Prevalence of virulence genes among Escherichia coli strains isolated from food and carcass swabs of different animal origins in Croatia

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Abstract

Introduction: Escherichia coli is present in the normal intestinal flora but some strains can cause intestinal and extraintestinal diseases, and research on its presence in food of animal origin is in the interests of public health. This study was designed to characterise E. coli strains according to their origin, their carriage of virulence genes specific for certain pathogroups, and phylogenetic group affiliation. Material and Methods: The study was carried out on 100 E. coli strains isolated from food samples of various animal origin as well as pig and cattle carcass swabs. Isolation of the strains was performed using two methods. One method included colony count and the other an overnight enrichment of the samples. Isolation was followed by DNA extraction and detection of virulence genes and phylogenetic group with conventional and multiplex PCRs. Results: In this study, the most prevalent gene was EAST1 (20%) and strains which carried it were identified as enteroadherent E. coli. Other pathogroups were represented in lower incidences. Phylogenetic group analysis revealed the prevalence of the A and B1 groups, with B1 mainly present in game and cattle strains, while the majority of pig and poultry strains were assigned to group A. Conclusion: This study provides an overview of the presence of potentially pathogenic strains and E. coli phylogenetic groups in Croatia, for which the data are limited. Further microbiological and molecular research is required to examine the epidemiological situation in the country.

Keywords: Escherichia coli, public health, pathogroup, phylogenetic group, Croatia.

Introduction

Escherichia coli (E. coli) strains are mostly present as intestinal commensal bacteria in the gastro-intestinal tract of humans and other animals; however, some can cause intestinal and extraintestinal infections (11). The pathology and expression of clinical symptoms classify intestinal pathogenic E. coli into six pathogroups: enterotoxigenic E. coli (ETEC); enteropathogenic E. coli (EPEC); enterohaemorrhagic E. coli (EHEC); enteroaggregative E. coli (EAEC); enteroinvasive E. coli (EIEC); and diffusely adherent E. coli (DAEC) (6). Strains of the bacteria that cause extraintestinal infection are called extraintestinal pathogenic E. coli (ExPEC) (41).

Human infections occur through the consumption of contaminated food such as undercooked meat, drinking contaminated water, or direct person-to-person contact. In developing countries, the major causes of infantile diarrhoea are ETEC, EPEC and EAEC, while EHEC and EAEC are mainly associated with food poisoning in the developed world (6). The presence of intestinal and extraintestinal pathogenic E. coli in food suggests a public health impact, and agencies such as the European Food Safety Authority (EFSA) report foodborne outbreaks associated with verotoxigenic E. coli (VTEC) and other pathogenic E. coli. In the 5-year period of 2015–2019 there was an increase in reported VTEC cases in the EU. This can be due to the
enhanced general awareness of VTEC following reports of large outbreaks in the EU and worldwide. It was shown by the EFSA that VTEC is the fourth most frequent bacterial agent causing foodborne outbreaks in the EU, with 34 outbreaks, 208 cases, 30 hospitalisations and 1 death reported in 2020 (15).

One of the most important human pathogens is EHEC, and the most significant virulence genes within this pathogroup are verotoxins (vtx). *Escherichia coli* which harbours these genes is called verotoxigenic *E. coli* (VTEC). This pathogroup can be present in the intestines of many animal species, but the main reservoirs are ruminants, especially cattle (21). The presence of vtx genes is closely related to the pathogen’s induction of haemorrhagic diseases, such as haemolytic-uremic syndrome (HUS) and bloody diarrhoea. The ability to cause disease also depends on the subtype (3). For EPEC, it is important to differentiate typical (tEPEC) from atypical (aEPEC) strains. There are two major diversities between tEPEC and aEPEC. The first includes the presence of the *E. coli* adherence factor plasmid that encodes the bundle-forming pili (bfp), present in tEPEC but not in aEPEC, and the second one is related to the host, because both humans and animals can equally be reservoirs for aEPEC and create a cycle of mutual infection, while only humans are believed to be the main reservoir for tEPEC (34, 37). As with tEPEC, humans were confirmed as the main reservoir for EIEC (37). Strains of EAEC are classified as typical EAEC (tEAEC) and atypical EAEC (aEAEC) based on the presence or absence of the aggR gene. Humans are the reservoir for tEAEC, while aEAEC has been isolated from different animal species, suggesting their role as its reservoir (37). Factors not regulated by aggR include the EAEC heat-stable toxin EAST-1, which is associated with EAEC causing diarrhoea (32). As for ETEC, heat-labile and heat-stable enterotoxins and colonisation factors are the pathogroups’ main characteristics (42), and humans are suggested as their main reservoir. However, young animals are susceptible to ETEC infections (17).

