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Inactivation of extraintestinal pathogenic *E. coli* clinical and food isolates suspended in ground chicken meat by gamma radiation☆

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* are common contaminants in retail poultry and involved inflammatory bowel disease, urinary tract infections and meningitis in both animals and humans. They cause significantly more illnesses and deaths in humans than Shiga toxin-producing *E. coli* (STEC). Ionizing radiation is used commercially for improving the safety and shelf-life of foods. In this study we inoculated ground chicken meat with 25 individual isolates of clinical uropathogenic *E. coli* (UPEC) and newborn meningitis causing *E. coli* (NMEC), isolates from retail chicken meat (CM), as well as retail chicken-skin isolates identified in our laboratory (CS). We then determined their gamma radiation inactivation kinetics (D_{10}-value). The mean D_{10}-value for all isolates (n = 25) was 0.30 kGy. The mean D_{10}-value for the UPEC, NMEC, CM, and CS isolates were 0.25, 0.29, 0.29, and 0.39 kGy, respectively. The mean D_{10}-value for the clinical isolates was 0.27 kGy vs. 0.34 kGy for the non-clinical isolates. There was no correlation between presence of virulence factors, antibiotic resistance, and radiation resistance. ExPEC were similar to that of STEC which were previously evaluated in our laboratory. The radiation doses needed to kill STEC poultry meat should also kill ExPEC.

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are foodborne pathogens associated with diseases in humans and animals including sepsis, inflammatory bowel disease (IBD), urinary tract infections (UTI), newborn meningitis and avian colibacillosis (Dowling, 2012; Liu et al., 2018; Mirsepasi-Lauridsen et al., 2016; Yamaji et al., 2018). ExPEC types include Uropathogenic *E. coli* (UPEC), Sepsis-associated *E. coli* (SEPEC), Neonatal Meningitis *E. coli* (NMEC) and Avian Pathogenic *E. coli* (APEC) (Mitchell et al., 2015). UPEC cause ca. 7 million UTI annually in the US at a cost of $11.6 billion (Yamaji et al., 2018), sepsis is costs ca. $23 billion annually (Hajj et al., 2018) and IBD affects more than 3 million each year with direct and people indirect costs of $6.3 and $5.5 billion annually (CDC, 2010; Dahlhamer et al., 2016). NMEC is a leading cause of neonatal bacterial meningitis (Wijetunge et al., 2015). APEC are responsible for a myriad of diseases in poultry including kidney infections, sepsis, and meningitis (Hyline, 2015) and may be a conduit for bacterial-associated antibiotic resistance and virulence factors from poultry to humans (Mellata, 2013). Poultry meat is a significant reservoir for foodborne ExPEC (Jakobsen et al., 2012; Johnson et al., 2006, 2005; Liu et al., 2018; Mitchell et al., 2015; Yamaji et al., 2018). ExPEC were isolated directly from food cause disease in animal model systems (Jakobsen et al., 2012; Stromberg et al., 2017; Vincent et al., 2010).

ExPEC were originally defined as containing two or more of five VF including *papA/C* (P-fimbiae), afa/dra (Dr binding adhesins), *sfa/foc* (S/F1C-fimbraie) *kpsMII* (K-capsular antigen), or *iutA* (aerobactin receptor-iron metabolism) (Russo and Johnson, 2000). While STEC tend to cluster in phylogroup B_1, ExPEC tend to cluster in phylogroups B_2 and D, although then are also found in phylogroups A and B_1 (Smith et al., 2007). Ca. 40 ExPEC VF are classified by function including adhesins, iron acquisition, protectins and invasins, toxins, and other miscellaneous factors (Dale and Woodford, 2015).

Gamma radiation, is a US Food and Drug Administration approved commercial process used to improve the safety and shelf-life of food including meat and poultry (FDA, 2007; Salvage, 2014). Irradiation kills bacteria by damage to its genetic material and oxidation of proteins and other cellular structures, and the effect of core genome genetic factors on radiation resistance has been studied extensively (Janion, 2008; Krisko and Radman, 2010). Virulence of ExPEC including

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survival in urine and invasion of urinary tract epithelial cells requires a functional RecA-mediated recombination system and active SOS response, often hyper-recombinogenic (Byrne et al., 2014; Harris et al., 2009; Li et al., 2010; Rodríguez-Beltrán et al., 2015).

The CDC recommends food treated with intervention technologies such as irradiation for at risk populations with preexisting medical conditions (CDC, 2013). In this study we had two objectives: 1) to determine the radiation resistance (D_{10}-value) of ExPEC; and 2) to examine the relationship between virulence factors and antimicrobial resistance and radiation resistance.

