The Role of Extraintestinal Foodborne Pathogens in Human Illness: Or How WGS Technology is Changing our Views on Foodborne Illness

Christopher H. Sommers
Lead Scientist
USDA-ARS-NEA-ERRC
600 E. Mermaid Lane
Wyndmoor, PA 19038
Christopher.sommers@ars.usda.gov

#Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

CIRMS 2016

THANKS

- ARS Co-authors

Shiowshuh Sheen and Butch Scullen

- Edinboro Univ. of PA.

Dr. Bill Mackay

- USDA-ARS Office of National Programs

(Dr. James Lindsay and Dr. Pamela Starke-Reed)

- Pioneers in the field of ExPEC

James R. Johnson, MD (Veterans Administration, MN)
Lance Price (George Washington University)
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

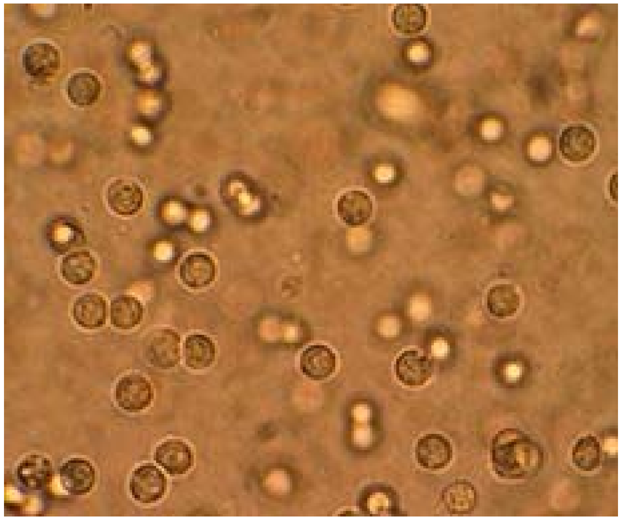
- Clostridium perfringens
- Clostridium botulinum
- Bacillus cereus

- Diarrhea, cramps, nausea, vomiting >>>>Sepsis, HUS, etc.

pixabay.com

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

•Nordstom, L., Liu, P., Price, L. 2013. Foodborne urinary tract infections: a new paradigm for antimicrobial-resistant
•foodborne illness. Front. Microbiol. 4: 1 - 6. http://www.frontiersin.org/Microbiology/editorialboard.

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?

Minardi, D., d'Anzeo, G., Cantoro, D., Conti, A., Muzzonigro, G. 2011. Urinary tract infections in women: etiology and treatment options. Int. J. Gen. Med. 4: 333-343.

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

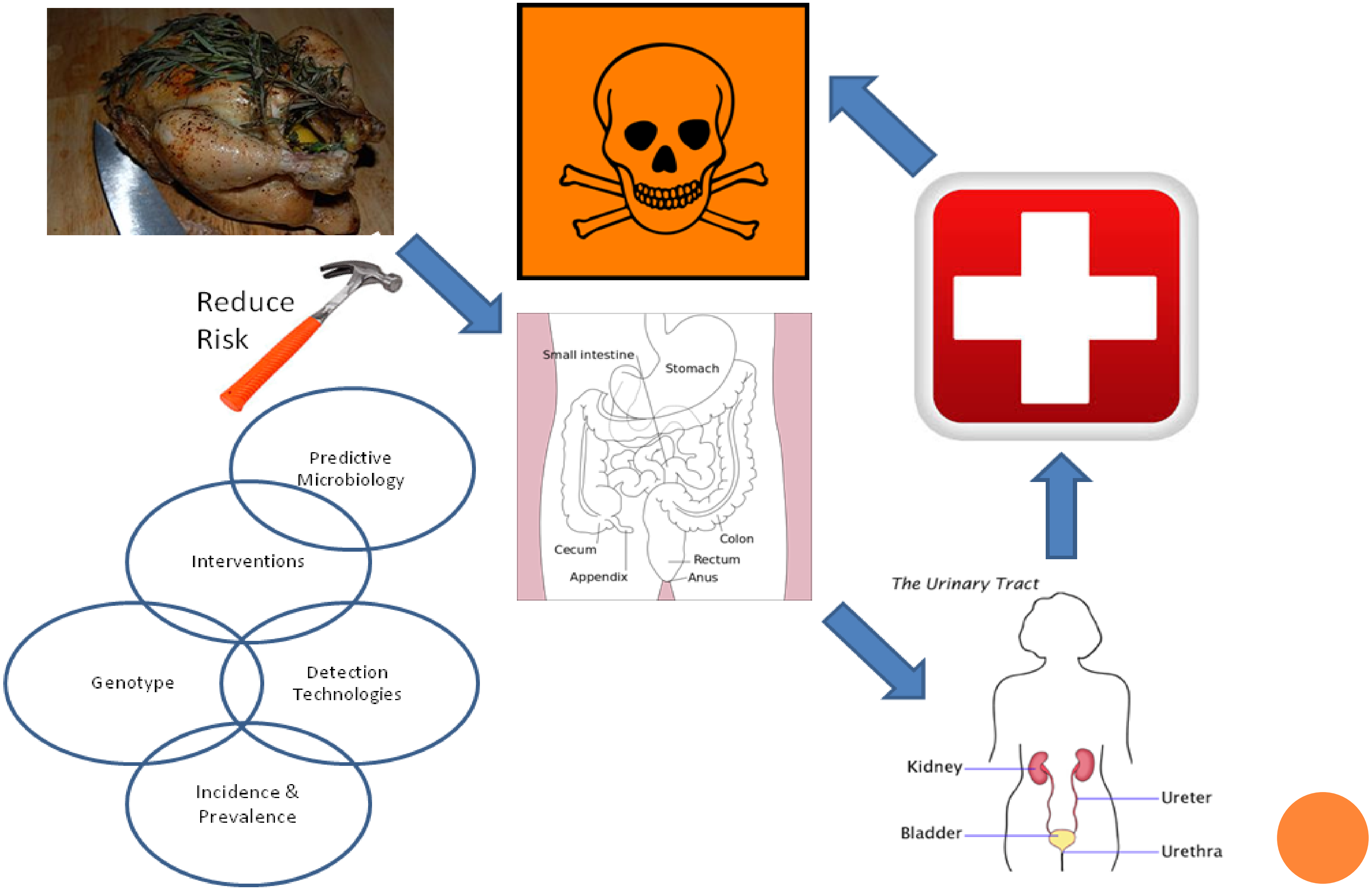
	Illnesses	Hospitalizations	Deaths
Diarrheal *E. coli* *(STEC, etc)*	ca. 306,000	ca. 3700	ca. 31
Uropathogenic	6 - 8 million	ca. 100,000	ca. 23,000
Meningococcal			ca. 500

