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Extraintestinal pathogenic *Escherichia coli* in poultry meat products on the Finnish retail market

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Abstract

Background: Extraintestinal pathogenic *Escherichia coli* bacteria (ExPEC) exist as commensals in the human intestines and can infect extraintestinal sites and cause septicemia. The transfer of ExPEC from poultry to humans and the role of poultry meat as a source of ExPEC in human disease have been discussed previously. The aim of the present study was to provide insight into the properties of ExPEC in poultry meat products on the Finnish retail market with special attention to their prevalence, virulence and phylogenetic profiles. Furthermore, the isolates were screened for possible ESBL producers and their resistance to nalidixic acid and ciprofloxacin was tested.

Methods: The presence of ExPEC in 219 marinated and non-marinated raw poultry meat products from retail shops has been analyzed. One *E. coli* strain per product was analyzed further for phylogenetic groups and possession of ten virulence genes associated with ExPEC bacteria (*kpsMT K1*, *ibeA*, *astA*, *iss*, *irp2*, *papC*, *iucD*, *tsh*, *vat* and *cva/cv*) using PCR methods. The *E. coli* strains were also screened phenotypically for the production of extended-spectrum β -lactamase (ESBL) and the susceptibility of 48 potential ExPEC isolates for nalidixic acid and ciprofloxacin was tested.

Results: *E. coli* was isolated from 207 (94.5%) of 219 poultry meat products. The most common phylogenetic groups were D (50.7%), A (37.7%), and B2 (7.7%). Based on virulence factor gene PCR, 23.2% of the strains were classified as ExPEC. Two ExPEC strains (1%) belonged to [O1] B2 *svg+* (specific for virulent subgroup) group, which has been implicated in multiple forms of ExPEC disease. None of the ExPEC strains was resistant to ciprofloxacin or cephalosporins. One isolate (2.1%) showed resistance to nalidixic acid.

Conclusions: Potential ExPEC bacteria were found in 22% of marinated and non-marinated poultry meat products on the Finnish retail market and 0.9% were contaminated with *E. coli* [O1] B2 *svg+* group. Marinades did not have an effect on the survival of ExPEC as strains from marinated and non-marinated meat products were equally often classified as ExPEC. Poultry meat products on the Finnish retail market may have zoonotic potential.

Keywords: Extraintestinal pathogenic *Escherichia coli*, ExPEC, Poultry meat, Retail market, Antibiotic resistance

Introduction

Escherichia coli strains can be classified into three major groups: commensal strains, intestinal pathogenic strains, and extraintestinal pathogenic *E. coli* (ExPEC) strains [1]. ExPEC strains derive predominantly from *E. coli* phylogenetic group B2, and to a lesser extent D, and were defined by Johnson et al. [2] as *E. coli* isolates containing two or more

of the following virulence markers: *papA* (P fimbriae structural subunit) and/or *papC* (P fimbriae assembly), *sfa/foc* (S and F1C fimbriae subunits), *afa/dr* (Dr-antigen-binding adhesins), *kpsMT II* (group 2 capsular polysaccharide units), and *iutA* (aerobactin receptor). ExPEC exist as commensals in the human intestines and can infect extraintestinal sites, such as the urinary tract, the meninges, the peritoneal cavity or lungs, and can also cause septicemia [3, 4]. The transfer of ExPEC from poultry to humans and the role of poultry meat as a source of ExPEC human disease have been discussed previously. Studies by Johnson et al. [2, 5-7]

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and Manges et al. [3] indicated that retail meat products are frequently contaminated with *E. coli*, and that poultry and pork meat may be a potential source of ExPEC strains.

There is a wide variety of poultry products available on the Finnish retail market, including fresh and modified-atmosphere-packaged (MAP) products with or without spices. Most of these products, approximately 80%, are sold marinated with salt and water- and/or oil-based, spiced sauces [8]. To the authors' knowledge, there is no information about the prevalence of ExPEC in marinated and non-marinated poultry meat products on the Finnish retail market.

