Extended-spectrum β-lactamase-producing *Escherichia coli* from extraintestinal infections in humans and from food-producing animals in Italy: a 'One Health' study

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ABSTRACT

Recently, *Escherichia coli* producing extended-spectrum β-lactamases (ESBLs) have become a serious public-health problem, and food-producing animals (FPAs) have been suggested as a potential reservoir/source. This study aimed to compare ESBL-producing *E. coli* isolates from different sources. ESBL-producing *E. coli* isolates were collected from humans ($n = 480$) and FPAs ($n = 445$) in Italy (2016–2017). Isolates were screened for the presence of ESBL and carbapenemase genes and were classified according to phylogenetic group and MLST genotyping. The genes *mcr-1* to *-5* were searched for in colistin-resistant isolates. CTX-M was the most frequent ESBL type both in human and animal isolates. CTX-M-15 prevailed in humans (75.0%) and cattle (51.1%) but not in poultry (36.6%). CTX-M-1 was common (58.3%) in pigs. SHV-type and CMY-2-like were found in FPAs, especially in poultry (17.0% and 29.9%, respectively). Additionally, 29 isolates were *mcr-1* carriers (3 from humans and 26 from FPAs). No carbapenemase genes were detected. 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 FPAs (three isolates from poultry). Nineteen STs were shared in both sources, with ST10, ST410 and ST69 being more frequently detected. Potential exchange of ESBL genes from animals to humans is feasible, underlying the need for strict monitoring based on a 'One Health' approach.

1. Introduction

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

During the last decades, ESBL-producing *E. coli* isolates have also been increasingly isolated from non-human sources, including food-producing animals (FPAs), suggesting that animals may be at least in part the source of ESBL-producing *E. coli* for humans [8,9].

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Although the major ST131 clone is very rarely detected in isolates from FPAs, 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 animal isolates can share the same ESBL types and other demonstrating distinctive ESBL gene patterns according to the source [10,11].

In human clinical settings, carbapenem resistance mediated by plasmid-encoded carbapenemases is a further public-health risk, reducing therapeutic options for *E. coli* isolates already resistant to third-generation cephalosporins [12,13]. Although the use of carbapenems is highly limited in animals, and carbapenemases have been poorly detected so far in animals in Europe [14,15], the possibility of non-human reservoirs should be surveyed. The presence both in human and veterinary isolates of plasmid-mediated resistance to colistin, which is amongst the last-choice antimicrobials used to treat MDR isolates, raises questions on the occurrence of mobile colistin resistance (*mcr*) genes associated with ESBL-producing *E. coli* [16,17].

In 2016, we set up a 'One Health' tailored pilot surveillance network to monitor the occurrence of ESBL-producing *E. coli* in humans and FPAs. In this study, we report the antimicrobial susceptibility patterns, characterisation of the ESBL genes, co-resistance to colistin and carriage of *mcr* genes in ESBL-producing *E. coli* isolates both from humans with extraintestinal infections and FPAs. We also compared the phylogenetic groups and sequence types (STs) of human and animal isolates in order 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 institutions in six Italian regions (Friuli Venezia Giulia, Trentino Alto Adige, Veneto, Lombardia, Lazio and Sicilia) so as 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 collect monthly the 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 routine diagnostic activity.

ESBL-producing *E. coli* from FPAs were isolated by selective culture of faeces or caecal intestinal contents from clinical/non-clinical cases in the frame of animal health surveillance activities conducted by three institutions for animal health (Istituti Zooprofilattici) operating within the Italian public health service. Only one isolate per herd was enrolled and the contribution of each institute (covering different Italian regions) to the overall number of isolates was proportional to the Italian production, thus regions with higher animal production contributed more to the final sample size.

