

Virulence factors in *Escherichia coli* isolated from chicken meat in Slovakia

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Summary

The aim of this study was to examine the presence of virulence factors and assignments to phylogenetic groups in *Escherichia coli* strains isolated during one year from chicken meat and broiler faeces before slaughtering. The five virulence genes *iutA*, *iss*, *cvaC*, *tsh* and *papC* were detected significantly more often amongst meat isolates than in faecal isolates from healthy broilers. Phylogenetic typing showed that all faecal isolates fell into pathogenic group D and group B2, while approximately one half of meat strains belonged to commensal phylogenetic groups A and B1. Majority of meat *E. coli* strains belonged to the pathogenic serotype O78. Results showed that retail broilers in Slovakian food markets could be the source of *E. coli* virulence factors for the human population.

Keywords

Escherichia coli; virulence factor; phylogenetic group; meat; poultry

With the implementation of Hazard Analysis Critical Control Point and Quality Management systems at poultry-processing plants, there are increasing requirements for the microbiological analysis of faecal and environmental contamination in the poultry carcasses. Bacteria from the intestines of chicken broilers may be transferred to retail meat products resulting in faecal contamination during various stages of the slaughter process (e.g. evisceration) and subsequent handling of the animal tissues. The presence of *Escherichia coli* indicator organisms not only indicates poor hygiene but also itself may be pathogenic.

Avian pathogenic *E. coli* (APEC) are known to possess a large number of potential virulence factors e.g. *iut*-receptor for aerobactin, *iss*-increased serum survival, *cvaC*-colicin V, *kps*-capsular polysialic acid virulence factor, *tsh*-temperature sensitive haemagglutinin and *ibeA*-invasive factor responsible for neonatal meningitis in humans [1]. APEC strains show similarities with human extraintestinal pathogenic *E. coli* (ExPEC) strains. Even though no specific set of virulence factors can be associated with APEC strains, most of the virulence genes are similar to those identified in human ExPEC strains. RODRIGUEZ-SIEK et al. revealed [2] that uropathogenic *E. coli* (UPEC) and

APEC have similarities in their serogroups, virulence genotypes and assignments to phylogenetic groups, which supported the hypothesis that poultry meat may be a vehicle for *E. coli* capable of causing human urinary diseases. Poultry meat contamination from microorganisms which cause deterioration in food safety and quality, in particular virulent *E. coli*, is a challenge for the improvement of food laboratory diagnostics. Mainly, quantification of the total numbers of *E. coli* cells is recommended in food quality assessment. However, it is necessary also to assess the presence of virulence factors. Considering the importance of *E. coli* strains as food-borne pathogens, this study aimed to identify the virulence factors in meat-associated *E. coli* isolated from chicken broilers bought in supermarkets in Slovakia.

MATERIALS AND METHODS

Poultry samples and identification of *Escherichia coli*

During one year, 175 samples of chicken broiler samples were investigated. The transport swabs from surface of meat (frozen broiler, frozen breast, frozen leg and frozen wing) from su-

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permarkets in Slovakia (producers: Podtatranská hydina Kežmarok, Slovakia and production facility in Košice-Napájdla; Hyza Topolčany, Slovakia; or broilers imported from Poland and Czech Republic) and rectal swabs from various broiler farms (Eastern and Western Slovakia) were resuscitated in buffered peptone water (Oxoid, Basingstoke, United Kingdom) and then were subcultured on MacConkey agar (Oxoid). A total of 28 meat and 72 faecal *E. coli* strains were selected. The identification was carried out using Triple sugar agar (Imuna, Šarišské Michalany, Slovakia) or Uriselect agar (Bio-Rad Laboratories, Hercules, California, USA), and by Entero test 24 (Pliva-Lachema, Brno, Czech Republic).

Serotyping was performed in meat strains by slide agglutination with polyvalent Anti-Coli A (O1, O2, O18, O78) and by monovalent O78, O1 and O2 antisera (Sifin, Berlin, Germany).