The use of antibiotic agents in food animal production has raised concern about the transmission of antibiotic-resistant bacteria to humans via the food supply. Acquired resistance to first-line antimicrobial agents has been found to increasingly complicate the management of ExPEC infections in humans. Resistance to fluoroquinolones and third- and fourth-generation cephalosporins is increasing in many countries [9-12]. Increasing numbers of extended-spectrum β -lactamase (ESBL)-producers are seen in Finnish uro-pathogenic *E. coli* (UPECs) (M. Vaara, personal communication). ESBL producing *E. coli* strains have emerged as a potential health hazard, as they have not only been identified from human clinical samples, but also from food producing animals [10,13].

The aim of the present study was to provide insight into the properties of ExPEC in poultry meat products on the Finnish retail market in order to evaluate their zoonotic potential. Special attention was given to their prevalence and the virulence and phylogenetic profiles. Furthermore, the isolates were screened for possible ESBL producers and their antibiotic resistance to nalidixic acid and ciprofloxacin was tested.

Materials and methods

Product description and sampling

A total of 219 retail raw chilled poultry meat products from different batches (199 chicken samples, 16 turkey samples and four samples including both chicken and turkey meat) were randomly selected between July 2006 and September 2006 from different local retail shops in Western Finland (88 samples) and between August 2006 and April 2007 in the Helsinki area (131 samples). Of all samples, 178 products were marinated and 41 were non-marinated. All products were packed in Finland, but in 31 (14.2%) samples the meat originated abroad. The term "marinated" refers to products with an oil- and/or water-based marinade and a blend of spices. All products were packed under a modified atmosphere consisting of carbon dioxide and nitrogen in different proportions, and had a shelf life of up to 10 days. All samples were transported immediately to the laboratory

and kept at $4 \pm 3^\circ\text{C}$ until being analyzed within 24 hours of purchase.

Culture method and identification

Each sample was aseptically removed from the package and placed in a Stomacher bag (Seward BA6041, Worthing, UK). Equal amounts of a weighed sample and Buffered Peptone Water (BPW) (LAB46, LabM, Lancashire, UK) were mixed with a minimal amount of 300 g of meat in 300 ml of BPW (LabM). The bag was shaken manually for 3 minutes and 100 μl of BPW was spread onto Chromocult[®] Coliform Agar (CCA) (Merck, Darmstadt, Germany) plates. All plates were incubated for 24 hours at 37°C . One presumptive *E. coli* colony isolated from each product was subcultured on Tryptic Soy Agar (TSA) agar (Merck, Darmstadt, Germany), and incubated as described above. For further identification, API 20E (bio Mérieux, Marcy l'Etoile, France) was used.

PCR methods

Virulence genotyping

A total of 207 *E. coli* isolates were characterized for their virulence factor genes using the polymerase chain reaction (PCR) method. Ten virulence genes were studied: *kpsMT* K1, *ibeA*, *astA*, *iss*, *irp2*, *papC*, *iucD*, *tsh*, *vat*, and *cva/cvi*. The single PCR reactions for *kpsMT* K1 and *ibeA* were performed as described by Johnson et al. [14]. The presence of eight virulence factor genes - *astA*, *iss*, *irp2*, *papC*, *iucD*, *tsh*, *vat*, and *cva/cvi* was studied using the multiplex PCR method by Ewers et al. [15]. All amplifications were performed in a 25 μl reaction volume, and a lysate of overnight culture in sterile water was used as a template.

Phylogenetic and O typing

E. coli isolates were assigned to phylogenetic groups using the PCR method documented earlier [16,17]. Based on the combination of genes (*svg*, *chuA*, *yjaA*, *uidA* and *TspE4.C2*) they possess, the isolates were assigned to one of five groups (A, B1, B2, D, B2₁/ST29). *E. coli* O antigen groups O1, O2 and O18 were determined according to Clermont et al. [18]. Amplifications were performed in a 25 μl reaction volume, and a lysate of overnight culture in sterile water was used as a template.

