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Characterization of Extraintestinal Pathogenic *Escherichia coli* isolated from retail poultry meats from Alberta, Canada

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ABSTRACT

Extraintestinal Pathogenic *Escherichia coli* (ExPEC) have the potential to spread through fecal waste resulting in the contamination of both farm workers and retail poultry meat in the processing plants or environment. The objective of this study was to characterize ExPEC from retail poultry meats purchased from Alberta, Canada and to compare them with 12 human ExPEC representatives from major ExPEC lineages. Fifty-four virulence genes were screened by a set of multiplex PCRs in 700 *E. coli* from retail poultry meat samples. ExPEC was defined as the detection of at least two of the following virulence genes: *papA*/*papC*, *sfa*, *kpsMT II* and *iutA*. Genetic relationships between isolates were determined using pulsed field gel electrophoresis (PFGE). Fifty-nine (8.4%) of the 700 poultry meat isolates were identified as ExPEC and were equally distributed among the phylogenetic groups A, B1, B2 and D. Isolates of phylogenetic group A possessed up to 12 virulence genes compared to 24 and 18 genes in phylogenetic groups B2 and D, respectively. *E. coli* identified as ExPEC and recovered from poultry harbored as many virulence genes as those of human isolates. In addition to the *iutA* gene, siderophore-related *iroN* and *fyuA* were detected in combination with other virulence genes including those genes encoding for adhesion, protectin and toxin while the *fimH*, *ompT*, *traT*, *uidA* and *vat* were commonly detected in poultry ExPEC. The *hemF*, *iss* and *cvaC* genes were found in 40% of poultry ExPEC. All human ExPEC isolates harbored *concnf* (cytotoxic necrotizing factor 1 altering cytoskeleton and causing necrosis) and *hlyD* (hemolysin transport) genes which were not found in poultry ExPEC. PFGE analysis showed that a few poultry ExPEC isolates clustered with human ExPEC isolates at 55–70% similarity level. Comparing ExPEC isolated from retail poultry meats provides insight into their virulence potential and suggests that poultry associated ExPEC may be important for retail meat safety. Investigations into the ability of our poultry ExPEC to cause human infections are warranted.

1. Introduction

Escherichia coli is a commensal bacterium generally found in the gastrointestinal tracts of humans and animals. Some strains can cause nosocomial and community-acquired infections including urinary tract, enteric and systemic post-surgical infections based on their virulence gene content (Pitout, 2012). Of particular concern are Extraintestinal Pathogenic *E. coli* (ExPEC). ExPEC are associated with human and animal infections that occur outside of the intestinal tract such as urinary tract and bloodstream infections. ExPEC ability to develop resistance to various antimicrobial agents contributes to an increase in human health risk and greater health care costs.

ExPEC isolated from avian and human sources contain overlapping features such as virulence factors and phylogenetic groupings (A, B1, B2, and D) suggesting the zoonotic pathogenic potential of avian ExPECs (Giufrè et al., 2012). However, zoonotic potential of these ExPEC strains is still controversial (Bélanger et al., 2011). The Avian Pathogenic *E. coli* (APEC) strains belonging to the ExPEC groups and causing colibacillosis in birds are reported to carry similar virulence attributes as human ExPEC. This suggests a possible route of ExPEC dissemination in the community through poultry and poultry products that may serve as a potential reservoir of ExPEC (Bergeron et al., 2012; Johnson et al., 2005b; Literak et al., 2013; Moulin-Schouleur et al., 2006, 2007; Tóth et al., 2012).

A number of reports have suggested a higher prevalence of ExPEC on retail chicken, beef and pork meat, however, the recovery of ExPEC has been greatest from chicken meat (Jakobsen et al., 2010; Johnson et al., 2005a, 2005b). These studies suggest that poultry meat could play a

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role in human infections (Johnson et al., 2005a, 2005c, 2009, 2003; Manges et al., 2007).

A detailed comparison of ExPEC strains isolated from poultry is needed to assess possible transmission (Jakobsen et al., 2010). Previously we have demonstrated that chicken is a reservoir for ExPEC carrying virulence and antibiotic resistance genes of animal and human importance (Bonnet et al., 2009; Lefebvre et al., 2009). These bacteria have the potential to spread through fecal waste potentially contaminating both farm workers and poultry carcasses in the processing plants or environment.

Obviously, monitoring of verotoxin producing *E. coli* is crucial for the meat industry but attention should also be paid to ExPEC, which cause serious extraintestinal infections in humans. In this study we analyzed and compared the phylogenetic grouping and the prevalence of ExPEC associated virulence genes in *E. coli* isolates recovered from retail poultry meats (turkey and chicken) and those from humans by using a set of multiplex PCR assays, serotyping and Pulsed Field Gel Electrophoresis (PFGE). The specific objective of the present study was to provide insight into the properties of ExPEC isolates recovered from retail poultry meats in order to understand their zoonotic potential. Detailed genomic analysis of ExPEC isolates of all possible sources including human and meat would help understanding the ecology of these pathogens.