Depending on the pathogroup, the symptoms range from heavy diarrhoea and dehydration in ETEC and EPEC infection, with possible chronic diarrhoea, vomiting and slight fever in EPEC-caused cases (2, 36), to watery diarrhoea in EIEC infection and watery secretory diarrhoea with mucus in EAEC-caused diseases (10). In contrast to strains causing intestinal infection, ExPEC possesses a wide range of virulence genes that enable it to invade and colonise organs and generate infections outside of the gastro-intestinal tract (5), causing extraintestinal infections in humans and animals. These strains can be isolated from food of animal origin, such as chicken and pig meat (25).

In recent years, there have been many studies regarding the association between pathogroups and phylogenetic groups. Commensal and enteric strains are mainly associated with the A and B1 groups, while extraintestinal strains are mainly identified in the B2 group. Research demonstrates that ETEC, EHEC and EIEC affiliate to the A, B1, C, and E phylogenetic groups, while EPEC, EAEC and DAEC are present in all groups (14). Furthermore, the dispersion of phylogenetic groups within *E. coli* strains is closely associated with the host species, and although each group can acquire any virulence gene, they survive in clones ‘best adapted’ to the host species, making them their reservoir (1).

In Croatia, information regarding the molecular characteristics of *E. coli* strains is deficient. To rectify the deficiency, this study aimed to investigate the presence of virulence genes and phylogenetic group affiliation of *E. coli* isolated from different food samples and carcass swabs of different animals originating from Croatia. The collected data will be compared with the origin of the strain and provide an insight into the presence of potentially pathogenic strains and phylogenetic groups of *E. coli* and a better understanding of their dissemination in Croatia.

**Material and Methods**

**Bacterial strains.** A total of 100 *E. coli* strains were analysed in this study. The strains were isolated from samples of different animal origins, including poultry (*n* = 27), game (*n* = 14), pigs (*n* = 30), and cattle (*n* = 29), delivered as routine laboratory samples. Samples of poultry origin included 23 of broiler meat, 2 of mechanically separated meat and 2 of meat preparations. The strains isolated from game came from fresh meat from three red deer, four fallow deer, one moufflon, four roe deer and two wild boar. Pig strains were isolated from 23 carcass swabs, 1 sample of fresh meat and 6 of packaged minced meat. Cattle-origin strains were isolated from 15 carcass swabs, 5 from samples of fresh meat, 6 from packaged minced meat and 3 from meat preparations. Isolation was performed using the ISO 16649-2:2001 method (22) and an additional one. The second method was enrichment of samples in buffered peptone water (BPW) at 37°C for 18–24 h according to the European Union Reference Laboratory (EURL) Methods 05, 07 and 08 recommended for the detection of virulence genes in *E. coli* (16), together with ISO/TS 13136:2012 (23) which proposes the use of BPW as a broth for selective enrichment. Incubation was followed by plating on a Tryptone Bile Glucuronie Agar (TBG) plate (Oxoid, Oxford, UK) and a Sorbitol MacConkey Agar (SMAC) plate (Merck, Darmstadt, Germany) and further incubation at 37°C for 18–24 h. Characteristic colonies were plated on blood agar (Merck) and identified as *E. coli* using the VITEK2 system (Biomérieux, Marcy-l’Étoile, France). After confirmation, the colonies were inoculated on Brain Heart Infusion (BHI) Broth (Merck) with 50% glycerol and stored at −80°C until further examination. DNA was isolated by heating the bacterial suspension at 95°C for 20 min, followed by centrifugation at 14,000 × g for 1 min. All amplified
PCR products were visualised with the QIAxcel capillary electrophoresis system (Qiagen, Hilden, Germany).