Scallan et al. (2011)
Nordstom et al. (2013)

Commonalities between iPEC and ExPEC

	iPEC	ExPEC
Meat and Poultry	x	x
Produce	x	x
Seafood	x	x
Soil	x	x
Groundwater	x	x
Foodborne	x	x
Animal to Animal	x	x
Animal to Human	x	x
Human to Human	x	x

PATH TO ILLNESS (UPEC)



Incidence and Prevalence of ExPEC in Foods

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

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

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

Johnson, J., Kuskowski, M., Smith, K., O'Bryan, T., Tatini, S. 2005. Antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in retail foods. 2005. J. Infect. Dis. 191: 1040 - 1049.

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.

Manges, A., Smith, S., Lau, B., Nuval, C., Eisenberg, J., Dietrich, P., et al. 2007. Retail meat consumption and the antimicrobial resistant Escherichia coli causing urinary tract infections: a case control study. Foodborne Path. Dis. 4: 419-431.

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.

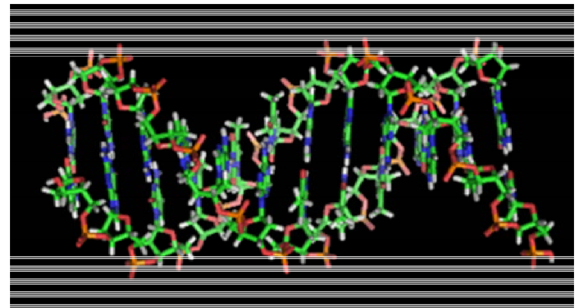
- Food isolates caused UTI in animal model systems.

- 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)

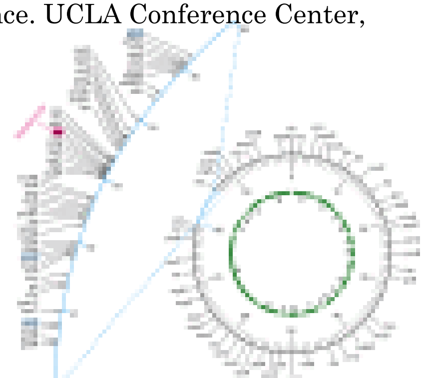
NOW: WHOLE GENOME SEQUENCING

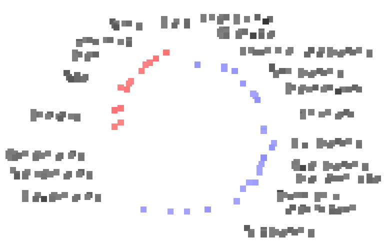
>95 % OF BACTERIAL GENOME
ASSEMBLE FRAGMENTS BY COMPUTER
PRODUCE CHROMOSOME MAP
PERFORM SNP AND PHYLOGENETIC ANALYSIS



Davis, G., Waits, K., Nordstrom, L., Weaver, B., Aziz, M., Gauld, M., Grande,H., Bigler,,R., Horwinski, J., Porter, S., Stegger, M., Johnson, J., Liu, C., Price, L. 2015. Intermingled Klebsiella pneumoniae populations between retail meats and human urinary tract infections. Clin. Infect. Dis. p. 1 - 8. DOI: 10.1093/cid/civ428.

