Extended spectrum beta-lactamase-producing *Escherichia coli* from extraintestinal infections in humans and from food producing animal, in Italy: a "One Health" study

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Abstract

Background: In recent years, *Escherichia coli* producing extended spectrum beta-lactamase (ESBL) has become a serious public health problem and food-producing animals (FPA) have been suggested as a potential reservoir/source.

Objective: To compare ESBL-producing *E. coli* isolates from different sources.

Methods: ESBL-producing *E. coli* was collected from humans (n.480) and FPA (n.445) in Italy (2016-2017). Isolates were screened for the presence of ESBL genes and classified according to phylogenetic group and MLST genotyping. *mcr*-1 to -5 genes were searched for in colistin resistant isolates.

Results: CTX-M was the most frequent ESBL-type in both human and animal isolates. CTX-M-15 prevailed in humans (75%) and cattle (51.1%) but not in poultry (36.6%). CTX-M-1 was common (58%) in pig. SHV-type and CMY-2-like were found in FPA, especially in poultry (17.0% and 29.9%, respectively). 29 isolates were *mcr*-1 carriers (3 from humans and 26 from FPA). Human isolates mostly belonged to phylogroup B2 (76.5%). Animal isolates were distributed among groups A (35.7%), B1 (26.1%) and C (12.4%). Few animal isolates (almost all from poultry) were classified into group B2 (4.3%). Most human isolates (83.4%) belonged to the pandemic ST131 clone and frequently carried CTX-M-15 (75.9%). ST131 was rarely detected in FPA (n.3 isolates from poultry). Nineteen STs were shared in both sources with ST10, ST410 and ST69 being more frequently detected.

Conclusions: According to our results the potential exchange of ESBL genes through plasmids or isolates from animal to humans is feasible, underlying the need for a strict monitoring based on an "One Health" approach.

1 Introduction

In humans, *Escherichia coli* (*E. coli*) is a member of the intestinal microbiota but also the leading cause of extraintestinal infections, mostly urinary tract infections (UTI) and sepsis. The successful treatments of these infections are more often tackled by antimicrobial resistance (AMR). [1,2] Since early 2000s, *E. coli* producing extended spectrum beta-lactamase (ESBL) has become a serious public health threat, causing severe infections in both hospital and community settings. [3,4] From 1% to 25-50% of all invasive *E. coli* isolates reported to the European Antimicrobial Resistance Network (EARS-Net) by the EU Member States are resistant to third-generation cephalosporins. The highest percentage is reported in Italy. [4] The occurrence and spread of high-risk multidrug-resistant (MDR) clones/lineages of *E. coli* causing extraintestinal infections such as the sequence type (ST)131 carrying resistance to third generation cephalosporin, fluoroquinolones and other antimicrobial groups is of concern. [5] A recent survey carried out in long-term care facilities (LTCFs) residents in Italy showed ST131 being predominant among *E. coli* isolates from both carriage and disease. [6,7]

During the last decades, ESBL-producing *E. coli* isolates have been also increasingly isolated from non-human sources including food-producing animals (FPA), suggesting that animals may be at least in part the source of ESBL-producing *E. coli* for humans. [8,9] Although the major ST131 clone was very rarely detected in isolates from FPA, ESBL genes carried by different clones may be horizontally acquired by human *E. coli* isolates. Conflicting results have been reported in the literature, with several investigations indicating that human and animals' isolates can share the same ESBL types and other demonstrating distinctive ESBL gene patterns according to the source.[10,11]

In human clinical setting, carbapenem resistance mediated by plasmid-encoded carbapenemases is a further public health risk, reducing therapeutic options in *E. coli* isolates already resistant to third generation cephalosporins. [12,13] Although the use of carbapenems is highly limited in animals and carbapenemases are poorly detected in animals so far, [14] the possibility of a non-human reservoirs should be surveyed. The presence in both human and veterinary isolates of a plasmid-mediated

resistance to Colistin, which is amongst the last choice antimicrobials used to treat multidrug-resistant isolates (MDR), raises questions regarding on the occurrence of Colistin resistance *mcr* genes associated to ESBL-producing *E. coli*. [15,16]

In 2016 year, we set up a "One Health" tailored pilot surveillance network to monitor the occurrence of ESBL-producing *E. coli* in humans and FPA. In this study, we report the antimicrobial susceptibility patterns, the characterization of the ESBL genes, the co-resistance to Colistin and the carriage of *mcr* genes, in ESBL-producing *E. coli* isolates from both humans with extraintestinal infections and FPA; we also compared genotypes of human and animal isolates according to the phylogenetic group and sequence type (ST), to identify shared or distinct molecular features.