**Detection of virulence genes.** A group of 15 virulence genes was used to determine the presence of six *E. coli* pathogroups, as presented in Table 1. A conventional PCR was performed for *ipah*, *aggR*, *aaiC*, *lt*, *bfp* and *EAST1*. The primers used and amplification conditions for *ipah*, *aggR*, *aaiC* and *lt* were those specified in EURL Methods 05, 07 and 08 (16). The *bfp* gene was detected according to Gunzburg et al. (19) and the *EAST1* gene was amplified as described by Yamamoto and Echeverria (45). A multiplex PCR was performed to determine the presence of *vtx1*, *vtx2*, *eae*, *saa* and *ehxA* according to Paton and Paton (40) and the *vtx1* and *vtx2* subtypes were determined using EURL Method 06 (16). Detection of *STI*, *STII*, *cnf1* and *cnf2* was achieved using multiplex and conventional PCRAs according to Pass et al. (39).

**Phylogenetic group determination.** The strains were assigned to a phylogenetic group by using a multiplex PCR according to Clermont et al. (7).

**Statistical analysis.** Statistical analysis was performed using Stata 13.1. (StataCorp., College Station, TX, USA) and expressed in the binary variable 0/1 (yes/no). The connection between certain values was verified with the chi-squared and Fisher’s exact tests. Multivariant statistical analysis was conducted using a logistic regression model. Results with *P* ≤ 0.05 were considered statistically significant.

### Table 1. Pathogroups, virulence factors and virulence genes

| Pathogroup (ETEC) | Virulence factor | Full name of virulence gene | Virulence gene |
|-------------------|-----------------|-----------------------------|----------------|
| VTEC (EHEC)       | toxin           | verotoxin 1, 2              | vtx1, vtx2     |
| VTEC (EHEC)       | haemolysin      | haemolysin                  | ehxA           |
| VTEC (EHEC)       | adhesin         | STEC autoagglutinating adhesin | saa          |
| EAEC              | adhesin         | aggregative adhesion regulation factor | aggR       |
| EAEC              | toxin           | heat-stable enterotoxin 1   | EAST1         |
| EPEC              | adhesin         | bundle forming pili         | bfp            |
| EPEC              | adhesin         | intimin                     | eae            |
| EIEC              | toxin           | heat-stable enterotoxin I, II | STI (sti, sth), STII |
| ETEC              | toxin           | heat-labile enterotoxin     | STI            |
| ExPEC             | cytotoxin       | cytotoxic necrotizing factor 1, 2 | cnf1, cnf2 |

VTEC – verotoxigenic *E. coli*; EHEC – enterohaemorrhagic *E. coli*; STEC – Shiga toxin–producing *E. coli*; EAEC – enteraggregative *E. coli*; EPEC – enteropathogenic *E. coli*; EIEC – enteroinvasive *E. coli*; ETEC – enterotoxigenic *E. coli*; ExPEC – extraintestinal pathogenic *E. coli*

### Results

#### Distribution of potentially pathogenic *E. coli* strains.**

The occurrence of virulence genes within the strains is presented in Table 2. The presence of virulence genes was established in 36 (36%) of the 100 tested *E. coli* strains. The presence of *EAST1*, *eae* and *cnf1/cnf2* virulence genes was detected in strains of various animal origins, with *EAST1* present in 20 of the 100 (20%), *eae* in 9 (9%) and *cnf1* in 6 (6%) and *cnf2* in 4 (4%) strains. Less varied by source, the *STII*, *vtx* and *ehxA* virulence genes were only detected in strains of game and pig origin. The *STII* gene was identified in 5 (5%) strains and *vtx* was detected in 2 (2%) strains and subtyped with the results *vtx1c* and *vtx1d* (game origin) and *vtx2e* (pig origin). The *ehxA* gene occurred in 2 of the 100 (2%) strains.