Price, L. 2014. Foodborne urinary tract infection studies. 20th International Microbial Genomes Conference. UCLA Conference Center, lake Arrowhead, CA. p 16.





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)
- 10^8-10^9 CFU/g, cm^2, or ml.
- UV-C intensity 2 mW/cm^2/s, 5 ^oC
- Cs-137 gamma ca. 0.07 kGy/min, 5 and -20 ^oC.
- HPP from 200 to 500 MPa/time/5^oC
- Recovered on E.coli petri films (ca. 24 h.)
- Calculate D_{10}s, 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 ar 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

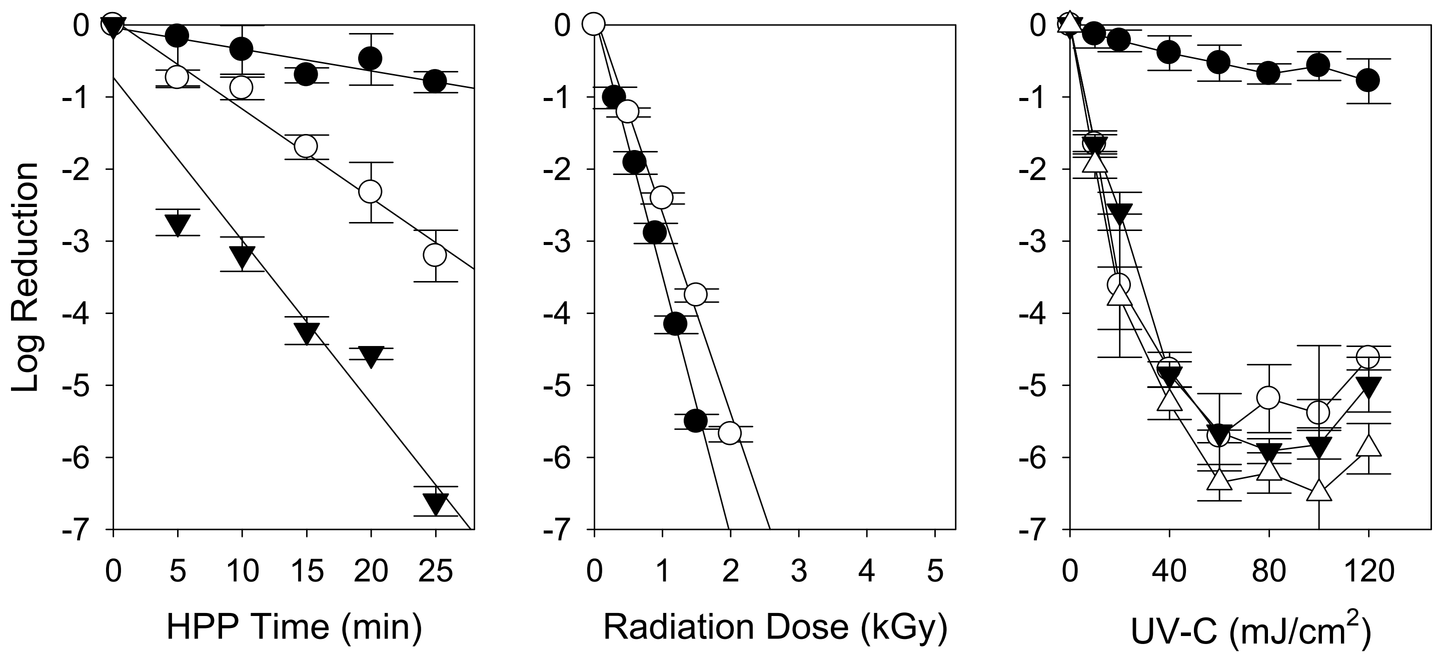
- Considered irradiation by FDA
- 254 nm UV-C (low pressure mercury lamps)
- Pulsed Light from xenon lamps
- Other technologies include excimer lamps (not in CFR).