2 Materials and methods

2.1 Study design and bacterial isolates

From March 2016 to September 2017, we conducted a multicentre cross-sectional study involving 15 partners from human and veterinary medicine in six Italian regions (Friuli Venezia Giulia, Trentino Alto Adige, Veneto, Lombardia, Lazio, Sicilia) so to distribute the sampling throughout Italy. Isolates of human origin were collected from urine or blood of outpatients and/or inpatients admitted to 12 different hospitals. Each hospital laboratory was asked to monthly collect first 3-10 (proportional to the number of urine and/or blood cultures tested in each hospital) consecutive and non-duplicate presumptive ESBL-producing *E. coli* isolates from urine or blood (4:1 ratio) detected during the routine diagnostic activity.

The commensal ESBL-producing *E. coli* from FPA were isolated by selective culture of feces or caecal intestinal content. The animals were selected by sampling among all FPA examined within the animal health surveillance of 3 Institute for Animal Health; only one isolate per herd was enrolled and the contribution of each Institute to the overall number of isolates was proportional to the Italian production, thus Regions with higher animal production were contributing more to the

final sample.

2.2 Detection of ESBL-producing *E. coli* and antimicrobial susceptibility testing (AST)

Human *E. coli* isolates were detected and identified to the species level and antimicrobial susceptibility tested according to standard laboratory procedures with automated methods in use in the participant laboratories (Vitek2, bioMerieux Italia SpA, Florence, Italy, and/or BD Phoenix™ Becton Dickinson Italia SpA, Milan, Italy). For the animal *E. coli* isolates, samples were cultured in selective enrichment broth (brain heart infusion, BHI) supplemented with 1mg/L Cefotaxime and subsequently isolated on MacConkey agar, supplemented with 1mg/L Cefotaxime. Once identified as *E. coli* by MALDI-TOF MS (Microflex Biotyper LT; Bruker Daltonics GmbH, Bremen, Germany), only one presumptive ESBL/AmpC-producing *E. coli* isolate per sample was randomly selected for further characterization. Antimicrobial susceptibility testing of the animal isolates was performed by the reference broth microdilution method, using TREK Sensititre custom panel ITGNEGF (Thermo-Fisher TREK Diagnostic Systems, Inc., Cleveland, OH, USA).

The interpretative breakpoints were based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria version 9.0 (https://eucast.org/clinical_breakpoints/). On the basis of the breakpoints, the *E. coli* isolates were then classified as resistant (R) or susceptible (S). The intermediate category, where present, was considered as susceptible.

For both sources, presumptive ESBL-producers were detected based on cephalosporins susceptibility: isolates resistant or with reduced susceptibility to third- and/or fourth-generation cephalosporins (MIC >1mg/L for at least one among Cefotaxime, Ceftazidime, and Cefepime) were selected and included in the study. ESBL production was confirmed by double-disc synergy testing (Total ESBL Confirm Kit, ROSCO Diagnostica A/S, Taastrup, Denmark).

In addition to cephalosporins, confirmed ESBL-producing isolates from human and animals were tested for susceptibility to Ampicillin/Sulbactam, Piperacillin/Tazobactam, Ertapenem, Imipenem, Meropenem, Ciprofloxacin, Levofloxacin, Amikacin, Gentamicin, Tigecycline, Colistin, Fosfomycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole. An isolate was defined as MDR

when it was resistant to at least three antimicrobial agents of different classes. [17]

2.3 Characterization of ESBL-encoding genes

Phenotypically confirmed ESBL-producing isolates were tested for the presence of the main ESBL and/or pAmpC gene types (*bla*$_{\text{CTX-M-}}$, *bla*$_{\text{SHV-}}$ and *bla*$_{\text{CMY-2}}$) by PCR and sequencing, as previously reported. [18] Comparative analysis of nucleotide and deduced amino acid sequences was performed by the advanced BLAST search program 2.2 at the National Center for Biotechnology Information site (www.ncbi.nlm.nih.gov/blast/).

2.4 Colistin resistance and *mcr* screening

The ESBL-producing *E. coli* isolates of human origin were initially tested for susceptibility to Colistin by automated systems (at the time of the study this method was one of the standard methods for Colistin). All human ESBL-producing *E. coli* isolates with Colistin MIC >1mg/L were confirmed by the reference broth microdilution method, using TREK Sensititre custom panel ITGNEGF. The ESBL-producing isolates of animal origin were directly tested for colistin susceptibility by the reference broth microdilution method, using the same custom panel reported above. Isolates exhibiting a Colistin MIC >1mg/L were screened for the presence of *mcr*-1 to -5 gene by multiple PCR, as previously described. [19]

2.5 Molecular typing of ESBL-producing *E. coli* isolates

ESBL-producing *E. coli* isolates from both human and animal sources were classified to seven major *E. coli* phylogenetic groups (A, B1, B2, C, D, E and F). [20] All isolates belonging to phylogroup B2 were tested by a rapid real-time PCR assay to detect the ST131 epidemic clone and the *bla*$_{\text{CTX-M-15}}$ gene, respectively. [21] A subset of the remaining *E. coli* isolates (including approximately 40% of the total number of the isolates not belonging to ST131 and all *mcr*-positive isolates) from both sources were tested by multilocus sequence typing (MLST) according to the MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

2.6 Data analysis

Data were collected, harmonized and stored. Correlations among variables were explored by Chi-square test, and multivariable analysis performed with logistic regression. Odds ratios and confidence intervals were provided for relevant variables with sufficient samples. Data analysis was performed using the R programme version 3.6.3 with basic data management functions.