2. Materials and methods

2.1. Chicken

Ground chicken (95% lean) was purchased from a local wholesaler. It was divided (90 g aliquots) into polynylon pouches (Uline, Inc., Philadelphia, PA). The pouches were then vacuum-sealed (50 mB) a Multi-Vac A300 packager (Multi-Vac Inc., Kansas City, MO) and frozen (−20 °C). The chicken was then irradiated (−20 °C) to 25 kGy for inactivation of background microflora, which was undetectable per the recovery protocol below (Gunther et al., 2019).

2.2. ExPEC

ExPEC (human clinical UPEC and NMEC isolates; food-source ExPEC isolates) were used to determine radiation resistance. UPEC clinical isolates 700928, 700336, BAA-1161, 700414, 700415, 700416, and 700417 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) (Xu et al., 2018a). NMEC clinical isolates SP-4, SP-5, SP-13, SP-16, SP-46, and SP-65 were obtained from the Netherlands Reference Laboratory for Bacterial Meningitis (Amsterdam, Netherlands) (Xu et al., 2018c). ExPEC isolates from retail chicken meat included DP254, WH333, WH398, F356, FEX675, and FEX725 which were obtained from the Department of Veterans Affairs, the FDA National Antimicrobial Monitoring System, and the University of Minnesota (Xu et al., 2018b). Isolates from retail chicken skin were isolated in our laboratory (Xu et al., 2018d, 2019a).

2.3. ExPEC growth and inoculation

ExPEC isolates were cultured independently in 20 ml Tryptic Soy Broth (TSB) without dextrose (BD-Difco, Sparks, MD) using 50 ml sterile tubes at 37 °C (150 rpm) for 18–24 h using a New Brunswick Model G34 Environmental Shaker (New Brunswick, Edison, NJ). The bacteria were then sedimented by centrifugation (1200 × *g*, Hermle Model Z206A, Hermle Labortechnik, Germany) and resuspended in 20 ml sterile 0.1% peptone water (SPW, BD-Difco).

Thawed ground chicken (90 g) was aliquoted into Nasco (Ft. Atkinson, WI) Whirl-Pak bags, inoculated with 10 ml of ExPEC, mixed manually for 1 min, and then sealed using the Multi-Vac A300 Packager. The final concentration of STEC in the ground beef was ca. 8 log CFU/g. The sample bags were then sealed in a second bag and stored at 4 °C until irradiation.

2.4. Gamma radiation

Our Lockheed Georgia Company (Marietta, GA, USA) ^{137}Cs irradiator, with a dose rate of 0.061 kGy/min was used for all exposures. Inoculated samples were placed vertically and centrally in the chamber. The temperature during irradiation was maintained 4 °C by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber which was monitored by thermocouple. Irradiation was performed at 0.3 kGy increments (0–1.5 kGy). The absorbed dose was verified using Bruker eScan EPR Analyzer alanine dosimeter system (Bruker, Billerica, MA). The dose uniformity ratio was less than 1.1 to 1.0.

2.5. Recovery of the surviving ExPEC

The individual chicken samples were added to 90 ml of 0.1% PW and then stomached for 2 min (Seward, Sussex, UK). Then 1.0 ml aliquots, after decimal dilution, was pipeted onto duplicate Aerobic Plate Count Petrifilms™ (3M Microbiology Products Co., St. Paul, MN). The Petrifilms were incubated for ca. 48 h at 37 °C and CFU determined with a calibrated 3M Petrifilm™ plate reader.

2.6. Statistical analysis

The mean plate counts of the treated samples (N) were divided by the control plate counts (No) and those log reductions were used for determination of D_{10}-value, e.g. reciprocal of the slope (Sommers et al., 2015). Each experiment (D_{10}-value determination) was conducted independently three times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations (D_{10}-value determination), descriptive statistics, analysis of variance (ANOVA, 95% confidence). D_{10}-values were grouped by AR/VF presence or absence and then analyzed using the Kruskal-Wallis nonparametric test, or student *t*-test MATLAB (METLAB, The MathWorks Inc., Natick, MA, ver. 2015b).