Polymerase chain reaction

Screening of *E. coli* isolates for APEC virulence genes was carried out by polymerase chain reaction (PCR) amplification of *iutA*-receptor for aerobactin (using primers GGC TGG ACA TGG GAA CTG G and CGT CGG GAA CGG GTA GAA TCG, annealing temperature of 63 °C, amplicon size being 300 bp), *cvaC*-colicin V (using primers CAC ACA CAA ACG GGA GCTGTT and CTTCCCGCAGCATAGTTCCAT, annealing temperature of 63 °C, amplicon size being 680 bp), and *kpsII*-capsular polysialic acid virulence factor (using primers GCG CAT TTG CTG ATA CTG TTG and CAT CCA GAC GAT AAG CAT GAG CA, annealing temperature of 63 °C, amplicon size being 272 bp) [3], *iss*-increased serum survival (using primers GTG GCG AAA ACT AGT AAA ACA GC and CGC CTC GGG GTG GAT AA, annealing temperature of 61 °C, amplicon size being 760 bp) [4], *tsh*-temperature sensitive haemagglutinin (using primers GGT GGT GCA CTG GAG TGG and AGT CCA GCG TGA TAG TGG, annealing temperature of 55 °C, amplicon size being 620 bp) [5], *papC*-P fimbrial adhesin (using primers GAC GGC TGT ACT GCA GGG TGT GGC G and ATA TCC TTT CTG CAG GGA TGC AAT A, annealing temperature of 65 °C, amplicon size being 328 bp) [6], *ibeA*-invasive factor of *E. coli* strains responsible for neonatal meningitis in humans (using primers TGA ACG TTT CGG TTG TTT TG and TGT TCA AAT CCT GGC TGG AA, annealing temperature of 55 °C, amplicon size being 814 bp) [7].

E. coli isolates were assigned to phylogenetic groups A, B1, B2 or D based on PCR with primers ChuA.1 (GAC GAA CCA ACG GTC AGG AT)

and ChuA.2 (TGC CGC CAG TAC CAA AGA CA), YjaA.1 (TGA AGT GTC AGG AGA CGC TG) and YjaA.2 (TGG AGA ATG CGT TCC TCA AC), and TspE4C2.1 (GAG TAA TGT CGG GGC ATT CA) and TspE4C2.2 (CGC GCC AAC AAA GTA TTA CG), which generated DNA fragments 279 bp, 211 bp and 152 bp, respectively, at the annealing temperature of 55 °C [8].

PCR was carried out in a total volume of 25 µl, containing 1 µl of template DNA solution, each of the primers at 20 pmol, the four deoxynucleoside triphosphates at 200 µmol·l⁻¹ each, PCR buffer, 1.5 mmol·l⁻¹ MgCl₂ and 1 U of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, California, USA). Temperature programme consisted of denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 1 min, at the annealing temperature specific for each pair of primers for 1 min, and at 72 °C for 1 min in a thermal cycler MJ Mini (Bio-Rad Laboratories). The amplified DNA fragments were separated in 1% agarose gel, stained with Gold View Nucleic Acid Stain (SBS Genetech, Beijing, China). A 100 bp ladder (Invitrogen) was used as a molecular weight standard.

RESULTS AND DISCUSSION

The five virulence genes *iutA*, *iss*, *cvaC*, *tsh* and *papC* were detected significantly more often amongst meat isolates than in faecal isolates from healthy broilers (Tab. 1). A typical member of the APEC pathotype is likely to contain several iron transporter-encoding genes like *irp2*, *fyuA*, *iutA*, *iroN* and *sitA*, and plasmid-associated genes, including *cvi/cvaC*, *tsh* and *iss* [2]. Moreover, *pap* genes, encoding for P fimbriae, were much more likely to be found in APEC strains than in faecal strains. The widespread trait of virulent avian *E. coli* strains is their resistance to serum comple-

Tab. 1. Frequency of virulence genes in 72 faecal and 28 meat-associated *E. coli* strains.

Virulence factors	Faecal <i>E. coli</i>	Meat <i>E. coli</i>
<i>iss</i>	39/72 (54.1%)	20/28 (71.4%)
<i>iutA</i>	54/72 (75%)	25/28 (89.2%)
<i>cvaC</i>	15/72 (20.8%)	12/28 (42.8%)
<i>tsh</i>	15/72 (20.8%)	16/28 (57.4%)
<i>papC</i>	12/72 (16.6%)	8/28 (28.5%)
<i>kpsII</i>	27/72 (37.5%)	8/28 (28.5%)
<i>ibeA</i>	6/72 (8.3%)	3/28 (10.7%)

Number of positive strains/total number of strains.

Tab. 2. Distribution of virulence factors and phylogenetic groups in 28 poultry meat-associated *E. coli* strains.