3 Results

3.1 Bacterial isolates

Overall, 925 phenotypically confirmed ESBL-producing *E. coli* isolates were selected and included in the study: 480 (51.9%) isolates were from humans and 445 (48.1%) from FPA. The 480 ESBL-producing *E. coli* isolates of human origin were collected from urine (n. 377) or blood (n. 103). The 445 ESBL-producing *E. coli* isolates of FPA origin were collected from 131 cattle, 120 pig and 194 poultry.

3.2 Antimicrobial susceptibility testing

Antimicrobial resistance profiles of the 925 ESBL-producing *E. coli* isolates are described in Figure 1. Most ESBL-producing *E. coli* isolates were resistant to Ciprofloxacin and Cefotaxime (413/480, 89.4% for human isolates and 199/445, 45.1% for FPA). Resistance to Gentamicin was detected in 37.6% of the human and in 26.3% of animal isolates. 42 isolates were resistant to Colistin (5 from humans and 37 from FPA). Few isolates, almost all from humans except one from FPA, were resistant to carbapenems, but none produced a carbapenemase.

Looking at combined resistance, most ESBL- producing *E. coli* isolates of human (269/480, 56%) and animal (307/405, 69%) origin exhibited an MDR phenotype (table 1). In human isolates, the most frequent phenotype was resistance to Cephalosporins, Fluoroquinolones and Aminoglycosides (70/480, 14.6%). This phenotype with additional resistance to Trimethoprim-sulfamethoxazole was observed in 9.6% of the isolates. In animal isolates, the predominant MDR phenotype was resistance to Penicillins, Cephalosporins, Trimethoprim/Sulfamethoxazole (84/445, 18.9%). This

phenotype with additional resistance to Fluoroquinolones was observed in 13.5% (60/445) of the isolates. Combined resistance to five antibiotics (Penicillins, Cephalosporins, Fluoroquinolones, Aminoglycosides and Trimethoprim/Sulfamethoxazole) was also detected (3.3% of human and 10.3% of animal isolates). All but one 42 colistin resistant isolates were MDR; nearly all were resistant to Penicillins and Cephalosporins.

3.3 Characterization of the ESBL genes

Among the 925 ESBL-producing *E. coli* isolates, 904 (97.7%) showed the presence of an ESBL gene. The remaining 21 isolates (2.3%) produced ESBLs not identified by the panel used (including the most frequent ESBLs, but not exhaustive). Distribution of the ESBL-types is detailed in Table 2. The CTX-M was the most frequent ESBL-type in both human and animal isolates. A $bla_{\text{CTX-M-}}$ gene was present in almost all human isolates (468/480, 97.5%) and in the 78.0% (347/445) of the animal isolates, regardless of whether the gene was present alone or in combination with other ESBL-types. The other ESBLs we detected included the SHV-type (4/480, 0.8%, and 34/445, 7.6%, in human and animal isolates, respectively) and CMY-2-like (5/480, 1.0%, and 67/445, 15.1%, in human and animal isolates, respectively).

The difference in the frequency of the CTX-M type between humans and animals was particularly marked (OR=11.0; CI = 6.2 – 21.4; $P < 0.001$). Among animals, isolates from poultry carrying CTX-M (107/194, 55.2%) were significantly lower than isolates from cattle (128/131, 97.7%) and pig (112/120, 93.3%) (OR=0.02; CI = 0.007 – 0.08; $P < 0.001$). Poultry isolates contained the widest spectrum of ESBL-types compared to isolates of other origin, with CMY-2-like (58/194, 29.9%) and SHV-12 (33/194, 17.0%) being common.

The predominant CTX-M group among isolates from both sources was CTX-M-1 (373/468, 79.7% and 331/347, 95.4 % in human and animal of CTX-M positive isolates, respectively, regardless of whether the CTX-M-1 group was present alone or in combination with other ESBL types) followed by CTX-M-9 group (table 2).

Gene variant was known for a large subset of CTX-M positive human isolates (392/468, 83.8%)

and for all 347 CTX-M positive isolates from animals (figure 2). The most common enzyme was CTX-M-15 representing the 75% (294/392) of all known CTX-M variants among human isolates and the 50.7% (176/347) of those among animal isolates. However, considering the total number of ESBL-producing *E. coli* isolates of animal origin, CTX-M-15 was found in the 39.6% (176/445) of the isolates. The second most common CTX-M enzyme was different between human and animal isolates, being CTX-M-27 in humans (41/392, 10.5%) and CTX-M-1 (149/347, 42.9%) in animals. Stratifying by animal species and considering as total the number of isolates per animal species, CTX-M-15 predominated in cattle (67/131, 51.1%) and to a lesser extent in poultry (71/194, 36.6%) but it was largely overcome by CTX-M-1 in pig (70/120, 58% vs. 38/120, 31.7% for CTX-M-1 and CTX-M-15, respectively) (figure 2).