Control strains

The following *E. coli* strains were used as positive PCR controls: RS 228 and A56 (K1, O18 *ibeA*); RS 228 (positive for all five genes), and ATCC 25922 (positive for all five genes except *svg*) for the phylogenetic groups; A110 (*astA*, *irp2*, *papC*); EC206 (*iss*, *irp2*, *papC*, *iucD*, *tsh*, *cva*); 252 (*iss*, *irp2*, *papC*, *iucD*, *vat*, *cva*, O2); IH11002 (O1).

Antimicrobial susceptibility testing

The susceptibility of 48 potential ExPEC isolates for nalidixic acid (30 µg) and ciprofloxacin (5 µg) was tested according to the standard Disk Susceptibility method [19]. The *E. coli* strains were also screened phenotypically for the production of ESBL with the double disk synergy test. Disks (Oxoid) containing cefotaxime, ceftazidime, and equivalent disks with clavulanic acid were used according to the standard method [20].

Statistical analysis

Comparisons of proportions for virulence factors detected and the distribution of the strains to phylogenetic groups were analyzed by Chi-square test. Statistical significance was set at a P value of < 0.001.

Results

E. coli was isolated from 207 (94.5%) of the 219 poultry meat products. One *E. coli* isolate from each product was characterized further. The majority of the isolates belonged to the phylogenetic group D (105; 50.7%), followed by groups A (78; 37.7%), and B2 (6; 7.7%) (Table 1). There were more phylogenetic group D and fewer group A isolates from marinated meat than from non-marinated meat, but the differences were not statistically significant. Two (1%) B2 isolates were positive for the *svg* gene specific for the clonal group B2₁/ST95 (Table 1). They both belonged to O1 serogroup.

In all isolates, we found at least one of the tested virulence genes (Table 2). Among the 123 strains of the phylogenetic groups B2 and D, the most common virulence genes were *iucD* (94 isolates, 76.4%), *irp2* (78 isolates, 63.4%), *iss* (40 isolates, 32.5%), and *astA* (75 isolates, 61%) (Table 2). The virulence factor genes were most frequent among isolates of phylogroup B2. Forty-eight (23.2%) of all isolates were positive for either two or all three of the genes classifying them as ExPEC: *iucD*, *papC* and *kpsMT* K1 (Table 3). ExPEC strains were as frequently detected from marinated (38/167; 22.8%) as from non-marinated (10/40; 25.0%) meat products.

The 48 isolates classified as ExPEC were susceptible to cephalosporins, ciprofloxacin and nalidixic acid, with the exception of one isolate (1/48; 2.1%) showing resistance to nalidixic acid. This ExPEC strain belonged to phylogenetic group D, and was positive for seven of the ten virulence factors studied.

Discussion

In the present study, almost all Finnish retail poultry meat products were found to be contaminated with *E. coli*. Twenty-two per cent of the products contained *E. coli* strains that could be classified as ExPEC. The analysis of these poultry meat samples was focused to the virulence genes that are most typical of avian pathogenic ExPEC, and thus most likely to prevail in poultry products. Inclusion of all *kpsMT* II group genes, instead of only that for K1 capsule, and some other ExPEC associated virulence factor genes, could have increased the observed prevalence of ExPEC. The findings from the Finnish poultry products are consistent with studies made in other countries on poultry meat at the retail level. Johnson et al. [2] found 21% of 110 *E. coli* isolates from chicken products to satisfy the criteria for ExPEC. Johnson et al. [6] reported a contamination rate of 46% for ExPEC in 189 retail poultry products. Xia et al. [21] found among different retail meats the highest rate of ExPEC strains in ground turkey (23.5%) and chicken breasts (20.2%). Kobayashi et al. [22] isolated 80 strains from 57 retail chicken carcasses, and grouped 21.25% of the strains into group B2 and 28.75% into group D. It has been recently shown that B2 strains positive for *svg* belong to a subset of ExPEC strains that contain avian pathogenic, uropathogenic as well as neonatal meningitis causing *E. coli* [23]. These strains often belong to serogroups O1, O2 or O18, and contain a high number of virulence genes. In this study, such ExPEC bacteria were found in 1% of Finnish poultry meat products. The isolates belonged to serogroup O1, and contained seven of the ten virulence genes studied.