2.2. Detection of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and antimicrobial susceptibility testing

Human *E. coli* isolates were detected and identified to species level and antimicrobial susceptibility testing was performed according to standard laboratory procedures with automated methods in use in the participating laboratories (VITEK®2, bioMérieux Italia SpA, Florence, Italy; and/or BD Phoenix™, Becton Dickinson Italia SpA, Milan, Italy). Presumptive ESBL-producing *E. coli* were detected based on cephalosporin susceptibility: isolates resistant or with reduced susceptibility to third- and/or fourth-generation cephalosporins [minimum inhibitory concentration (MIC) >1 mg/L for at least one among cefotaxime, ceftazidime and cefepime] were selected and included in the study. For the presumptive ESBL-producing animal *E. coli* isolates, samples were cultured in selective enrichment broth (brain–heart infusion) supplemented with 1 mg/L cefotaxime and subsequently isolated on MacConkey agar supplemented with 1 mg/L cefotaxime. Once identified as *E. coli* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (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 characterisation.

For both origins of presumptive ESBL/AmpC-producing *E. coli* isolates, ESBL production was confirmed by the double-disk synergy test (Total ESBL Confirm Kit; ROSCO Diagnostica A/S, Taastrup, Denmark).

Antimicrobial susceptibility testing of 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).

In addition to cephalosporins, confirmed ESBL-producing isolates from humans 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 (SXT). An isolate was defined as MDR when it was resistant to at least three antimicrobial agents of different classes [18].

Interpretative breakpoints were based on the 2019 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria v.9.0 (https://eucast.org/clinical_breakpoints/). On the basis of the breakpoints, the *E. coli* isolates were then classified as resistant or susceptible. The intermediate category, where present, was considered as susceptible. *Escherichia coli* ATCC 25922 was used as a control strain for antimicrobial susceptibility testing.

2.3. Characterisation of extended-spectrum β-lactamase (ESBL)- and carbapenemase-encoding genes

Phenotypically confirmed ESBL-producing isolates were tested for the presence of the main ESBL and/or plasmid AmpC (pAmpC) gene types ($bla_{\text{CTX-M}}$, bla_{SHV} and $bla_{\text{CMY-2}}$) by PCR and sequencing as reported previously [19]. 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 (NCBI) website (www.ncbi.nlm.nih.gov/blast/). Carbapenemase-encoding genes (bla_{VIM}, bla_{IMP}, bla_{NDM}, $bla_{\text{OXA-48}}$ and bla_{KPC}) were identified by PCR and sequencing [6]. Reference isolates present in our institute were used as ESBL- and/or carbapenemase-producing control strains for PCR, as used already in our previous works [6,19].

2.4. Colistin resistance and mcr screening

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 > 1 mg/L were confirmed by the reference broth microdilution method using TREK Sensititre custom panel ITGNEGF. *Escherichia coli* NCTC 13846 was used as a control for colistin resistance testing. 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 > 1 mg/L were screened for the

presence of the genes *mcr-1* to *-5* by multiplex PCR as previously described [20].

2.5. Molecular typing of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli isolates

ESBL-producing *E. coli* isolates were classified into seven major *E. coli* phylogenetic groups (A, B1, B2, C, D, E and F) following the PCR-based method described in details by Clermont et al. [21]. Briefly, a quadruplex PCR targeting *arpA* (400 bp), *chuA* (288 bp), *yjaA* (211 bp) and *TspE4.C2* (152 bp) genes was performed. Based on the quadruplex PCR results, an isolate was immediately assigned to B1, B2 or F phylogroup or required additional PCR for group A, C, D and E assignment with primers for *arpA* (301 bp) and *trpA* (219 bp) genes and *trpA* internal control primers (489 bp). 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 [22]. A random subset of the remaining *E. coli* isolates (including ~40% of the total number of 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). The subset chosen was representative of each phylogenetic group and of all hospitals/farms.

2.6. Data analysis

Data were collected, harmonised and stored. Correlations among variables were explored by χ^2 test, and multivariable analysis was performed with logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were provided for relevant variables with sufficient samples. Data analysis was performed using the R program v.3.6.3. Statistical significance was defined as a *P*-value of $\leq$0.05.