3.4 Isolates carrying *mcr* genes

By the reference broth microdilution method, 5 isolates from humans and 37 from FPA were found resistant to Colistin (MIC range: 4- ≥8 mg/L) according to the EUCAST clinical breakpoint (MIC>2mg/L). Among them, 29 isolates were *mcr*-1 carriers (3 from humans and 26 of animal origin) (table 3). Two of these isolates from cattle contained more than one *mcr* gene, namely one isolate with both *mcr-1* and *mcr-3* and the other with both *mcr-1* and *mcr-4*. No other *mcr* genes (*mcr-2* or *mcr-5*) were detected. Overall, the proportion of *mcr* gene carrying isolates was 0.6% (3/480) for human isolates and 5.8% (26/445) for animal isolates.

The distribution of the *mcr* genes stratified by sources and associated ESBL genes are shown in table 3. The most frequently associated ESBL was CTX-M-15, followed by CTX-M-1.

3.5 Molecular typing

Distribution of all 925 ESBL-producing *E. coli* isolates stratifying by source into the phylogenetic groups is shown in table 4. Human ESBL-producing *E. coli* isolates mostly (367/480, 76.5%) belonged to phylogenetic group B2, while animal isolates were mainly distributed among groups A (159/445, 35.7%), B1 (116/445, 26.1%) and C (55/445, 12.4%). Only few animal isolates were classified into group B2 (19/445, 4.3%). Differences in phylogenetic distribution among animal

isolates according to the source were detected. Almost all B2 isolates of animal origin were recovered from poultry where they represented the 8.3% (16/194) of the total isolates. Besides, group A isolates were more frequent among cattle and pig isolates (57/131, 43.5% and 48/120, 40%, respectively) then in poultry (54/194, 27.8%).

Overall, ST was defined for almost 63% (579/920) of all isolates included in this study (supplementary table 1). Out of 367 human isolates of the phylogroup B2, 327 (327/367, 89.1%) belonged to the pandemic ST131 clone, while among B2 isolates of animal origin only 3 were ST131 (3/19, 15.8%), all recovered from poultry. The majority of ST131 isolates of human origin carried CTX-M-15 (208/274, 75.9%), although CTX-M-27 (37 isolates), CTX-M-14 (13 isolates), CTX-M-1 (9 isolates), and other CTX-M variants were also found (data not shown). Conversely, all ST131 recovered from poultry contained SHV-12 but not a CTX-M enzyme.

Regarding the remaining non-ST131 isolates, a ST was assigned for a subset of isolates (66 human and 184 animal isolates). Non-ST131 isolates were disseminated among a number of different STs (n. 30 STs for human and n. 90 STs for animal isolates) with a few isolates within each ST (number of isolates per ST ranging from 1 to 12) (supplementary table 1). Notably, 19 STs (19/31 of human STs, 61.3%, and 19/91 of animal STs, 20.9%) including ST131 were shared by human and animal isolates with ST10, ST410, ST38, ST69, ST167 and ST1431 more frequently detected in both sources (table 5).

Distribution of STs in the 29 *mcr*-positive isolates by source is shown in supplementary table 2. We found 21 different STs, with all 3 human isolates belonging to ST131, and animal isolates having high diversity of STs (26 isolates belonged to 21 different STs). However, a poultry isolate belonged to ST131. The 2 isolates carrying both *mcr*-1 and *mcr*-3 or *mcr*-4 belonged to ST10 and ST1011, respectively.

4 Discussion

Antimicrobial resistance is now considered as a one of the most worrying global problem in both

public and animal health. The World Health Organization (WHO) foresees that the annual worldwide number of deaths caused by antimicrobial resistance will rise from 700,000 to 10 million, by year 2050. [22] The high prevalence of ESBL-producing *E. coli* in isolates from human infections is of concern and it is recommended to recognise their reservoirs and transmission routes. The debate on possible animal origin of antibiotic-resistant isolates in human infections is still open, but few studies have focused on this problematic with a "One Health" approach. In this study, we had the opportunity to investigate the AMR, the distribution of the ESBL types, the phylogenetic group and the MLST in 925 ESBL-producing isolates deriving from different source, humans and animals, to describe their characteristics and shared features.

Comparison of the resistance phenotypes of the human ESBL-producing *E. coli* isolates from UTI and sepsis with those isolated from FPA revealed that most ESBL-producing isolates from both sources were MDR (56% and 69%, respectively) and can share the major resistance patterns, although with different prevalence according to the source. Resistance to Cephalosporins, Fluoroquinolones and Aminoglycosides was the most frequent phenotype observed in human isolates, in line with data from the European Antimicrobial Resistance Surveillance Network (14.6% vs 6.2% of EU/EEA population weighted mean in the period 2015-2018). [23] In animal ESBL-producing *E. coli* isolates, resistance to Penicillins, Cephalosporins, Trimethoprim-Sulfamethoxazole was observed more frequently.