3. Results and discussion

Our previous research with these ExPEC suspended in GCM demonstrated a correlation between ExPEC VF and thermal (heat) resistance, including *fdeC*, *sinH*, *cnf1*, *gad*, *ompT, iha, fimH* and *sat* (Xu et al., 2019b). Twenty-five ExPEC (UPEC, NMEC, FS, CS) including 13 serovars, 18 Sequence Types (ST), 10 antibiotic resistances (including multidrug extended beta-lactam resistant) were inoculated into GCM and their D_{10}-value determined (Xu et al., 2018a-d, 2019a). Graphic representation of radiation resistance is shown in Fig. 1. The D_{10}, serovar, and ST information are shown in Table 1. There was no difference in D_{10} based on VF/AR as determined by Kruskal-Wallis nonparametric test or student *t*-test (Xu et al., 2019b). A Table of VF for the isolates may be obtained by emailing our laboratory. Our research on radiation resistance of STEC indicated the Leukocyte Effacing-Pathogenicity Island might lower D_{10}-value (Sommers et al., 2015).

The mean D_{10}-value for all ExPEC isolates (n = 25) was 0.30 (± 11) kGy with a range of 0.18–0.68 kGy. The mean D_{10}-value for the UPEC, NMEC, CM, and CS isolates were 0.25 (± 0.05), 0.29 (± 0.05), 0.29 (± 0.16), and 0.39 (± 0.17) kGy, respectively, with CS > CM = NMEC > UPEC ($p < 0.05$). The mean D_{10}-value for the clinical isolates was 0.27 kGy vs. 0.34 kGy for the non-clinical isolates ($p < 0.05$). As expected, the ExPEC D_{10}-values were greater than those of *recA* mutants, which are typically < than 0.05 kGy (Sommers and Rajkowski, 2008).

Sommers et al. (2016) found the D_{10}-value of a multiisolate cocktail of UPEC suspended in ground chicken meat to be 0.27 kGy. Sommers et al. (2015) found the D_{10}-value for 40 individual STEC ranged from 0.16 to 0.48 kGy with a mean D_{10}-value of 0.31 kGy. In that study nonclinical isolates (0.36 kGy), on average, were more radiation resistant than the clinical isolates (0.27 kGy). The reason for the difference between clinical and nonclinical isolates is currently unknown. Thayer et al. (1995) found the D_{10}-value of *E. coli* O157:H7 multiisolate cocktail suspended in low-fat ground turkey breast meat, turkey leg meat, beef, pork and lamb to be 0.30, 0.29, 0.30, 0.30, 0.32 kGy, respectively. López-González et al. (1999) found the D_{10}-value of *E. coli* O157:H7 suspended in ground beef to be 0.27 kGy. Black and Jaczynski (2006) obtained D_{10}-values of 0.22–0.33 kGy for *E. coli* O157:H7 suspended in chicken, beef, and trout fillet meat. Turgis et al. (2008) found the D_{10}-value of *E. coli* O157:H7 suspended in ground beef to be 0.21 kGy. Our results for ExPEC are consistent with those obtained for