Fecal contamination of carcasses at slaughter is presumed to be the source of contamination of meat with

Table 1 Phylogenetic distribution of the 207 *Escherichia coli* isolates originating from marinated and non-marinated retail poultry meat at Finnish retail market

Phylogenetic group	Non-marinated meat (n = 40)	Marinated meat (n = 167)	Total (n = 207)
uidA negative ^{ns}	0	1 (0.6%)	1 (0.5%)
A ^{ns}	21 (52.5%)	57 (34.1%)	78 (37.7%)
B1 ^{ns}	1 (2.5%)	4 (2.4%)	5 (2.4%)
D ^{ns}	15 (37.5%)	90 (53.9%)	105 (50.7%)
B2 ^{ns}	3 (7.5%)	13 (7.8%)	16 (7.7%)
B2 ₁ /ST29 ^{ns}	0	2 (1.2%)	2 (1%)

^{ns} statistically non-significant.

Table 2 Distribution of virulence factor genes (%) among 207 *Escherichia coli* –isolates belonging to major phylogenetic groups and isolated from poultry meat at Finnish retail market

Virulence factor genes	Prevalence of trait within phylogenetic groups, no. (%)			Total (n = 207)
	A + B1 (+uidA neg.) (n = 84)	D (n = 105)	B2 (+B2 ₁ /ST29) (n = 18)	
<i>cva</i> *	26 (31%)	12 (11.4%)	10 (55.6%)	48 (23.2%)
<i>vat</i> *	1 (1.2%)	3 (2.9%)	10 (55.6%)	14 (6.8%)
<i>tsh</i> *	16 (19%)	2 (1.9%)	7 (38.9%)	25 (12.1%)
<i>iucD</i> *	29 (34.5%)	77 (73.3%)	17 (94.4%)	123 (59.4%)
<i>irp2</i> *	19 (22.6%)	62 (59%)	16 (88.9%)	97 (46.9%)
<i>iss</i> *	44 (52.4%)	28 (26.7%)	12 (66.7%)	84 (40.6%)
<i>astA</i> *	20 (23.8%)	73 (69.5%)	2 (11.1%)	95 (45.9%)
<i>papC</i> ^{ns}	7 (8.3%)	9 (8.6%)	5 (27.8%)	21 (10.1%)
<i>ibeA</i> *	0	2 (1.9%)	10 (55.6%)	12 (5.8%)
<i>kpsMT</i> K1*	0	40 (38.1%)	6 (33.3%)	46 (22.2%)

* $P < 0,001$; statistically significant.

^{ns} statistically non-significant.

potential ExPEC bacteria [24-26]. However, our previous studies (unpublished) detected *E. coli* fulfilling ExPEC criteria only in 2.3% of fecal samples from healthy broilers. All these isolates belonged to phylogroup D, and none to B2. Our studies have shown that colibacillosis caused by avian pathogenic *E. coli* is not an uncommon disease in Finnish broiler houses. The study shows that such ExPEC bacteria can efficiently contaminate poultry meat and enter the food chain. In Finland, poultry meat is usually cut into pieces or very thin slices. It can be speculated that cutting may provide favorable or even selective conditions for the survival of ExPEC bacteria in the product consisting of meat and meat juice. The

conditions may mimic those in the host tissue, thus supporting the survival of bacteria possessing protective virulence mechanisms. Meat processing may favor the survival of other types of *E. coli* as well, such as ESBL-producing strains, in the meat products.

Marinades and especially their spices may potentially serve as sources of food pathogens, and furthermore promote pathogen survival. This has been a matter of concern in Finland, where most of the poultry meat is sold in marinades. In this study, we did not observe any differences between *E. coli* strains from marinated and non-marinated products with regard to prevalence of potential ExPEC and other virulence factor genes and phylogenetic groups.