Few ESBL-producing *E. coli* isolates from both sources were resistant to carbapenems. It is already reported that resistance to carbapenems in *E. coli* is still uncommon in Europe; [4,23] furthermore, isolates from this study did not produce any carbapenemase, suggesting that other mechanisms, such as reduced outer membrane permeability by porin loss, might be involved.

Our analysis of ESBL genes revealed that the CTX-M types strongly prevailed in both human and animal isolates, this is consistent with previous studies highlighting that the CTX-M is now the prevalent ESBL in *E. coli*, partially substituting the SHV- and TEM-types. [24,25] However, while almost all human isolates carried a CTX-M ESBL (mainly of CTX-M-1 group), animal isolates also

harboured relevant proportions of CMY-2 and SHV enzymes. Compared to isolates from other sources poultry had a lower proportion of CTX-M types and showed a broader variety of ESBL enzymes. This might suggest that pig and cattle had a certain level of similarity in distribution of ESBL genes as in humans, whilst poultry differ, as reported already. [26,27] Nevertheless, considering the CTX-M variants, the CTX-M-15 enzyme, which largely predominated in human isolates, was detected in consistent percentages (ranging from about 30% to 50%, according to the animal species) of animal isolates including poultry. Similarly, the CTX-M-1 enzyme and other minor CTX-M variants were found in both human and animal isolates although with different frequencies, indicating that ESBL genes can be shared between the different sources. The possible horizontal transfer of ESBL gene placed on plasmids from animal to human isolates was not investigated since no plasmid analysis was carried out. This represents a limitation of our study and deserves future attention.

Phylogenetic distribution was different by source. Human isolates were prevalently classified in phylogroup B2 (76.5%), mainly for the predominance of the pandemic clone ST131, harbouring ESBL of CTX-M-15 type and showing additional resistance to several antibiotics. Conversely and as reported already, [18,28] one out of three animal isolates belong to phylogroup A and one out of four to B1. B2 isolates were found in few animal isolates (16 in poultry and 3 in pig), and ST131 was detected in 3 isolates from poultry. According to our results, the phylogroup and the genotype (ST131) more strongly associated with infections in humans was recovered exclusively in poultry isolates. Else from ST131, MLST analysis shows some shared STs although each including a few isolates. ST10 and ST410, both being relatively common in both sources, should be carefully followed up [18,29,30] as the latter has been recently suggested as a new high-risk clone capable to patient-to-patient transmission, causing hospital outbreaks. [31] In agreement with the results in this study, an investigation recently conducted in Germany on ESBL-producing *E.coli* isolates demonstrated clonal dissemination of ST410 in human and animal populations. [32]

The spread of ESBL-producing *E. coli* frequently associated to resistance to several commonly used

antimicrobial agents led to the use of old antibiotics, such as Colistin, [33] as the last-resource antibiotic for the treatment of MDR Enterobacterales in humans. The recent emergence of plasmid-mediated colistin resistance is a challenge in human medicine since it can reduce the therapeutic options for MDR caused infections. [15] As result of this study the occurrence of the plasmid-located colistin resistance genes *mcr* in both human and animal isolates phenotypically resistant to colistin is of greatest concern. All *mcr* gene-carrying isolates of human origin belonged to ST131 while the 26 *mcr*-positive animal isolates were included in several STs, indicating that this mobile element is now associated with the pandemic ST131 clone in humans and FPA may become a dangerous reservoir for this resistance. As previously described in other studies, [34] we found ST10 and ST1011 in animal isolates harboring *mcr* genes. Since its first description in China in 2016, *mcr*-1 gene and its variants have been reported worldwide in human and FPA, especially associated with ESBL production in *E. coli* isolates. [35] The proportion of *mcr*-1-carrying isolates detected in ESBL-producing *E. coli* from FPA in this study represents a serious public health threat that requires strict surveillance. Although no evidence of transmission of *mcr-1*-carrying isolates from animals to humans has been demonstrated, the occurrence of a *mcr*-1 ST131 isolate from poultry is of concern.

In conclusion, this work's findings suggest that, although ST131 clone dominating in human isolates was rarely found in isolates of animal origin, subgroups of ESBL-producing *E. coli* isolates from FPA may share genotypes (STs) and/or ESBL genes with isolates from humans. In addition, the high proportions of *mcr*-carrying isolates detected in *E. coli* from FPAs, including one ST131 isolate, represents a serious public health threat that requires strict surveillance.