The animal origin and sample type in which virulence genes were detected is also presented in Table 2. Of the 27 poultry origin strains, the presence of virulence genes was established in 10 (9 isolated from broiler meat and 1 from mechanically separated meat), while in the other 17 strains (14 isolated from broiler meat, 1 from mechanically separated meat and 2 from meat preps) none of the virulence genes were detected. Among the 14 strains isolated from game meat, 11 harboured virulence genes (2 strains being red deer isolates, 3 fallow deer, 3 roe deer, 2 wild boars and 1 moufflon) while the other 3 strains (isolated from red deer, fallow deer and roe deer) were found not to carry any of the target genes.

#### Table 2. Occurrence of virulence genes and phylogenetic groups in different animal species

| Virulence gene | Number of positive strains, phylogenetic group and type of sample | Total gene-positive strains (%) |
|---------------|---------------------------------------------------------------|---------------------------------|
| *EAST1*       | 4 (2A, 1B1, 1F); 3, 1 MSM                                    | 13 (36.11)                       |
| *eae*         | 4 (3A, 1E); BM                                               | 7 (19.44)                        |
| *EAST1, STI*  | 4 (3A, 1B1); DE, 2 FM, M                                     | 5 (13.89)                        |
| *EAST1, eae*  | 1 (B1); BM                                                  | 1 (2.78)                         |
| *EAST1, ehxA* | --                                                           | 1 (2.78)                         |
| *eae, ehhA*   | --                                                           | 1 (2.78)                         |
| *cnf1, cnf2*  | --                                                           | 1 (2.78)                         |
| *vtx1*        | --                                                           | 1 (2.78)                         |
| *vtx2*        | --                                                           | 1 (2.78)                         |
| *bfp* (%)     | 10 (27.78)                                                   | 8 (22.22)                        |

A, B1, B2, C, D, E, F – phylogenetic group; BM – broiler meat; MSM – mechanically separated meat; DE – fresh red deer meat; FD – fresh fallow deer meat; RD – fresh roe deer meat; WB – fresh wild boar meat; M – fresh mouflon meat; CS – carcass swab; MM – minced meat; MP – meat preparation; FM – fresh meat
Table 3. The frequency of virulent genes compared to the origin of the sample

| Strain origin | Number of strains (%) | Virulence gene presence | Number of virulence genes in strains |
|---------------|-----------------------|-------------------------|-------------------------------------|
|               |                       | Negative (%) | Positive (%) | One (%) | Two (%) |
| Poultry       | 27 (27)               | 17 (62.96)   | 10 (37.04)   | 8 (80)  | 2 (20)  |
| Game          | 14 (14)               | 3 (21.43)    | 11 (78.57)   | 5 (45.45)| 6 (54.55)|
| Pig           | 30 (30)               | 23 (76.67)   | 7 (23.33)    | 4 (57.14)| 3 (42.86)|
| Cattle        | 29 (29)               | 21 (72.41)   | 8 (27.59)    | 7 (87.5)| 1 (12.5)|
| **Total (%)** | **100 (100)**         | **64 (64)**   | **36 (36)**  | **24 (24)**| **12 (12)**|
| **P**         | **0.004**             | **0.003**    |             |          |          |

Table 4. Distribution of E. coli phylogenetic groups in different animal species

| Strain origin | Phylogenetic group | Total number of strains |
|---------------|--------------------|-------------------------|
|               | A | B1 | B2 | C | D | E | F |
| Poultry       | 11 | 6  | 0  | 0 | 2 | 2 | 6 | 27 |
| Game          | 4  | 5  | 2  | 0 | 2 | 1 | 0 | 14 |
| Pig           | 13 | 12 | 0  | 1 | 3 | 1 | 0 | 30 |
| Cattle        | 10 | 13 | 2  | 2 | 2 | 0 | 0 | 29 |
| **Total (%)** | **38 (38)** | **36 (36)** | **4 (4)** | **3 (3)** | **9 (9)** | **4 (4)** | **6 (6)** | **100** |
| **P**         | **0.039**           |             |          |          |          |          |          |          |