3. Results

3.1. Bacterial isolates

Overall, 925 phenotypically confirmed ESBL-producing *E. coli* isolates were selected and included in the study: 480 isolates (51.9%) were from humans and 445 (48.1%) were from FPAs. The 480 ESBL-producing *E. coli* isolates of human origin were collected from urine (n = 377; 78.5%) or blood (n = 103; 21.5%). The 445 ESBL-producing *E. coli* isolates of FPA origin were collected from cattle (n = 131; 29.4%), pigs (n = 120; 27.0%) and poultry (n = 194; 43.6%).

3.2. Antimicrobial susceptibility testing

Antimicrobial resistance profiles of the 925 ESBL-producing *E. coli* isolates are shown in Fig. 1. Most ESBL-producing *E. coli* isolates from both sources were resistant to cefotaxime (97.7% in humans and 99.3% in FPAs). Resistance to ciprofloxacin ranged between 45.1% in FPAs and 89.4% in humans. Resistance to gentamicin was detected in 37.6% of human isolates and 26.3% of animal isolates. A total of 42 isolates were resistant to colistin (5 from humans and 37 from FPAs). A few isolates (13 from humans and 1 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; 56.0%) and animal (307/445; 69.0%) origin exhibited a MDR phenotype (Table 1). Some combinations of resistance phenotypes appearing in humans did not appear or were uncommon in animals, and vice versa. In human isolates, the most frequent phenotype was resistance to cephalosporins, fluoroquinolones and aminoglycosides (70/480; 14.6%). This phenotype with additional resistance to SXT was observed in 9.6% (46/480) of the isolates. In animal isolates, instead the predominant MDR phenotype was resistance to penicillins, cephalosporins and SXT (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 antimicrobial classes (penicillins, cephalosporins, fluoroquinolones, aminoglycosides and SXT) was also detected (3.3% of human isolates and 10.3% of animal isolates). All but 1 of the 42 colistin-resistant isolates were MDR; nearly all were resistant to penicillins and cephalosporins.

3.3. Characterisation of extended-spectrum β-lactamase (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). The distribution of the ESBL types is detailed in Table 2. CTX-M was the most frequent ESBL type both in human and animal isolates. A $bla_{\text{CTX-M}}$ gene was present in almost all human isolates (468/480; 97.5%) and in 78.0% (347/445) of animal isolates, regardless of whether the gene was present alone or in combination with other ESBL types. Other ESBLs 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 ESBL between human and animal isolates was particularly marked (OR = 11.0, 95% CI 6.2–21.4; $P < 0.001$). Among animals, the percentage of isolates from poultry carrying CTX-M (107/194; 55.2%) was significantly lower than isolates from cattle (128/131; 97.7%) and pigs (112/120; 93.3%) (OR = 0.02, 95% CI 0.007–0.08; $P < 0.001$). Poultry isolates contained the widest spectrum of ESBL types compared with 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 (79.7% and 95.4% in human and animal 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 sequencing was completed for a large subset of CTX-M-positive human isolates (392/468; 83.8%) and for all 347 CTX-M-positive isolates from animals (Fig. 2). The most common enzyme was CTX-M-15 representing 75.0% (294/392) of all known CTX-M enzymes among human isolates and 50.7% (176/347) of those among animal 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 the 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%). CTX-M-1 was the most common CTX-M enzyme in pigs [70/120 (58.3%) vs. 38/120 (31.7%) for CTX-M-1 and CTX-M-15, respectively] (Fig. 2).

3.4. Isolates carrying mcr genes

By the reference broth microdilution method, 5 isolates from humans and 37 isolates from FPAs were found to be resistant to colistin (MIC range, 4 to $\geq$8 mg/L) according to the EUCAST clinical breakpoint (MIC > 2 mg/L). Among them, 29 isolates carried *mcr-1* (3 from humans and 26 of animal origin) (Table 3). Two of these isolates contained more than one *mcr* gene, namely one isolate with both *mcr-1* and *mcr-3* from cattle and the other isolate with both *mcr-1* and *mcr-4* from pig. No other *mcr* genes (*mcr-2* or