2. Material and methods

2.1. Bacterial strains

Ten *E. coli* strains (J96/JJ079, 2H25/BUTI3-1-4, L31/Low31, V27/BUTI 1-5-1, PM9/BUTI 1-7-6, 2H15?16?/BUTI 3-1-2, 31A/JJ1166, 1A/JJ1167, 536/JJ425 and JJ055) kindly provided by Dr. James R. Johnson (Department of Medicine, University of Minnesota, USA) were used as controls for the detection of virulence genes. Three APEC strains (D0602195 Barn 3 Pool, D0602195 Barn 4, and 0606519) provided by M. Ngeleka (University of Saskatchewan, SK, Canada) as well as the non-pathogenic *E. coli* K12 were included (Forgetta et al., 2012). Twelve *E. coli* isolates from human infections (two strains from stool: GMS002A and GMS009B, two from blood: S20323 and H15 and eight from UTI: UTI PI 141 and UTI PI 500, MSHS 95, MSHS 161, MSHS 258, MSHS 472, MSHS 769 and MSHS 1014A) previously described by Bergeron et al. (2012) were used for comparisons.

2.2. Sampling and E. coli isolation

The sampling plan used by Canadian Integrated Program for Antimicrobial Resistance Surveillance was followed which involved continuous weekly sampling from randomly selected census divisions, weighted by population (Sheikh et al., 2012). A total of 297 retail poultry (206 chickens and 91 turkeys) samples were purchased and 700 *E. coli* isolates recovered from retail chicken (502 isolates) and turkey (198 isolates) meats were characterized. After initial processing, samples were transferred to double strength EC broth and incubated at 44 °C for 24 h. Then a loop full from EC broth was inoculated onto the MacConkey agar plate followed by incubation at 35 °C. Typical lactose fermenting, pink *E. coli* colonies (up to n = 3) were selected from each sample for further analysis. *E. coli* were confirmed using standard biochemical methods and by PCR (Aslam et al., 2004).

2.3. Analysis of virulence genes and phylogenetic groups

Confirmed *E. coli* isolates were tested for the presence of 54 virulence genes using specific primer sets (Life Technologies Inc., Burlington, ON) in multiplex PCRs as previously described (Johnson and Stell, 2000). Classification of isolates as ExPEC was based on the presence of two or more of the following virulence genes: *pap* (P fimbriae), *sfa* or *foc* (S/F1C fimbriae), *afa* or *dra* (binding, adhesions), *iutA* (aerobactin receptor), and *kpsM II* (group II capsule synthesis) as described (Johnson et al., 2003, 2005a, 2008, 2009). The ExPEC strains were assigned to one of the four designated phylogenetic group (A, B1, B2, or D) based on the pattern of *chuA*, *yjaA*, and TSPE4.C2 genes presence as described by Clermont et al. (2000).

2.4. Serotyping and pulsed-field gel electrophoresis

All isolates classified as ExPEC were serotyped at the Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada. Identification of somatic (O) and flagellar (H) antigens were performed by standard agglutination methods that identified O1 to O173 and H1 to H56 by following the procedures described previously (Ewing, 1986). Confirmed ExPEC isolates were subtyped to assess their genetic relatedness using PFGE technique by following the standard protocols as described by Ribot et al. (2006). Briefly, agarose plugs were prepared with *E. coli* cell suspension and lysed with proteinase K. Genomic DNA in gel matrix was digested using *Xba*I restriction enzyme and DNA fragments were separated on 1.5% agarose gel by following the conditions as described by Ribot et al. (2006).

2.5. Statistical analyses

All data were entered into Excel spreadsheets and frequencies of genes, serotypes and ExPEC were calculated. Statistical analysis of the data was performed using SAS software 9.2 (SAS Institute, Inc., Cary, NC). The association test of Cochran–Mantel–Haenszel was used to determine the relationship between meat types and genotype using the FREQ procedures as well as associations between ExPEC pathotype and genotype (Bonnet et al., 2009). A P value of 0.05 was used to declare significance.

3. Results

3.1. ExPEC prevalence

Overall 8.4% of the isolates (59 isolates) from retail poultry including chicken and turkey were classified as ExPEC based on the criteria established by Johnson et al. (2003). Among the detected ExPEC, 8.7% (44/502) of the isolates were from retail chicken meat and 7.5% (15/198) of isolates were from retail turkey meat (data not shown). Fifty-five (93.2%), three (5.1%) and one (1.7%) of the 59 defined ExPEC isolates harbored two, three, and four of the ExPEC-defining markers, respectively. The remaining 641 of the 700 (91.6%) *E. coli* isolates were defined as non-ExPEC. However, 236 of these 641 (36.8%) isolates harbored one of the ExPEC-defining markers, and 405 out of 641 (63.8%) isolates harbored none. The 236 isolates harboring one ExPEC-defining marker may be other ExPEC such as avian pathogenic *E. coli*. This study focused only on the 59 isolates classified as ExPEC based on the two gene criterion.

3.2. Serotypes and phylogenetic groups of ExPEC

One chicken meat and one turkey meat isolate could not be serotyped. Among the remaining 57 ExPEC isolates, 28 different serotypes were identified with 18 and 9 serotypes being found in chicken and turkey meats, respectively. Statistically significant ($P < 0.05$) association between a serotype and a source (chicken or turkey meats) of ExPEC was observed. In chicken ExPEC isolates, the serotypes O71:H10 (8 isolates), O6:H16 (7 isolates), O7:H18 (4 isolates), O22:H2 (3 isolates), O11:H25 (3 isolates) and O2:H42 (3 isolates) were common (Table 1). None of these serotypes were found in turkey isolates where serotype O?:H7 was more common (Table 2). The serotypes O21:H16, O25:H4, O2:H1 and O2:H6 were found in both chicken (Table 1) and turkey (Table 2). Serotype O25:H4 was found in chicken, turkey and human isolates (Tables 1–3).