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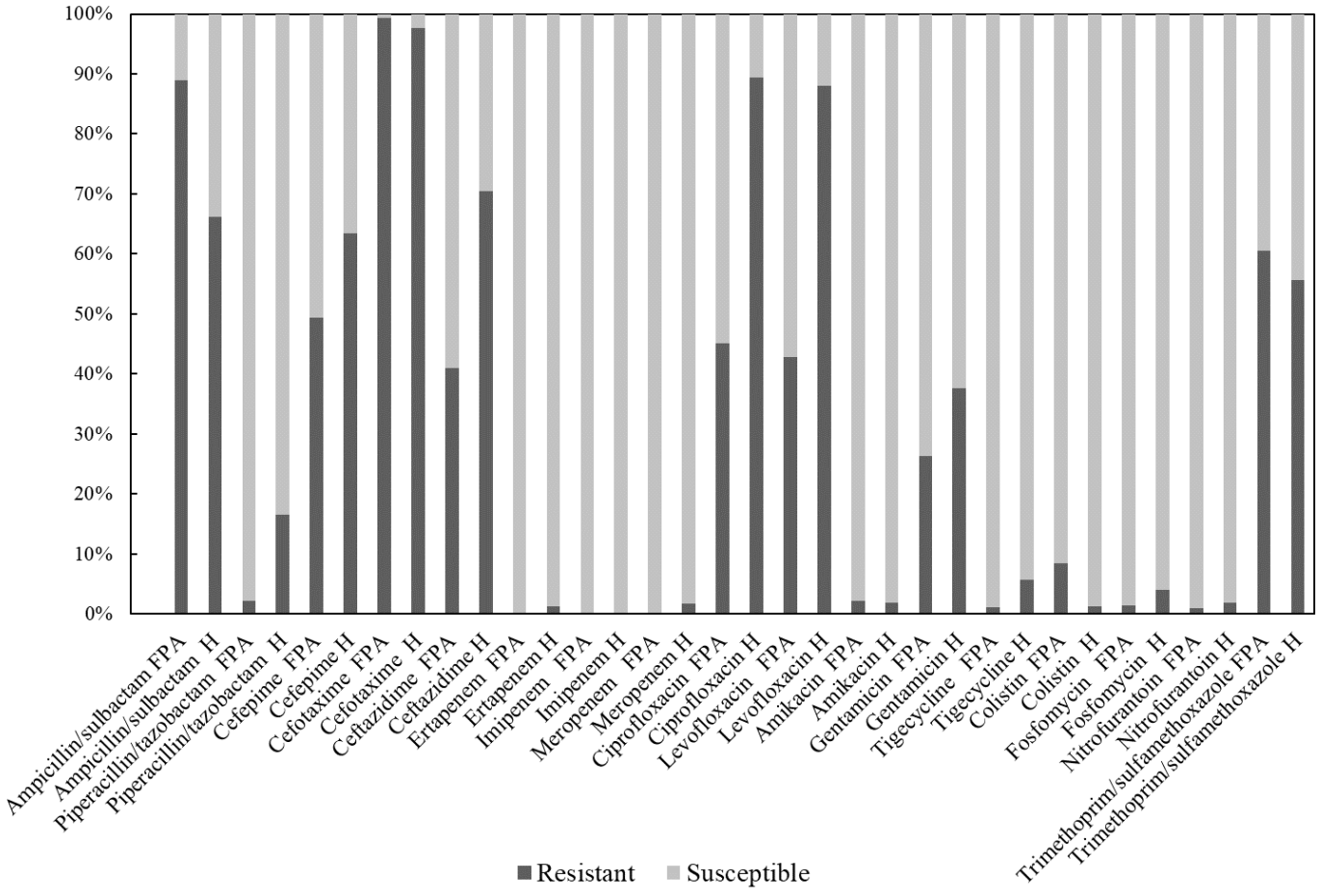
Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at JAC online.

Figure 1. Antimicrobial resistance profiles of 925 ESBL-producing *E. coli* isolates (480 from humans and 445 from food-producing animals)



376 Figure 2. Distribution of CTX-M-type enzymes among ESBL-producing *E. coli* isolates from

377 humans and food-producing animals (divided by species).