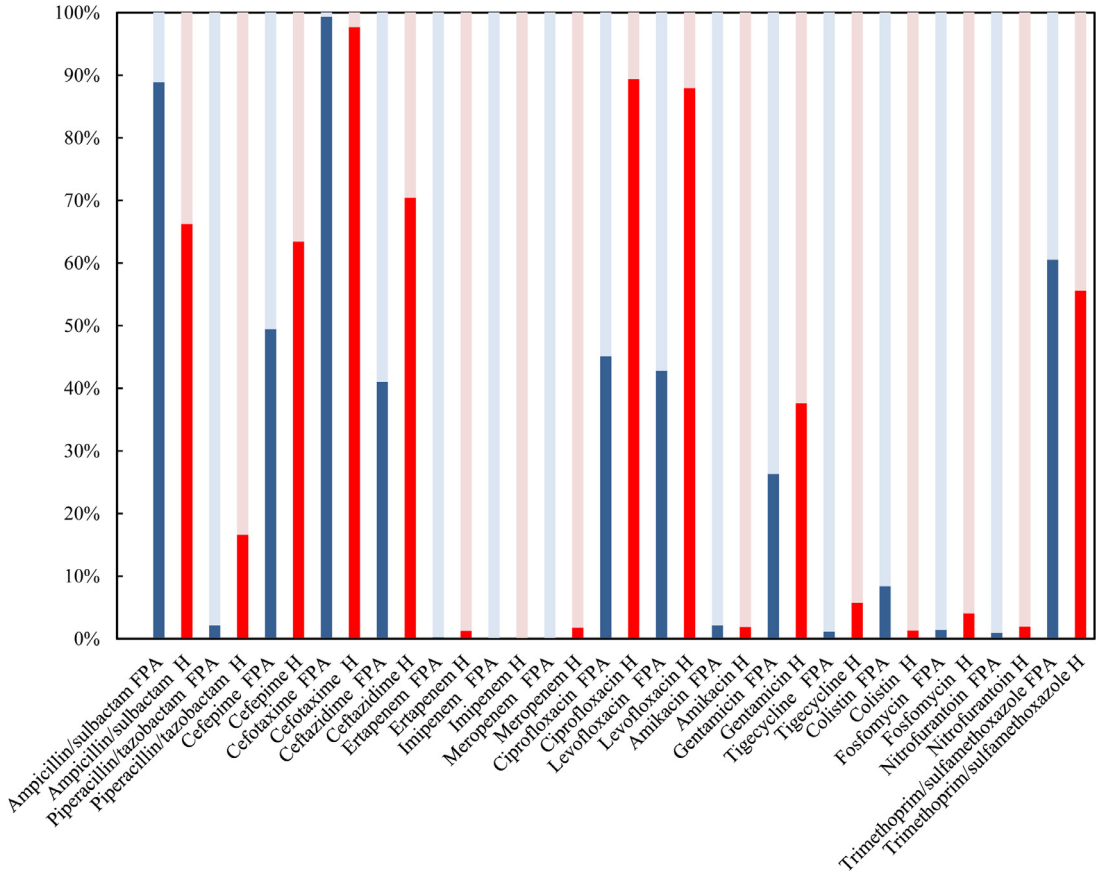

Fig. 1. Antimicrobial resistance profiles of 925 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates [480 from humans (H) and 445 from food-producing animals (FPA)]. Red/blue colour in each column indicate percentage resistant isolates for H and FPA isolates, respectively; light red/light blue colour indicate percentage susceptible isolates for H and FPA isolates, respectively.