Virulence factor	Number of strains	Commensals / Number of strains	Pathogens / Number of strains
none	1	Group A / 1 strain	
<i>iut</i>	1	Group A / 1 strain	
<i>kps</i> , <i>pap</i>	1		Group B2 / 1 strain
<i>iut</i> , <i>iss</i>	3	Group A / 1 strain, Group B1 / 1 strain	Group B2 / 1 strain
<i>iut</i> , <i>tsh</i>	1		Group B2 / 1 strain
<i>iut</i> , <i>ibe</i>	2		Group B2 / 2 strains
<i>kps</i> , <i>tsh</i>	1	Group A / 1 strain	
<i>iut</i> , <i>iss</i> , <i>cva</i>	2	Group A / 2 strains	
<i>iut</i> , <i>cva</i> , <i>tsh</i> , <i>pap</i>	1	Group A / 1 strain	
<i>iut</i> , <i>iss</i> , <i>kps</i>	2		Group B2 / 2 strains
<i>iut</i> , <i>iss</i> , <i>cva</i> , <i>tsh</i>	3	Group A / 2 strains	Group B2 / 1 strain
<i>iut</i> , <i>iss</i> , <i>kps</i> , <i>tsh</i>	3		Group B2 / 3 strains
<i>iut</i> , <i>iss</i> , <i>kps</i> , <i>tsh</i> , <i>ibe</i>	1		Group B2 / 1 strain
<i>iut</i> , <i>iss</i> , <i>cva</i> , <i>tsh</i> , <i>pap</i>	6	Group B1 / 5 strains	Group B2 / 1 strain

ment (*iss*), which facilitates distinction between virulent strains and non-virulent strains [4]. However, virulent *E. coli* need not be the typical pathogens. Properties of our meat-associated *E. coli* strains clearly correlated with the previous findings, since about a half of strains were commensals with virulence factors.

Phylogenetic typing showed that all faecal isolates from healthy chicken broilers fell into pathogenic group D (62.5%) and group B2 (37.5%), while 53% of meat-associated strains belonged to commensal phylogenetic groups A and B1. However, the nine meat strains from both groups (i. e. commensals and pathogens) agglutinated with the polyvalent antiserum detecting the most frequent poultry serotypes (O1, O2, O18, O78). Majority of strains belonged to the pathogenic serotype O78.

Uropathogenic isolates are known to primarily belong to one of two pathogenic groups, namely, B2 or D [8, 9]. Although all faecal strains of *E. coli* in this study also fell into group B2 (37.5%) and D (62.5%), one half (53%) of meat-associated *E. coli* belonged to commensal groups A and B1. However, our study suggested that isolates from A and B1 groups may possess virulence gene profiles similar to B2 isolates (Tab. 2). Moreover, meat-associated strains belonged to the pathogenic serotype O78. APEC probably serve as a source or as a reservoir of virulence genes for human ExPEC [9]. Poultry meat is an important vehicle for virulent gastrointestinal pathogens such as *Campylobacter jejuni* [10], *Salmonella enterica* [11] and *E. coli*. It is widely argued that as meat products are cooked, there is little probability that virulent and antibi-

otic resistant bacteria present in the raw material will colonize the human gut. There is a substantial overlap between the phylogroups, serotypes and virulence factors of *E. coli* from human urinary infections and those of poultry strains of *E. coli* associated with the disease of avian colibacillosis [12]. WARREN et al. [13] revealed that quinolone-resistant *E. coli* with various beta lactamase genes that are common in human infections worldwide were found in imported chicken breasts, indicating a possible source for gut colonization. A high level of quinolone resistance in *E. coli* was detected in poultry-associated strains also in Slovakia [14]. Similarly, JOHNSON et al. [16] found that retail foods, particularly poultry products, from the Minneapolis–St. Paul region, were frequently contaminated with antimicrobial-resistant *E. coli* and/or ExPEC with virulence factors *papC*, *kpsM* and *iutA*, belonging to the pathogenic group B2. Ongoing testing of domestically produced meat commodities will help provide information needed to better understand risks associated with virulence and antimicrobial resistance of enteric pathogens in the domestic retail meat supply, including the detection of new resistant genotypes should they arise [15].

CONCLUSIONS

Individual Slovakian poultry meat samples were found to contain *E. coli* strains with five virulence factors *iutA*, *iss*, *cvaC*, *tsh* and *papC* related to avian pathogenic or human uropathogenic *E. coli*.

About a half of meat-associated strains belonged to the commensal phylogenetic groups A and B1. Retail foods may be an important vehicle for community-wide dissemination of virulent *E. coli*, which may represent a newly-recognized group of medically significant food-borne pathogens.

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