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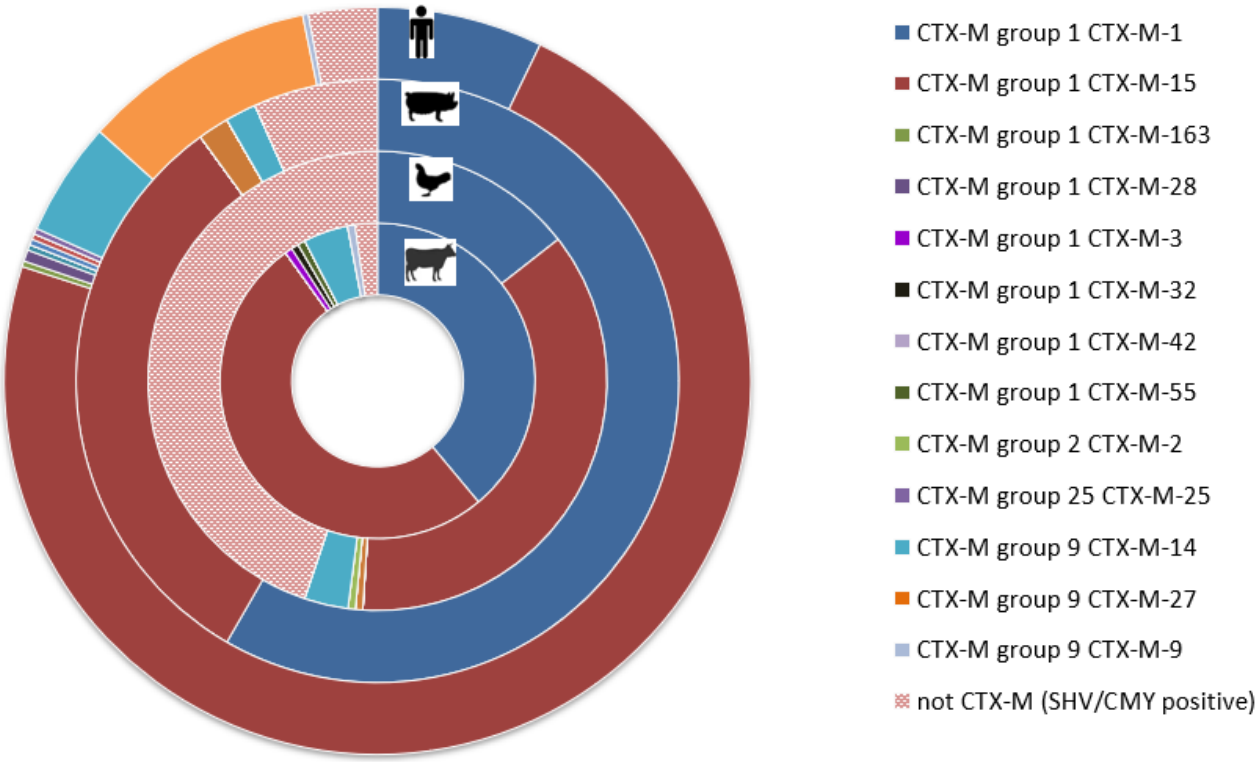

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380 Table 1. Resistance combinations among ESBL- *E. coli* isolates showing a MDR phenotype

381 stratified by source and tested against antimicrobial agents of different classes

Resistance patterns		**Human (No=480)**
Resistance to three antimicrobial agents of different classes		
Penicillins+Cephalosporins+Fluoroquinolones		23
Penicillins+Cephalosporins+Trimethoprim/Sulfamethoxazole		5
Penicillins+Cephalosporins+Aminoglycosides		0
Penicillins+Fluoroquinolones+Aminoglycosides		0
Penicillins+Trimethoprim/Sulfamethoxazole+Aminoglycosides		0
Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides		0
Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole		54
Cephalosporins+Fluoroquinolones+Aminoglycosides		70
Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides		0
Penicillins+Cephalosporins+Colistin		0
Cephalosporins+Fluoroquinolones+Colistin		1
	total	153
Resistance to four antimicrobial agents of different classes		
Penicillins+Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole		16
Penicillins+Cephalosporins+Fluoroquinolones+Aminoglycosides		24
Penicillins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides		0
Penicillins+Cephalosporins+Trimethoprim/Sulfamethoxazole+Aminoglycosides		9
Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides		46
Penicillins+Cephalosporins+Trimethoprim/Sulfamethoxazole+Colistin		0
Penicillins+Cephalosporins+Fluoroquinolones+Colistin		1
Cephalosporins+Fluoroquinolones+Aminoglycosides+Colistin		1
Cephalosporins+Fluoroquinolones+Colistin+Tigecycline		0
	total	97
Resistance to five antimicrobial agents of different classes		
Penicillins+Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides		16
Penicillins+Cephalosporins+Trimethoprim/Sulfamethoxazole+Aminoglycosides+Colistin		0
Penicillins+Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Colistin		0
Penicillins+Cephalosporins+Fluoroquinolones+Aminoglycosides+Colistin		0
Penicillins+Cephalosporins+Fluoroquinolones+Colistin+Tigecycline		0
Cephalosporins+Fluoroquinolones+Aminoglycosides+Trimethoprim/Sulfamethoxazole+Colistin		2
	total	18
Resistance to six antimicrobial agents of different classes		
Penicillins+Cephalosporins+Fluoroquinolones+Trimethoprim/Sulfamethoxazole+Aminoglycosides+ Colistin		1
Penicillins+Cephalosporins+Trimethoprim/Sulfamethoxazole+Aminoglycosides+Colistin+Tigecycline		0
	total	1
total number of MDR isolates		269