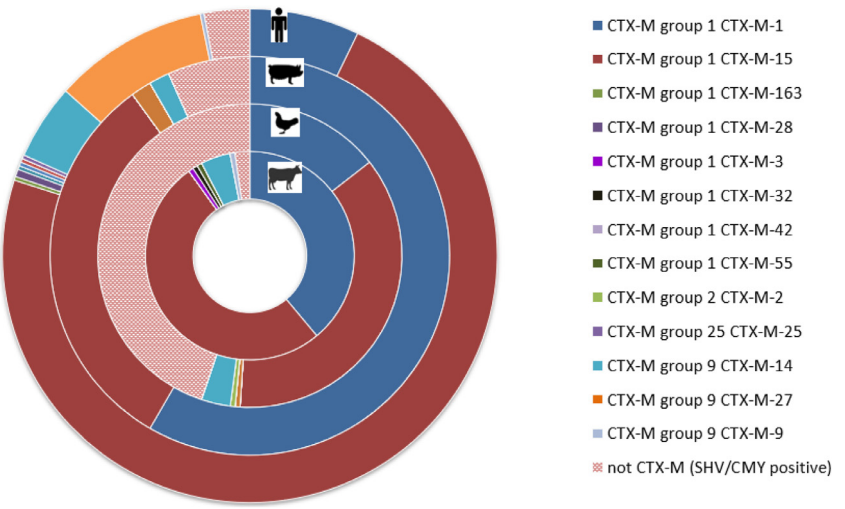

Fig. 2. Distribution of CTX-M-type enzymes among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from humans and food-producing animals (divided by species).

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. Mechanisms of colistin resistance other than *mcr* were not investigated. The distribution of *mcr* genes stratified by sources and associated ESBL genes are also shown in Table 3. The most frequently associated ESBL was CTX-M-15 followed by CTX-M-1 both in human and animal isolates. In two cases, both from animal sources, *mcr-1* was found in association with CMY-2-like or SHV-12.

3.5. Molecular typing

The distribution of all 925 ESBL-producing *E. coli* isolates stratified by source into phylogenetic groups is shown in Table 4. Hu-

Table 1
Resistance patterns among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates showing a multidrug-resistant (MDR) phenotype stratified by source and tested against antimicrobial agents of different classes

Resistance pattern	Humans ($N = 480$)		Animals ($N = 445$)	
	n	%	*n*	%
Resistance to three antimicrobial agents of different classes				
Penicillins + cephalosporins + fluoroquinolones	23	4.8	27	6.1
Penicillins + cephalosporins + SXT	5	1.0	84	18.9
Penicillins + cephalosporins + aminoglycosides	0	0.0	12	2.7
Penicillins + fluoroquinolones + aminoglycosides	0	0.0	0	0.0
Penicillins + SXT + aminoglycosides	0	0.0	0	0.0
Fluoroquinolones + SXT + aminoglycosides	0	0.0	0	0.0
Cephalosporins + fluoroquinolones + SXT	54	11.3	8	1.8
Cephalosporins + fluoroquinolones + aminoglycosides	70	14.6	7	1.6
Fluoroquinolones + SXT + aminoglycosides	0	0.0	0	0.0
Penicillins + cephalosporins + colistin	0	0.0	1	0.2
Cephalosporins + fluoroquinolones + colistin	1	0.2	0	0.0
Total	153	31.9	139	31.2
Resistance to four antimicrobial agents of different classes				
Penicillins + cephalosporins + fluoroquinolones + SXT	16	3.3	60	13.5
Penicillins + cephalosporins + fluoroquinolones + aminoglycosides	24	5.0	11	2.5
Penicillins + fluoroquinolones + SXT + aminoglycosides	0	0.0	0	0.0
Penicillins + cephalosporins + SXT + aminoglycosides	9	1.9	12	2.7
Cephalosporins + fluoroquinolones + SXT + aminoglycosides	46	9.6	3	0.7
Penicillins + cephalosporins + SXT + colistin	0	0.0	9	2.0
Penicillins + cephalosporins + fluoroquinolones + colistin	1	0.2	1	0.2
Cephalosporins + fluoroquinolones + aminoglycosides + colistin	1	0.2	0	0.0
Cephalosporins + fluoroquinolones + colistin + tigecycline	0	0.0	1	0.2
Total	97	20.2	97	21.8
Resistance to five antimicrobial agents of different classes				
Penicillins + cephalosporins + fluoroquinolones + SXT + aminoglycosides	16	3.3	46	10.3
Penicillins + cephalosporins + SXT + aminoglycosides + colistin	0	0.0	6	1.3
Penicillins + cephalosporins + fluoroquinolones + SXT + colistin	0	0.0	5	1.1
Penicillins + cephalosporins + fluoroquinolones + aminoglycosides + colistin	0	0.0	2	0.4
Penicillins + cephalosporins + fluoroquinolones + colistin + tigecycline	0	0.0	1	0.2
Cephalosporins + fluoroquinolones + aminoglycosides + SXT + colistin	2	0.4	0	0.0
Total	18	3.8	60	13.5
Resistance to six antimicrobial agents of different classes				
Penicillins + cephalosporins + fluoroquinolones + SXT + aminoglycosides + colistin	1	0.2	10	2.2
Penicillins + cephalosporins + SXT + aminoglycosides + colistin + tigecycline	0	0.0	1	0.2
Total	1	0.2	11	2.5
Total no. of MDR isolates	269	56.0	307	69.0

