
- Delay sprouting (garlic and onions)

- Kill bacterial foodborne pathogens:
  - Red meat and poultry
  - Spices
  - Mollusks (oysters) and crustaceans (shrimp)
  - Food for astronauts

- Approved by the World Health Organization
- Approved in over 60 countries
- Approved by FDA and USDA

- You eat irradiated foods every day.

Images from wikipedia
HIGH PRESSURE PROCESSING

• Pascalization, or high pressure processing (HPP), is a method of preserving and sterilizing food, in which a product is processed under very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food.

• 100 to 500 MPa.

• High pressure squeezes bacteria until they are crushed. Denatures proteins.

• Good for inactivation of viruses.

• Can shuck oysters and crustaceans

• Commercially used for guacamole, salsas, fruit juices, sliced luncheon meats, oysters, ground meat, etc.

• FDA & USDA approved

www.stanstedfluidpower.com
ULTRAVIOLET LIGHT

- Considered irradiation by FDA
- 254 nm UV-C (low pressure mercury lamps)
- Pulsed Light from xenon lamps
- Other technologies include excimer lamps (not in CFR).

- Fruit juices (water)
- Low fat foods (surface decontamination)
- Food contact surfaces
- Environmental decontamination

- Kills bacteria through formation of cyclobutane pyrimidine dimers,
  6-4 photoproducts, oxidation of proteins.

- Non-penetrating for solid foods.
- Can penetrate packaging depending on composition
Inactivation of Uropathogenic E.coli on chicken meat and chicken purge by nonthermal processing technologies. HPP 300 (●), 400 (○) and 500 (▼) MPa are shown as well as gamma radiation at 4 (●) and -20 (○) oC. For UV-C inactivation of UPEC on chicken breast meat (●), and chicken exudates on SS (○), HDPE (▼) and HDPP ( ). Each experiment was conducted independently three times (n=3).

# Uropathogenic E. Coli

<table>
<thead>
<tr>
<th>Technology</th>
<th>Parameter</th>
<th>D_{10} (SEM)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Pressure Processing</td>
<td>300 MPa</td>
<td>30.6 (±0.12) min</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>400 MPa</td>
<td>8.37 (±1.06) min</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>500 MPa</td>
<td>4.43 (±0.12) min</td>
<td>0.96</td>
</tr>
<tr>
<td>Gamma Radiation</td>
<td>4 ºC</td>
<td>0.28 (±0.01) kGy</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>-20 ºC</td>
<td>0.36 (±0.01) kGy</td>
<td>0.99</td>
</tr>
<tr>
<td>Ultraviolet Light (Chicken Purge)</td>
<td>Stainless Steel</td>
<td>9.16 (±2.54) mJ/cm²</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>HDPP</td>
<td>12.5 (±0.32) mJ/cm²</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>HDPE</td>
<td>13.8 (±0.73) mJ/cm²</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Inactivation of S. saprophyticus on chicken meat and chicken purge by nonthermal processing technologies. HPP 300 (●), 400 (○) and 500 (▼) MPa are shown as well as gamma radiation at 4 (●) and -20 (○) oC. For UV-C inactivation of UPEC on chicken breast meat (●), and chicken exudates on SS (○), HDPE (▼) and HDPP ( ). Each experiment was conducted independently three times (n=3).

## S. saprophyticus Table

<table>
<thead>
<tr>
<th>Technology</th>
<th>Parameter</th>
<th>$D_{10}$ (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Pressure</strong></td>
<td>200 MPa, 5 °C</td>
<td>15.5 (±0.65) min</td>
</tr>
<tr>
<td></td>
<td>300 MPa, 5 °C</td>
<td>9.43 (±0.22) min</td>
</tr>
<tr>
<td></td>
<td>400 MPa, 5 °C</td>
<td>3.54 (±0.21) min</td>
</tr>
<tr>
<td><strong>Gamma Radiation</strong></td>
<td>5 °C</td>
<td>0.64 (±0.01) kGy</td>
</tr>
<tr>
<td></td>
<td>-20 °C</td>
<td>0.77 (±0.01) kGy</td>
</tr>
<tr>
<td><strong>Ultraviolet Light on Coupons</strong></td>
<td>SS</td>
<td>18.5 (±1.27) mJ/cm²</td>
</tr>
<tr>
<td></td>
<td>HDPE</td>
<td>16.6 (± 1.54) mJ/cm²</td>
</tr>
<tr>
<td></td>
<td>HDPE</td>
<td>14.9 (±1.88) mJ/cm²</td>
</tr>
</tbody>
</table>
CONCLUSIONS

• ExPEC (UPEC) are present in foods, with poultry and poultry meat being the most significant reservoir.

• There is a link between consumption of meat and poultry contaminated with extraintestinal pathogens and illness in humans.

• Link was made possible through molecular characterization.

• There are multiple causes of urinary tract infections in humans:
  - nosocomial infections (hospitals, nursing homes)
  - sexually transmitted disease
  - familial and animal to human transmission

• What is the percentage associated with food consumption? 10%?

• What about *S. saprophyticus*? Present in many foods. What portion are human pathogens? Link between food and illness?
CONCLUSIONS

• Inactivation kinetics for ExPEC are similar to historical data for iPEC (HPP, irradiation, and UV-C).

• Inactivation kinetics for *S. saprophyticus* are similar to historical data for *S. aureus* (HPP, irradiation, and UV-C).

• ExPEC were more resistant to HPP than *S. saprophyticus*.

• *S. saprophyticus* was more resistant to gamma radiation and UV-C than ExPEC.

• Current experiments are examining thermal inactivation of ExPEC and *S. saprophyticus*.

• What about radiation resistance of ExPEC and *S. saprophyticus* in:
  - spices
  - leafy greens
  - seafood?