382 Table 2. Distribution of ESBL types among *E. coli* isolates stratified by source.

ESBL/pAmpC type	Source of isolates				
	Humans (N=480) n (%)	**Animals**[a] (N=445) n (%)	**Cattle** (N=131) n (%)	**Pig** (N=120) n (%)	**Poultry** (N=194) n (%)
CTX-M[b]	468 (97.5)	347 (78.0)	128 (97.7)	112 (93.3)	107 (55.2)
CTX-M group 1	367 (76.5)	318 (71.5)	120 (91.6)	105 (87.5)	93 (47.9)
CTX-M group 2	-	1 (0.2)	-	-	1 (0.5)
CTX-M group 9	94 (19.6)	15 (3.4)	7 (5.3)	2 (1.7)	6 (3.1)
CTX-M group 25	1 (0.2)	-	-	-	-
SHV[b]	4 (0.8)	34 (7.6)	-	1 (0.8)	33 (17.0)
SHV-12	2 (0.4)	30 (6.7)	-	-	30 (15.5)
pAmpC[b]	5 (1.0)	67 (15.1)	1 (0.8)	8 (6.7)	58 (29.9)
CMY-2-like	1 (0.2)	54 (12.1)	-	4 (3.3)	50 (25.8)
Combined ESBL					
CTX-M group 1-SHV-5	1 (0.2)	-	-	-	-
CTX-M group 1-SHV-12	1 (0.2)	2 (0.4)	-	1 (0.8)	1 (0.5)
CTX-M group 1- CMY-2-like	4 (0.8)	11 (8.1)	1 (0.8)	4 (3.3)	6 (3.1)
SHV-12- CMY-2-like	-	2 (0.4)	-	-	2 (1.0)

387 [a] Total animal isolates irrespective of the animal species
388 [b] Total number of isolates positive for the gene, regardless of whether the gene was present alone or in combination with other ESBL
389 types
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391 Table 3. Isolates carrying mobile colistin resistance *mcr*-genes described according to the source,

392 colistin resistance phenotype and associated ESBL gene

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Source	Samples (No.)	Colistin R (%)	*mcr*-1 (%)	*mcr-1*+ *mcr*-3 (%)	*mcr1*+ *mcr*-4 (%)	CTX-M-1	CTX-M-15	CTX-M-32	CTX-M-2	CTX-M-14	CMY-2-like	SHV-12
Humans	480	5 (1.0)	3 (0.6)[a]	0	0	1	2	0	0	0	0	0
Urine	377	4 (1.1)	2 (0.5)	0	0	1	1	0	0	0	0	0
Blood	103	1 (1.0)	1 (1.0)	0	0	0	1	0	0	0	0	0
Animals	445	37 (8.3)	24 (5.4)	1 (0.2)	1 (0.2)	5	16	1	1	1	1	1
Cattle	131	8 (6.1)	6 (4.6)	1	0	0	6	1	0	1	0	0
Pig	120	13 (10.8)	8 (6.7)	0	1	3	6	0	0	0	0	0
Poultry	194	16 (8.2)	9 (4.6)	0	0	1	4	0	1	0	1	1

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403 Table 4. Phylogenetic group distribution for 925 ESBL-producing *E. coli* isolates stratified by
404 source
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Source	Phylogenetic group														Total
	A	(%)	B1	(%)	B2	(%)	C	(%)	D	(%)	E	(%)	F	(%)	
Humans	41	8.5	28	5.8	367	76.5	16	3.33	13	2.7	6	1.3	9	1.9	480
Animals	159	35.7	116	26.1	19	4.3	55	12.4	29	6.5	28	6.3	39	8.7	445
Cattle	57	43.5	36	27.5	0	0	15	11.5	10	7.6	6	4.6	7	5.3	131
Pig	48	40.0	26	21.7	3	2.5	19	15.8	9	7.5	5	4.2	10	8.3	120
Poultry	54	27.8	54	27.8	16	8.3	21	10.8	10	5.2	17	8.8	22	11.3	194

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409 Table 5. Sequence types shared by ESBL-producing *E. coli* isolates from humans and food-

410 producing animals

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Sequence Type	Humans (392)[a]		Animals (187)[b]	
	n	%	n	%
ST131	327	83.4	3	1.6
ST410	12	3.1	9	4.8
ST10	8	2.0	11	5.9
ST38	4	1.0	3	1.6
ST69	3	0.8	6	3.2
ST167	3	0.8	3	1.6
ST1431	3	0.8	3	1.6
ST224	2	0.5	2	1.1
ST453	2	0.5	1	0.5
ST648	2	0.5	2	1.1
ST744	2	0.5	5	2.7
ST23	1	0.3	6	3.2
ST46	1	0.3	1	0.5
ST88	1	0.3	4	2.1
ST90	1	0.3	1	0.5
ST117	1	0.3	7	3.7
ST162	1	0.3	1	0.5
ST345	1	0.3	2	1.1
ST457	1	0.3	4	2.1

413 [a] total number of human isolates tested for ST

414 [b] total number of animal isolates tested for ST

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