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## RESEARCH ARTICLE



# Occurrence of *Escherichia coli* producing extended spectrum $\beta$ -lactamases in food-producing animals

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## ABSTRACT

Multidrug resistance due to the production of extended-spectrum beta-lactamases (ESBLs) is a major problem in human as well as in veterinary medicine. These strains appear in animal and human microbiomes and can be the source of infection both in animal and in human healthcare, in accordance with the One Health theorem. In this study we examined the prevalence of ESBL-producing bacteria in food-producing animals. We collected 100 porcine and 114 poultry samples to examine the prevalence of ESBL producers. Isolates were identified using the MALDI-TOF system and their antibiotic susceptibility was tested using the disk diffusion method. ESBL gene families and phylogroups were detected by polymerase chain reactions. The prevalence of ESBL producers was relatively high in both sample groups: 72 (72.0%) porcine and 39 (34.2%) poultry isolates were ESBL producers. *Escherichia coli* isolates were chosen for further investigations. The most common ESBL gene was CTX-M-1 (79.3%). Most of the isolates belong to the commensal *E. coli* phylogroups. The porcine isolates could be divided into three phylogroups, while the distribution of the poultry isolates was more varied. In summary, ESBL-producing bacteria are prevalent in the faecal samples of the examined food-producing animals, with a dominance of the CTX-M-1 group enzymes and commensal *E. coli* phylogroups.

## KEYWORDS

ESBL producers, poultry, swine, asymptomatic carriage

## INTRODUCTION

Bacteria producing extended spectrum  $\beta$ -lactamases (ESBLs) cause serious problem in human and veterinary healthcare, as these bacteria are resistant to penicillins, broad-spectrum cephalosporins and monobactams. Beta-lactam antibiotics are used frequently in human and veterinary medicine against infections caused by Enterobacterales (Nüesch-Inderbinen et al., 2016). Correlation between the consumption of 3rd and 4th generation cephalosporins and cephalosporin resistance of *Escherichia coli* was reported in human medicine and in food-producing animals by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) in a

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joint report (ECDC, EFSA and EMA, 2017). In this manner, ESBL enzymes, found frequently in the background of resistance to multiple beta-lactam antibiotics, became ubiquitous in Enterobacterales, especially in *E. coli*, spreading on mobile genetic elements and occurring frequently in the gut microbiome of humans or animals. The microbiome thus serves as a reservoir of ESBL producers and ESBL genes, and the human and animal microbiomes are interconnected through the food chain (Silva et al., 2019). Recognising the importance of this relationship, the World Health Organization (WHO), the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO) established the One-Health concept 'to achieve optimal health and well-being outcomes recognising the interconnections between people, animals, plants and their shared environment' ([https://www.onehealthcommission.org/en/why\\_one\\_health/what\\_is\\_one\\_health/](https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/)).

The aim of this study was to investigate the occurrence of ESBL producers in food-producing animals, and to determine their human pathogenic potential through phylogroup analysis. We also compared these isolates to contemporary isolates from asymptomatic human carriage.

## MATERIALS AND METHODS

### Samples and culturing

A total of 100 porcine and 114 poultry faecal samples were investigated. The samples were cultured on eosin-methylene blue agar supplemented with 2 mg L<sup>-1</sup> cefotaxime. This culturing method helped screen the ESBL strains. The antibiogram (antibiotic susceptibility testing) was determined using the Kirby-Bauer disk diffusion method based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations against carbapenems (ertapenem, meropenem, imipenem), amoxicillin/clavulanic acid, cefotaxime, cefepime, ciprofloxacin, sulphamethoxazole/trimethoprim (Sumetrolim, SXT) amikacin, tobramycin, and gentamicin; colistin susceptibility was tested by microdilution (Merlin GmbH, Berlin, Germany). The identification of bacterial colonies was performed using Matrix-Assisted Laser Desorption/Ionization Time-of Flight (MALDI-TOF) Biotyper (Bruker Daltonics, Bremen, Germany). The ESBL phenotype was examined using the double-disk synergy test, the cefotaxime and amoxicillin/clavulanic acid disks placed at 30 mm apart. The double-disk synergy test was positive when the inhibition zone was enhanced between the two disks (synergy between cefotaxime and the lactamase-inhibitor clavulanic acid). Animal-derived isolates were compared to 17 contemporary human isolates originating from asymptomatic faecal or oral carriage collected at the University of Debrecen.