- 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

Uropathogenic E.coli



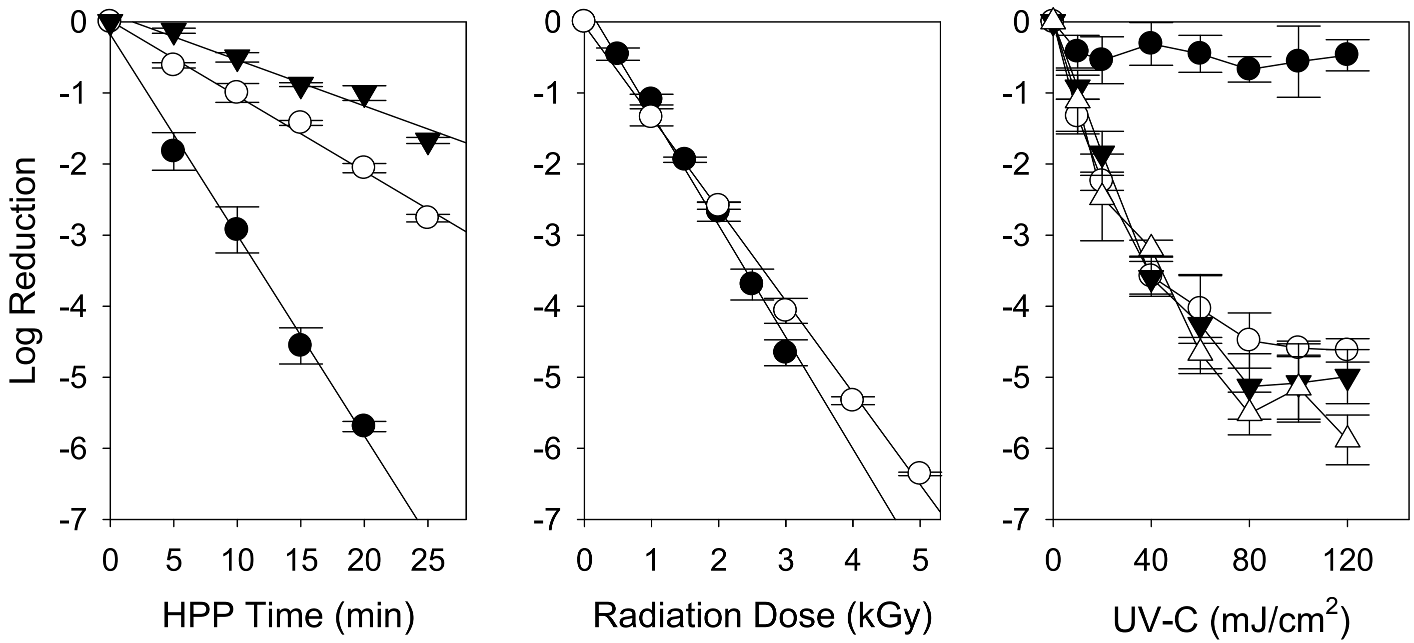
Inactivation of Uropathogenic E.coli on chicken meat and chicken purge by nonthermal processing technologies. HPP 300 (●), 400 (○) and 500 (▼) MPa are shown as well as gamma radiation at 4 (●) and -20 (○) oC. For UV-C inactivation of UPEC on chicken breast meat (●), and chicken exudates on SS (○), HDPE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

Front. Microbiol., 14 April 2016 | http://dx.doi.org/10.3389/fmicb.2016.00413

Uropathogenic E.coli

Technology	Parameter	D_{10} (SEM)	R^2
High Pressure Processing	300 MPa	30.6 (±0.12) min	0.99
	400 MPa	8.37 (±1.06) min	0.98
	500 MPa	4.43 (±0.12) min	0.96
Gamma Radiation	4 ^{o}C	0.28 (±0.01) kGy	0.99
	-20 ^{o}C	0.36 (±0.01) kGy	0.99
Ultraviolet Light (Chicken Purge)	Stainless Steel	9.16 (±2.54) mJ/cm^2	0.98
	HDPP	12.5 (±0.32) mJ/cm^2	0.99
	HDPE	13. 8 (±0.73) mJ/cm^2	0.99

S. SAPROPHYTICUS



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).

In Review: Front. Microbiol.

S. saprophyticus Table

Technology	Parameter	D_{10} (SEM)
High Pressure	200 MPa, 5 ^{o}C	15.5(±0.65) min
	300 MPa, 5 ^{o}C	9.43 (±0.22) min
	400 MPa, 5 ^{o}C	3.54 (±0.21) min
Gamma Radiation	5 ^{o}C	0.64 (±0.01) kGy
	-20 ^{o}C	0.77 (±0.01) kGy
Ultraviolet Light on Coupons	SS	18.5 (±1.27) mJ/cm^2
	HDPE	16.6 (± 1.54) mJ/cm^2
	HDPE	14.9 (±1.88) mJ/cm^2

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?