SXT, trimethoprim/sulfamethoxazole.

Table 2
Distribution of extended-spectrum β-lactamase (ESBL) types among *Escherichia coli* isolates stratified by source

ESBL/pAmpC type	Humans ($N = 480$) [*n* (%)]	Animals[a] ($N = 445$) [*n* (%)]	Cattle ($N = 131$) [*n* (%)]	Pigs ($N = 120$) [*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 ESBLs					
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 (2.5)	1 (0.8)	4 (3.3)	6 (3.1)
SHV-12 + CMY-2-like	–	2 (0.4)	–	–	2 (1.0)

pAmpC, plasmid AmpC.
[a] Total animal isolates irrespective of the animal species.
[b] Total number of isolates positive for the gene, regardless of whether the gene was present alone or in combination with other ESBL types.

man ESBL-producing *E. coli* isolates mostly (76.5%) belonged to phylogenetic group B2, while animal isolates were mainly distributed among groups A (35.7%), B1 (26.1%) and C (12.4%). Only a few animal isolates were classified into group B2 (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 8.2% (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 (40.0%), respectively] than in poultry [54/194 (27.8%)].

Overall, the ST was defined for almost all (62.6%; 579/925) of the isolates included in this study (Fig. 3; Supplementary Table S1). Of 367 human isolates in phylogroup B2, 327 (89.1%) belonged to the pandemic ST131 clone, while among B2 isolates of animal

Table 3
Isolates carrying mobile colistin resistance (*mcr*) genes described according to the source, colistin resistance phenotype and associated extended-spectrum β-lactamase (ESBL) gene

Source	No. of samples	Colistin R [*n* (%)]	*mcr-1* [*n* (%)]	*mcr-1* + *mcr-3* [*n* (%)]	*mcr-1* + *mcr-4* [*n* (%)]	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)	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	0	0	1	0	0
Pigs	120	13 (10.8)	9 (6.7)	0	1	3	6	1	0	0	0	0
Poultry	194	16 (8.2)	9 (4.6)	0	0	2	4	0	1	0	1	1

R, resistant.

Table 4
Phylogenetic group distribution for 925 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates stratified by source

Source	Phylogenetic group [*n* (%)]							Total
	A	B1	B2	C	D	E	F	
Humans	41 (8.5)	28 (5.8)	367 (76.5)	16 (3.3)	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 (8.8)	445
Cattle	57 (43.5)	36 (27.5)	0 (0)	15 (11.5)	10 (7.6)	6 (4.6)	7 (5.3)	131
Pigs	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 (8.2)	21 (10.8)	10 (5.2)	17 (8.8)	22 (11.3)	194