Table 5. The relationship between phylogenetic groups and virulence genes

| Phylogenetic group | Absent (%) | Present (%) | n (%) | P     |
|--------------------|------------|-------------|-------|-------|
| A                  | 24 (37.5)  | 14 (38.89)  | 38 (38)|       |
| B1                 | 24 (37.5)  | 12 (33.33)  | 36 (36)|       |
| B2                 | 2 (3.13)   | 2 (5.56)    | 4 (4) | 0.833 |
| C                  | 1 (1.56)   | 2 (5.56)    | 3 (3) |       |
| D                  | 7 (10.94)  | 2 (5.56)    | 9 (9) |       |
| E                  | 2 (3.13)   | 2 (5.56)    | 4 (4) |       |
| F                  | 4 (6.25)   | 2 (5.56)    | 6 (6) |       |
| **Total (%)**      | **64 (64)**| **36 (36)** | **100 (100)** |       |

Fig. 1. Distribution of virulence genes within phylogenetic groups

Of the 30 pig-origin strains, 7 (isolated from carcass swabs) harboured virulence genes, while these genes’ presence was not established in the other 23 strains (16 isolated from carcass swabs, 6 from minced meat and 1 from fresh meat). The 29 strains of cattle origin included 8 containing virulence genes (4 detected in carcass swabs, 2 in minced meat, 1 in a meat preparation and 1 in fresh meat) and 21 not carrying any of the tested virulence genes (11 yielded from carcass swabs, 4 from fresh meat, 4 from minced meat and 2 from meat preparations).

The distribution of virulence genes was uneven between strains of different origins and some harboured two such genes (Table 3), this state being frequently observed in game origin strains. Additionally, most of the virulence genes were detected in game origin strains (78.57%).
Phylogenetic grouping of *E. coli* strains. The distribution of the strains within phylogenetic groups is presented in Table 4, which shows A (38%) and B1 (36%) as the most frequent groups, followed by D (9%), F (6%), B2 (4%), E (4%) and C (3%). The largest affiliation of strains isolated from poultry (11 out of 27; 40.74%) and pigs (13 of 30; 43.33%) was to phylogenetic group A, while for strains isolated from game (5 of 14; 35.71%) and cattle (13 of 29; 44.83%) it was to the B1 group. Group F was only present in strains of poultry origin, the B2 phylogenetic group was not confirmed in poultry or pig strains and C was not found in strains isolated from poultry and game.

Relationship between pathogroups and phylogenetic groups. In the 36 strains carrying virulence genes, A and B were the most prevalent phylogenetic groups (Fig. 1). The results revealed 14 out of 36 (38.89%) of the strains to be assigned to phylogenetic group A and 12 out of 36 (33.33%) to group B1, with the others (B2, C, D, E and F) represented equally (Table 5). Fourteen strains assigned to A group harboured *EAST1*, *eae* and *STII* virulence genes, while all of the analysed virulence genes except *vtx* were present in strains assigned to the B1 group. The results comprise two strains from the B2 group carrying the *cnf1* and *cnf2* genes, two strains from the F group with the *EAST1*, *cnf1* and *cnf2* genes, two strains from the E group carrying *eae* and *vtx1*, two strains from C group harbouring the *vtx2* and *EAST1* and two strains from group D with the *EAST1*, *ehxA* and *eae* genes. The distribution of the virulence genes within phylogenetic groups is displayed in Table 2.

Statistical analysis. The distribution of the results revealed a relationship between the distribution of the virulence genes and the origin of the sample (*P* = 0.004) (Table 3). Compared to strain from poultry, pigs and cattle, most of the strains isolated from game harboured more than one virulence gene and the frequency of this difference was significant (*P* = 0.003) (Table 3). Statistical analysis also confirmed significance in the distribution of phylogenetic groups in host organisms, with *P* = 0.039 (Table 4). However, there was no significant correlation between the presence of virulence genes and phylogenetic group affiliation (*P* = 0.833) (Table 5).