### Resistance genes and phylogenetic analysis

The *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub> genes were detected by PCR, as described previously (Ebrahimi et al., 2014). The *bla*<sub>CTX-M</sub> genes in the CTX-M-1 group were sequenced using

group-specific PCR primers. Sequences were analysed by means of CLC DNA Workbench v4.0 (CLC Bio, Aarhus, Denmark).

The phylogenetic analysis of *E. coli* isolates was performed by the multiplex PCR method of Clermont et al. (2013). This method defines eight phylogroups: phylogroups A1, B1, C, E, F and clade I contain mainly commensal strains, while phylogroups B2 and D are responsible for extraintestinal infections in humans. The pandemic ST131 clone and its subclades C1-M27 and the C2 carrying *bla*<sub>CTX-M-15</sub> were detected using the method of Matsumura et al. (2017).

## RESULTS

### Prevalence of ESBL producers

The prevalence of ESBL-producing isolates was markedly different between the porcine and poultry samples, 72 (72.0%) and 39 (34.2%) ESBL-producing isolates were found, respectively. All poultry and 43 porcine isolates were *E. coli*; 21 *Proteus* spp., 7 *Myroides odoratimimus*, 5 *Citrobacter freundii*, 2 *Morganella morganii* and 1 *Providencia rettgeri* were also found among the porcine isolates. Only *E. coli* isolates were investigated further.

### Phenotypic resistance

The phenotypic resistance was similar among the porcine and the poultry *E. coli* isolates: colistin (0.0% vs. 2.6%), amikacin (39.5% vs. 35.9%), tobramycin (39.5% vs. 35.9%), and all *E. coli* isolates were resistant to cefotaxime and cefepime. In the case of gentamicin, the resistance was higher within porcine isolates (79.1% vs. 12.8%). The SXT resistance showed similar results (81.4% vs. 35.9%). All of the poultry isolates were resistant to ciprofloxacin, while 68.9% (30/43) of the porcine *E. coli* were resistant to it. All isolates were susceptible to carbapenems (ertapenem, meropenem, imipenem), the drug group of first choice against ESBL-producers in human medicine (Fig. 1).

### Prevalence of ESBL gene families

The distribution of ESBL gene families differed between the two groups of isolates. Group CTX-M-1 was the most common; its prevalence was higher in porcine than in poultry isolates (39/43, 90.7% and 27/39, 69.7%, respectively). All but one porcine isolates and all poultry isolates carried the *bla*<sub>CTX-M-1</sub> gene, the remaining one porcine isolate harboured *bla*<sub>CTX-M-3</sub>; the *bla*<sub>CTX-M-15</sub> gene was not found in the animal isolates. The CTX-M-2 gene family was found only in porcine isolates (4.7%), while the CTX-M-9 was found in poultry *E. coli* (7.7%). The CTX-M-8 family was totally absent (Fig. 2). The genes of these groups were not sequenced.

### Prevalence of *E. coli* phylogroups

The occurrence of *E. coli* phylogroups was different in the two types of isolates. Most of the isolates belonged