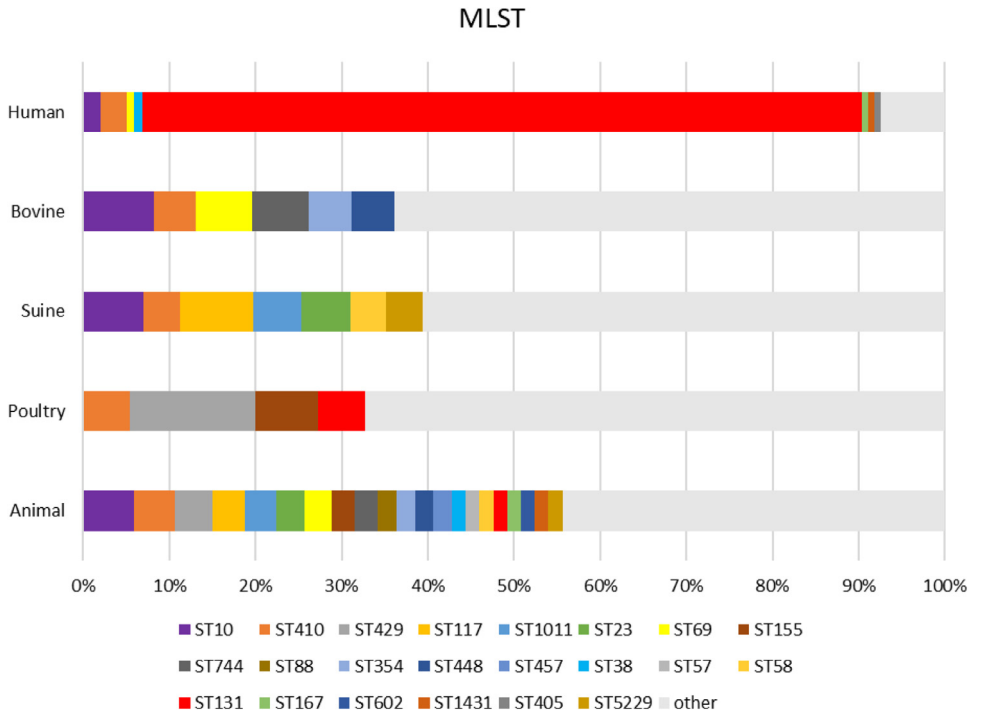

Fig. 3. Distribution of the most frequent sequence types (STs) amongst 579 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates recovered from humans and food-producing animals. Refer to Supplementary Table S1 for a more detailed list of the STs.

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 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 random subset of isolates (66 human and 184 animal isolates). Fig. 3 shows the most frequent STs for the ESBL-producing *E.coli* isolates along with their distribution according to the source. Non-ST131 isolates were disseminated among a number of different STs (n = 30 STs for human isolates and n = 90 STs for animal isolates) with a few isolates within each ST (number of isolates per ST ranging from 1–12) (Fig. 3; Supplementary Table S1). ST131 was the most frequent ST in *E. coli* isolates from humans; ST10, ST69 and ST744 were most represented STs among isolates from cattle; ST117, ST10, ST23 and ST1011 from pigs; and ST429 and ST155 from poultry. Notably, 19 STs [19/31 (61.3%) human STs and 19/91 (20.9%) animal STs] including ST131 were shared by human and animal isolates, with ST10, ST410, ST38, ST69, ST167 and ST1431 more frequently detected in both sources (Fig. 3; Table 5).

The distribution of STs in the 29 *mcr-1*-positive isolates by source is shown in Supplementary Table S2. We found 21 different STs, with all 3 human isolates belonging to ST131 and animal isolates having a high diversity of STs (26 isolates belonged to 21 different STs). However, a poultry isolate belonged to ST131. The

Table 5
Sequence types (STs) shared by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from humans and food-producing animals (FPAs)

	Humans (N = 392)[a]		FPAs (N = 187)[b]	
ST	*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

[a] Total number of human isolates tested for ST.
[b] Total number of FPA isolates tested for ST.

two 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 one of the most worrying global public-health problems both in human and animal health sectors. 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 the year 2050 [23]. 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 the possible animal origin of antibiotic-resistant isolates in human infections is still open, but few studies have focused on this problem with a 'One Health' approach. In this study, we had the opportunity to investigate the antimicrobial resistance, distribution of ESBL types, phylogenetic groups and MLST STs in 925 ESBL-producing isolates derived from human and animal sources in order to describe their characteristics and shared features.