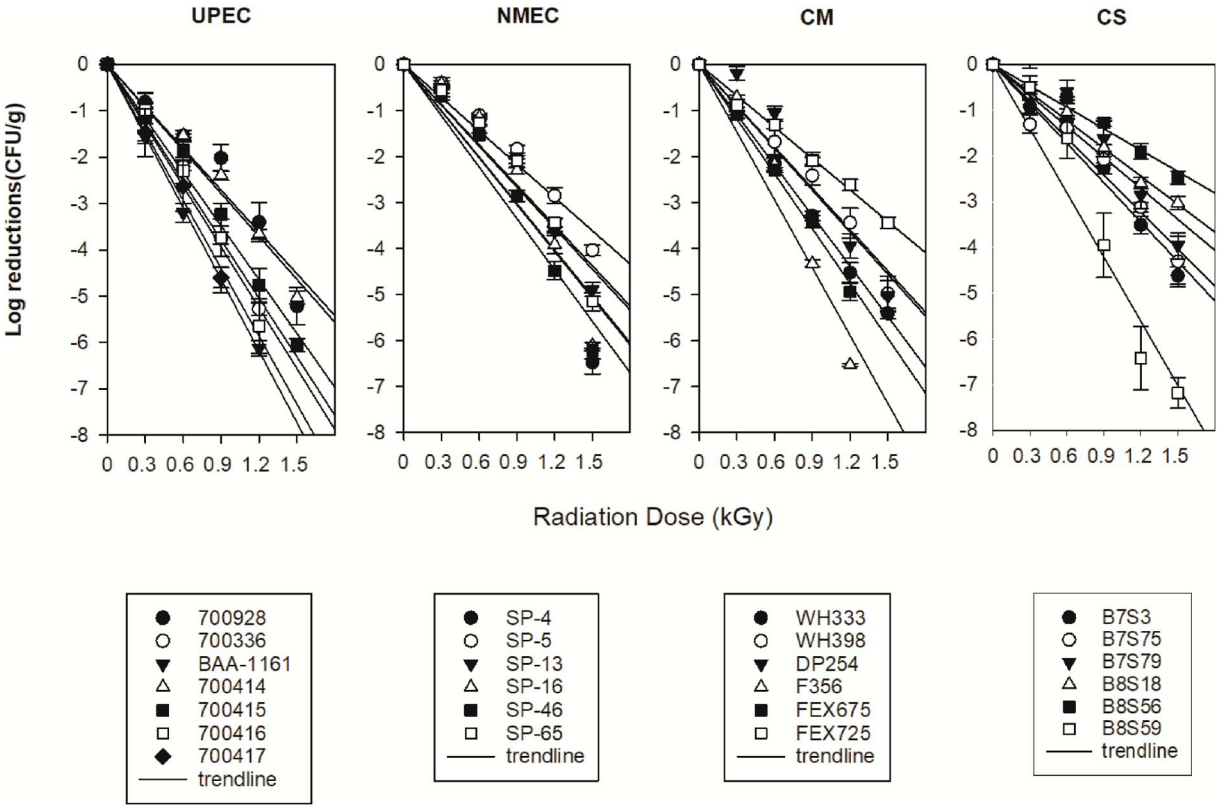

Fig. 1. Irradiation of Extraintestinal Pathogenic *Escherichia coli* including UPEC, NMEC, isolates from retail chicken meat (CM), and isolates from retail chicken skin (CS). Each experiment was conducted independently three times (n = 3). Error bars: standard error of the mean for the log reductions. Solid lines: linear regression curves.

Table 1
Serotype, Sequence Type (ST) and Radiation resistance of ExPEC isolates suspended in ground chicken meat. Uropathogenic *E. coli* (UPEC), Newborn Meningitis-associated *E.coli* (NMEC), isolates from retail chicken meat (CM), and isolates from retail chicken skin (CS). D_{10}-value (kGy) is the mean of three independent experiments (n = 3). Standard Error of the Mean (SEM) is shown in parenthesis.

Group	Isolate	Serotype	ST	D_{10} kGy (SEM)
UPEC	700336	O4:K6	12	0.23 (± 0.01)
	BAA-1161	O17:H18	597	0.20 (± 0.01)
	700414	O6:H31	127	0.31 (± 0.01)
	700415	O4:H5	12	0.25 (± 0.01)
	700416	O4:H5	599	0.22 (± 0.02)
	700417	O4:H5	12	0.20 (± 0.02)
	700998	O6:H1	73	0.31 (± 0.03)
NMEC	SP-4	O18:K1	390	0.25 (± 0.01)
	SP-5	O7:K1	62	0.37 (± 0.01)
	SP-13	O18:K1	416	0.30 (± 0.01)
	SP-16	O83:K1	Unknown	0.25 (± 0.01)
	SP-46	O7:K1	62	0.24 (± 0.01)
	SP-65	O83:K1	567	0.30 (± 0.01)
CM	WH333	O120: H4	428	0.27 (± 0.01)
	WH398	O24: H4	117	0.32 (± 0.03)
	DP254	O1: H7	95	0.28 (± 0.01)
	F356	O2: H6	141	0.18 (± 0.01)
	FEX675	O120: H4	428	0.25 (± 0.01)
	FEX725	O1:H45	2171	0.45 (± 0.18)
CS	B7S3	102:H6	115	0.36 (± 0.02)
	B8S56	102:H6	7284	0.68 (± 0.05)
	B8S18	102:H6	770	0.48 (± 0.02)
	B8S59	O11:H25	1674	0.20 (± 0.01)
	B7S75	O25:H4	131	0.27 (± 0.01)
	B7S79	O24:H4	117	0.36 (± 0.02)

STEC over the last 20 years. The radiation resistance of the ExPEC were less than that for *Salmonella* spp. when compared to historical data obtained in our laboratory (Thayer et al., 1995).

4. Conclusions

Unlike STEC, we found no correlation between VF and radiation resistance. The USFDA currently allows refrigerated red meat and poultry meat to be treated with radiation doses up to 4.5 kGy (FDA, 2007). Radiation doses lower than 4.5 kGy would be required for ExPEC elimination. Poultry meat treated with intervention technologies such as irradiation may lessen the risk of IBD, sepsis, or UTI in people with pre-existing medical conditions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103264.

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