**Discussion**

This study analysed the occurrence and distribution of virulence genes and phylogenetic groups in *E. coli* strains isolated from food of different animal species and carcass swabs originating from Croatia. The results presented in this study confirmed the presence of various intestinal and extraintestinal *E. coli* strains. The presence of virulence genes in this bacteria in food of animal origin is a public health issue, and food contaminated with pathogenic *E. coli* could lead to illness. The research provides an insight into the diversity of *E. coli* strains isolated from food specimens as well as carcass swabs from different animal species originating from Croatia, for which the data are limited.

The most frequently occurring virulence genes were the ones associated with the EAEC, aEPEC and ExPEC pathogroups. These genes were detected in strains retrieved from samples of which the animal origins were varied, which could be explained by the genes’ broad distribution within different animal hosts and the environment (12, 24). In this study, *EAST1* was the most prevalent gene, but its contribution to the virulence of EAEC remains unclear (6). However, previous research associated *EAST1* toxin with severe cases of child diarrhoea in India (32), confirming its pathogenicity and indicating its significant association with EAEC causing diarrhoea. Furthermore, the zoonotic potential of the aEPEC strains confirmed in this investigation can also be assumed based on previous research. Moura *et al.* (34) described the relationship between aEPEC strains of human and animal origin, indicating that strains isolated from animals have the potential to cause diarrhoea in humans. As for ExPEC, previous studies indicate food of animal origin as a source of infection for humans (25). The presence of *cnf1* and *cnf2* detected in this study indicates a need for further research, since *cnf1* is mainly associated with diarrhoea and extraintestinal infections in humans, and *cnf2* with septicaemia or diarrhoea in cattle and sheep (26). Unlike that pathogroup, ETEC and VTEC were only present in strains of game and pig origin and harbouring the vtx genes identified as *vtx2* (pig origin) and *vtx1c* and *vtx1d* (game origin). Based on the subtype, these strains cannot cause severe clinical illness in humans such as HUS or bloody diarrhoea. Furthermore, none of the VTEC strains harboured other vtx-related virulence genes analysed in this study (*saa*, *ehxA* or *eae* and certain other gene combinations and the percentage of hospitalisations and HUS cases in humans (29). As a contrary indication however, *vtx1c* has often been isolated in patients with milder infections and, in combination with *vtx1a*, in patients with bloody diarrhoea (3). As for STII, its presence is almost exclusively linked to pigs (13) and further research is needed to establish possible role of the gene as a zoonotic pathogen. Furthermore, some genes (*ipaH*, *bfp*, *aggR*, *aaIC* and *STII*) were not confirmed in any of the strains, which was expected because of their adaptation to humans.

The results demonstrate that most of the target genes were present in strains of game origin. The connection between the source of the strain and the presence of virulence genes was confirmed with *P* ≤ 0.05, suggesting game meat as a significant source of pathogenic *E. coli*. This could be the effect of many factors, including poor shot placement by hunters, their association with EAEC, aEPEC and ExPEC pathogroups present in game meat, especially
deer species, indicating the need for further research. These data are important, especially with regard to VTec, because game is considered to be a reservoir and source of human pathogenic VTec and EHEC strains. Examples are noted from studies from other European countries such as Spain, in which 40% of wildlife-isolated VTec strains shared the same characteristics as the ones isolated from human patients from that geographic area (33). Research conducted in Germany also linked 32.8% (46/140) of VTec strains isolated from wildlife meat with high-level virulence genes for humans (30). A report published by the EFSA also disclosed a high prevalence of VTec in deer meat (26.7%), indicating it as one of the most contaminated foods of animal origin (15).