Table 3 Virulence gene profiles and phylogeny groups in ExPEC isolates (n = 48) and their distribution in marinated and non-marinated poultry meat at Finnish retail market

Combinations of genes	Phylogeny group	Non-marinated meat (n = 40)	Marinated meat (n = 167)
<i>cva, vat, iucD, papC, irp2, iss, kpsMT</i> K1	B2svg ⁺	0	2
<i>cva, vat, tsh, iucD, irp2, iss, ibeA, kpsMT</i> K1	B2	0	3
<i>cva, vat, tsh, iucD, papC, iss, astA</i>	D	0	1
<i>cva, iucD, irp2, iss, astA, kpsMT</i> K1	D	1	0
<i>cva, iucD, irp2, astA, kpsMT</i> K1	D	0	1
<i>vat, iucD, papC, irp2, iss, astA, kpsMT</i> K1	D	1	0
<i>iucD, papC, irp2, iss, astA, kpsMT</i> K1	D	0	6
<i>iucD, irp2, iss, astA, kpsMT</i> K1	D	2	4
<i>iucD, irp2, astA, kpsMT</i> K1	D	3	17
<i>iucD, iss, astA, kpsMT</i> K1	D	0	2
<i>iucC, papC, irp2, iss</i>	B2	2	0
<i>iucC, irp2, kpsMT</i> K1	D	1	0
<i>iucD, papC</i>	B2 and D	0	2
negative	various	30	129

The studies on survival of *E. coli* in marinated meat products have focused on verocytotoxigenic strains. It has been stated that acidification of a traditional marinade resulted in greater lethality of *E. coli* O157:H7 compared with otherwise similar marinades that were not further acidified [27,28]. The pH of commercial marinades used in Finnish retail poultry meat products ranges from pH 4.16 ± 0.03 [29] to pH 4.5 [30]. It has been speculated that the buffering capacity of meat might neutralize the acidic components in marinades, leading to a decreased antimicrobial effect [8]. Although this study did not indicate that marinades are a risk factor for ExPEC contamination of the product, it is necessary to study the effect of marinades on survival of zoonotic *E. coli*, such as ESBL-producing strains.

In the present study, all potential ExPEC isolates ($n = 48$) were susceptible to ciprofloxacin and cephalosporins, and only one isolate exhibited resistance to nalidixic acid. These results illustrate the very low level of antimicrobials used in poultry production in Finland. Meat producing flocks, in particular, are usually reared without any antimicrobial treatment. The percentage of flocks treated has varied between 0.0 and 0.37% in broiler flocks and 3.79–4.97% in turkey flocks during 2007–2010 (<http://www.ett.fi>). No fluoroquinolones or cephalosporins are used for poultry. Cephalosporin resistant *E. coli* isolates have emerged in numerous locations around the world after the sampling time of this study. This study, covering the Finnish retail market in 2006–2007, serves as a starting point for further studies. It is essential to gain more information about the antibiotic resistance and the genetic properties of the *E. coli* isolates present in retail poultry meat in Finland.

Conclusions

Potential ExPEC bacteria were found in 22% of marinated and non-marinated poultry meat products on the Finnish retail market. Of the products, 0.9% were contaminated with *E. coli* [O1] B2 *svg*⁺ group, which has been implicated in multiple forms of ExPEC disease. There were no differences between marinated and non-marinated products with regard to the prevalence of potential ExPEC bacteria, other virulence factor genes and phylogenetic groups of the *E. coli* isolates. Poultry meat products on Finnish retail market may have zoonotic potential.

Competing interests

The authors declared that they have no competing interests.

Authors' contribution

UL, PPM participated in the discussion on the study design. PPM, UL participated in the collection of samples, analysis and interpretation of the data. UL carried out the microbiological analyses of the samples. TP, KR and II carried out all used PCR methods and the antimicrobial susceptibility testing. Analysis and interpretation of the PCR and the antimicrobial susceptibility testing were carried out by TP, SP, II and KR. II and TP carried

out the statistical analyses. UL, II, TP and SP wrote the manuscript. All authors read and approved the final manuscript.

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