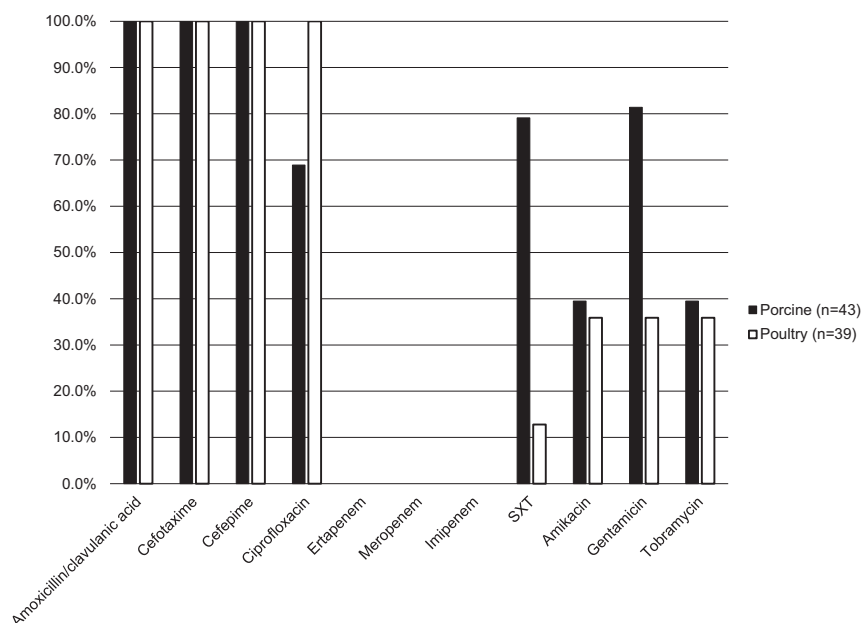


Fig. 1. Proportion of isolates resistant to different antibiotics (SXT = sulphamethoxazole/trimethoprim)

to commensal phylogroups, but the distribution was different, the porcine isolates could be distributed into three phylogroups, while the poultry isolates were more varied, and only phylogroup D was absent from both types of samples (Fig. 3). Of the porcine isolates, 67.4% (29/43) belonged to phylogroup C, while 11.6% (5/43) and 14.0% (6/43) of these isolates were members of phylogroups A and B1, respectively. Most of the poultry isolates belonged to phylogroups B1 and E (30.8% and 30.8%, 12/39, respectively), while 7.7% (3/39) and 12.8% (5/39) of them could be classified into two other commensal phylogroups, C and F (Fig. 3).

### Human isolates

The antibiotic resistance of human isolates was higher against all examined antibiotics. SXT and aminoglycoside resistance rates were 16/17 and 14/17, respectively; one human isolate was resistant against ertapenem.

The most common ESBL gene was the CTX-M-15 (11/17); as expected, four of the isolates proved to be of the clone ST131 clade C2. A single isolate carried blactxm1, another carried CTX-M27 and belonged to the clade ST131/C1-M27. The occurrence of the TEM and SHV gene families was similar among the human isolates (5/17).

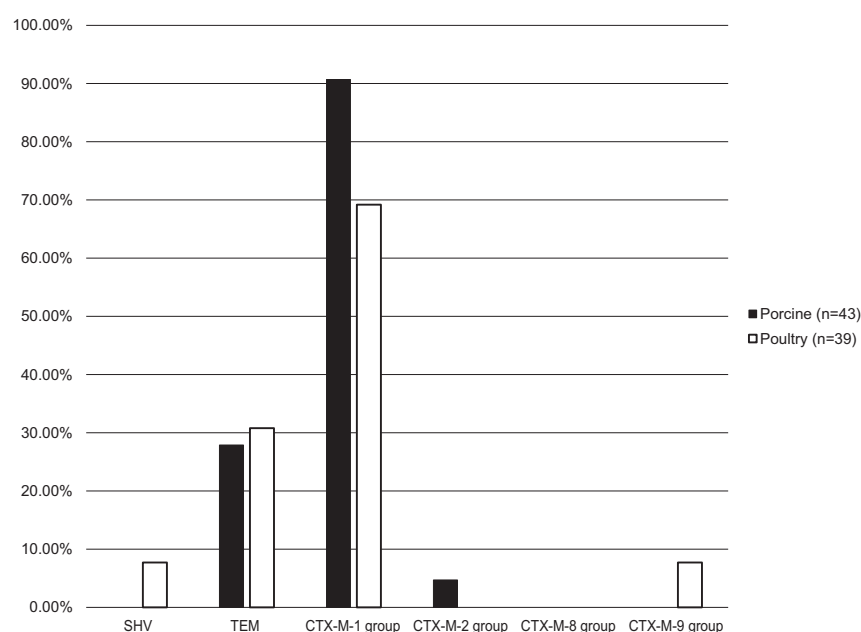


Fig. 2. Result of the PCR examination of ESBL gene families – comparison of porcine and poultry *E. coli* isolates