Comparison of the resistance phenotypes of the human ESBL-producing *E. coli* isolates from urine and blood with those isolated from FPAs revealed that most ESBL-producing isolates from both sources were MDR (56.0% and 69.0%, respectively) and shared 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 EARS-Net (14.6% vs. 6.2% of EU/EEA population-weighted mean in the period 2015–2018) [24]. In animal ESBL-producing *E. coli* isolates, resistance to penicillins, cephalosporins and SXT was observed more frequently.

Fourteen ESBL-producing *E. coli* isolates from both sources were resistant to carbapenems. It has been previously reported that resistance to carbapenems in *E. coli* is still uncommon in Europe [4,24]; furthermore, isolates from this study did not produce any carbapenemase, suggesting that isolates could harbour carbapenemase genes not included in the panel used or that other resistance mechanisms, such as reduced outer membrane permeability owing to porin loss, might be involved.

Our analysis of ESBL genes revealed that the CTX-M types strongly prevailed both in human and animal isolates, which is consistent with previous studies highlighting that CTX-M is now the prevalent ESBL in *E. coli*, partially replacing the SHV and TEM types [25,26]. 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 with isolates from other sources, poultry isolates had a lower proportion of CTX-M types and showed a broader variety of ESBL enzymes. This might suggest that pigs and cattle had a certain level of similarity in distribution of ESBL genes as in humans, whilst poultry differ as previously reported [27,28]. Nevertheless, considering CTX-M type, the CTX-M-15 enzyme, which largely predominated in human isolates, was detected in consistent percentages (ranging from ~30% to 50% according to the animal species) of animal isolates including poultry. Similarly, the CTX-M-1 enzyme and other minor CTX-M enzymes were found both in human and animal isolates although with different frequencies, indicating that ESBL genes can be shared between the different sources. Possible horizontal transfer of ESBL genes harboured 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.

The phylogenetic distribution was different by source. Human isolates were prevalently classified in phylogroup B2 (76.5%), mainly due to the predominance of the pandemic clone ST131, harbouring ESBLs of CTX-M-15 type and showing additional resistance to several antibiotics, in line with previous findings [6,28]. Conversely, 35.7% of FPA isolates belonged to phylogroup A and 26.1% to phylogroup B1, as recently reported by Ibekwe et al. where phylogroups A and B1 were most prevalent amongst animal isolates (A = 30% and B1 = 29% of the isolates tested) [29]. Other papers reported similar results [19,30]; B2 isolates were found in only 4.3% of animal isolates (16 in poultry and 3 in pigs), and ST131 was detected exclusively in the 3 isolates from poultry. According to our results, the phylogroup (B2) and the genotype (ST131) more strongly associated with infections in humans were recovered exclusively in poultry isolates, as previously found [31]. Apart 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 [19,32,33] as the latter has been recently suggested as a new high-risk clone capable of patient-to-patient transmission, causing hospital outbreaks [34]. 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 [35].

The spread of ESBL-producing *E. coli* frequently associated with resistance to several commonly used antimicrobial agents has led to the use of old antibiotics such as colistin [17] as a 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 infections [16]. As result of this study, the occurrence of the plasmid-mediated mobile colistin resistance *mcr* genes both in human and animal isolates phenotypically resistant to colistin is of great concern. All *mcr* gene-carrying isolates of human origin belonged to ST131, while the 26 *mcr*-positive animal isolates were included in several STs. As previously described in other studies [36], we found ST10 and ST1011 in animal isolates harbouring *mcr* genes. Since its first description in China in 2016, the *mcr-1* gene and its variants have been reported worldwide in humans and FPAs, especially associated with ESBL production in *E. coli* isolates [37]. The proportion of *mcr-1*-carrying isolates detected in ESBL-producing *E. coli* from FPAs in this study represents a serious public-health threat that requires strict surveillance. Al-

though no evidence of transmission of *mcr-1*-carrying isolates from animals to humans has been demonstrated, the occurrence of an *mcr-1* ST131 isolate from poultry is of concern.

In conclusion, the findings of this work suggest that although the ST131 clone dominating in human isolates was rarely found in isolates of animal origin, subgroups of ESBL-producing *E. coli* isolates from FPAs 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.

Declaration of Competing Interests

None declared.

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Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2021.106433.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

TS: add in link

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