Other results mostly show broiler chicken meat contaminated with E. coli, which was probably because of poor sanitation standards of water used in the cutting and processing of chicken meat (28). All the positive pig-origin strains were isolated from carcass swabs. This is probably the result of cross-contamination during slaughter, which can occur with intestinal damage, faecal contamination, or contamination from the environment, equipment, or workers’ hands (43). Similarly, cattle carcass swabs were probably contaminated with E. coli during hide removal and evisceration (20). Other samples such as minced meat and meat preparations were contaminated during the slaughter process or subsequent handling (35). No differences investigated in the comparison of different samples from the same animal species were proved to be statistically significant, indicating the need for future research with a larger number of samples.

In the 100 E. coli strains analysed in this study, most were assigned to the A and B1 phylogenetic groups, including virulence gene-positive strains. These data are in agreement with the prevalence of the A and B1 groups in commensal strains isolated from different food and animal species (4, 9, 38) and the occurrence of enteric E. coli within these groups (8). The results of the study also indicate the phylogenetic group tendency toward a specific host (P = 0.039), with group A more frequently distributed in strains of pig origin and B1 more prevalent in game and cattle strains. The results confirm previous research suggesting that strains isolated from omnivores are mainly associated with group A, while B1 is more prevalent in strains isolated from herbivores (4). The analysed poultry strains had A as the dominant group, with group F appearing only in poultry, confirming the phylogenetic groups association with the species origin of the sample (9). Ultimately, the gathered information confirmed a connection between the source of the strain and phylogenetic group affiliation, verifying the groups’ adaptation to certain host species. This information could have a practical application as most virulence genes in survive phylogenetic groups best adapted to certain host species.

Escherichia coli are very diverse bacteria, and sometimes it is very difficult to place a strain in only one pathogroup. For example, EAST1, which was the most prevalent virulence gene in this study, is commonly associated with the EAEC pathogroup but is also present in others, such as EHEC, EPEC and ETEC (32, 37). Furthermore, strains carrying the EAST1 gene were present in all phylogenetic groups except for B2 and E, confirming its distribution over the entire phylogenetic tree (14) and in food of diverse origins. Similarly to EAST1, the eae gene is hosted by different animals (27) and has broad phylogenetic group distribution as a consequence. Unlike EAST1 and eae, the STII and vtx genes were identified in game- and pig-origin strains. Strains positive for STII were assigned to the A and B1 groups and those positive for vtx to the E and C groups, which is consistent with the outcomes described by Escobar-Páramo et al. (14). Previous reports emphasised the importance of assigning strains to certain phylogenetic groups, because they can be evaluated for their pathogenicity accordingly. For instance, Wang et al. (44) indicated an association between the strains isolated from cattle, humans with diarrhea and the B1 group. This association could indicate a greater zoonotic potential of the eae-positive cattle strains isolated in this study. Along with enteric E. coli, the presence of ExPEC was also confirmed in strains isolated from samples of different animal origins. This pathogroup can be distributed in all phylogenetic groups, but is commonly associated with B2 and D (31), which harbour more virulence genes (25). Furthermore, previous studies confirm the connection between the B2 group and the occurrence of extraintestinal infections (8), which suggests that the ExPEC strains analysed in this study and identified as B2 (game and cattle) may possess greater zoonotic potential than those assigned to B1 (pig, cattle) and F (poultry) group.

In conclusion, the study revealed the presence of intestinal and extraintestinal pathogenic E. coli in food of various animal origin as well as carcass swabs, indicating a potential public health risk. This study confirmed the wide distribution of certain pathogroups (EAEC and aEPEC) within different animal hosts and the importance of game meat as a source of potentially pathogenic E. coli, indicating the need for further research. Additionally, this research provides information on the presence of E. coli phylogenetic groups in domestic and wild animals in Croatia, which has not been exhaustively analysed. The study also confirms a connection between the source of a strain and phylogenetic group affiliation, verifying the groups’ adaptation to certain host species. However, further research is needed to assess the epidemiological association between strains obtained from this study and the strains isolated from the environment, animals, other food and humans suffering from E. coli infections.

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