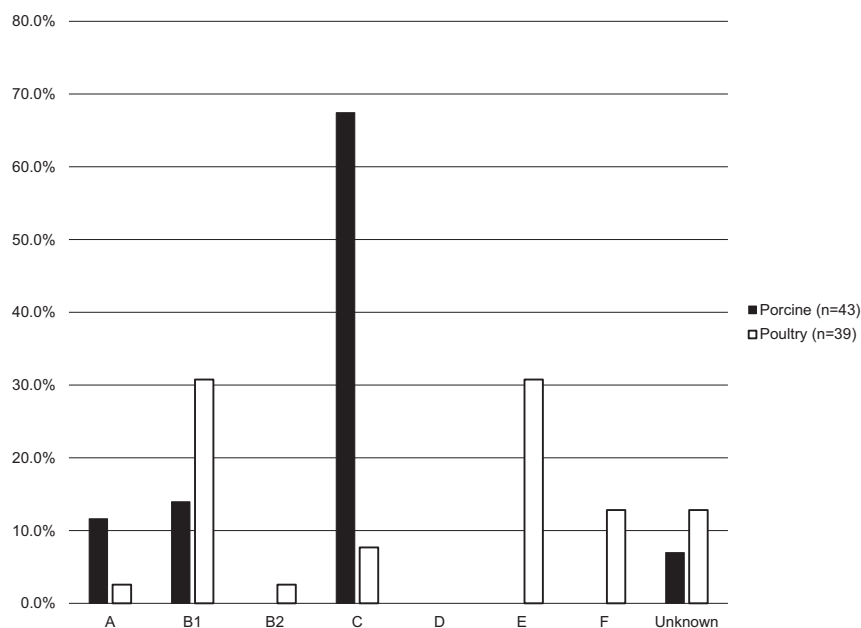


Fig. 3. Result of *E. coli* extended phylogroup multiplex PCR based on the method of Clermont et al. (2013)

Most isolates belonged to the B2 and D phylogroups (10/17, 58.9% and 4/17, 23.5%, respectively); five isolates from the B2 group were of sequence type 131. The remaining isolates belonged to the A and E phylogroups.

## DISCUSSION

A high prevalence of ESBL-producing bacteria was found among the poultry and pork isolates. Although the prevalence of ESBL producers was different, the gene distribution was highly similar. These gene patterns did not differ from data of the literature; the vast majority of animal isolates carried *bla*<sub>CTX-M-1</sub> among both types of animal isolates, while in the human isolates *bla*<sub>CTX-M-15</sub> dominated (Bush, 2013; Abraham et al., 2018). This is in line with a previous Hungarian study surveying the faecal carriage of ESBL producers in animals (Tóth et al., 2013) and reporting *bla*<sub>CTX-M-1</sub> as the most common gene.

Very similar results have been reported worldwide, with the dominance of *bla*<sub>CTX-M-1</sub> in poultry (Girlich et al., 2007; Saliu et al., 2017; Gundran et al., 2019), which is horizontally transmitted among different strains (Zurfluh et al., 2014) and may reach humans through the food chain (de Been et al., 2014). Similar dominance and transmission were reported in the case of porcine samples (Abraham et al., 2018). Interestingly, among pig slaughterhouse workers, direct animal contact was associated with a higher risk of carrying ESBL producers (Dohmen et al., 2017), pointing out the importance of transmission through the pork food chain.

Human isolates contemporary with those investigated by Tóth et al. (2013), derived from asymptomatic individuals including animal keepers and abattoir workers, showed a marked dominance of *bla*<sub>CTX-M-1</sub> (Ebrahimi et al., 2014), the

other frequent gene was *bla*<sub>CTX-M-15</sub>. This proportion shifted towards dominance of the pandemic *bla*<sub>CTX-M-15</sub> carrying the ST131 clone (Ebrahimi et al., 2016a, 2016b), which was also detected in human isolates but, importantly, not in animal isolates in this study.

In general, commensal phylogroups were frequently found among ESBL carriers in animals. In poultry, the phylogroups B2 and D containing extraintestinal pathogens were rare, in line with earlier results (Saliu et al., 2017; op. cit.). In contrast, among porcine isolates the dominant phylogroups among ESBL producers were those associated with intestinal (C) and extraintestinal (A and B1) infections, in the same way as among human isolates (B2 and D).

In animal-derived isolates, *bla*<sub>CTX-M-1</sub> has been the dominant gene in ESBL-producing *E. coli* for more than a decade, which is also detected continuously, but only in a relatively small proportion of human isolates. In contrast, the gene most important in human isolates, *bla*<sub>CTX-M-15</sub> is found exclusively in human isolates and has failed to cross the species barrier. This suggests that the gene flow is continuous between humans and animal isolates but the proportion of shared genes or isolates is small and unimportant in terms of public health epidemiology in Hungary.

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