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Escherichia coli of human and avian origin: detection of clonal groups associated with fluoroquinolone and multidrug resistance in Italy

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Objectives: Poultry have been suggested as a reservoir for fluoroquinolone-resistant extraintestinal pathogenic *Escherichia coli* (ExPEC). Our aim was to investigate whether genotypes associated with ciprofloxacin and multidrug resistance were shared among human and avian *E. coli*.

Methods: We compared 277 human ExPEC isolates from urinary tract infection (UTI) and sepsis (142 susceptible and 135 ciprofloxacin resistant) and 101 avian isolates (68 susceptible and 33 ciprofloxacin resistant) by antimicrobial resistance phenotype, phylogenetic group and multilocus sequence type (ST).

Results: Most ciprofloxacin-resistant isolates from both human and avian sources were multidrug resistant. Human and avian isolates strongly differed in phylogenetic group assignment (B2 and A predominated among human and avian isolates, respectively), but a shift towards group A associated with ciprofloxacin resistance was observed among human isolates (8/100, 8.0% versus 17/87, 19.5%, $P=0.021$ for UTI and 5/42, 11.9% versus 15/48, 31.3%, $P=0.028$ for sepsis). Heterogeneity of ST clones was observed, with ST131 strongly predominant in human ciprofloxacin-resistant strains (58/135, 43.0%), but not in avian strains. However, two major ST clonal complexes (CCs; CC10 and CC23, both belonging to group A) associated with ciprofloxacin resistance and multiresistance were shared by human and avian isolates.

Conclusions: The major human and avian *E. coli* ST clones associated with multidrug resistance were identified. A subset of ST clones belonging to CC10 and CC23 poses a potential zoonotic risk.

Keywords: zoonosis, urinary tract infections, MLST, molecular epidemiology

Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) represents a huge public-health burden in human diseases, since it is the most common cause of community- and hospital-acquired urinary tract infections (UTIs) as well as bloodstream infections caused by Gram-negative bacteria.[1–3] The management of infections due to ExPEC has been increasingly complicated by the emergence and dissemination of resistance to commonly used antimicrobial agents such as trimethoprim/sulfamethoxazole, fluoroquinolones (FQs) and β-lactams, including extended-spectrum cephalosporins.[4,5] The speed with which FQs are losing their efficacy against ExPEC is an alarming public-health concern, especially considering that FQ-resistant ExPEC often exhibits a multidrug-resistant (MDR) phenotype. The worldwide dissemination of the *E. coli* sequence type (ST) 131 clone underlines the importance of clonal spread in the diffusion of FQ resistance.[6–9] Other epidemic and/or endemic drug-resistant clones or clonal groups have been identified, which are associated with resistance to FQs, β-lactams and/or trimethoprim/sulfamethoxazole, such as clonal complex (CC) 69, CC31 and ST393.[10,11] The source of these clones has yet to be established: have they arisen in the community or have they been acquired from an external source, e.g. food supply? Chickens have been suggested as reservoirs for FQ-resistant ExPEC strains.[12] FQs are widely used in farm animals, mainly in poultry, and FQ-resistant *E. coli* strains are frequently isolated from healthy and diseased birds.[13] Evidence supporting the hypothesis of the possible avian origin of FQ-resistant human ExPEC was found in several investigations, but other researchers reported partially contrasting results, including a previous study of our own.[14–17] Therefore, despite great efforts to shed light on the issue, a firm conclusion has yet to be reached. In almost all

studies on this subject, potential similarities or differences among *E. coli* isolates from both human and avian sources have been assessed by comparing distributions in the phylogenetic groups and prevalence of virulence genes. However, phylogenetic groups are entities too large and genetically heterogeneous to define by themselves the genetic background of strains. In addition, virulence typing results depend on the panel of virulence genes chosen to be tested, but no universal panels have been employed. Moreover, the data obtained by these kinds of studies are not easy to translate in terms of specific clones, such as *E. coli* ST131, universally recognized by the scientific community. Multilocus sequence typing (MLST) appears an appropriate tool to characterize the clonal structure within phylogenetic groups; the application of this technique can allow the identification and comparison of genotypes associated with antimicrobial resistance phenotypes.

In the present study, we compared *E. coli* isolates from both human extraintestinal infections and healthy avian sources, stratified by FQ resistance phenotype, with respect to multidrug resistance profile, phylogenetic group and MLST type. The aim of the investigation was to identify genotypes associated with resistance to FQ and other antimicrobial agents, and to determine whether these genotypes were shared or not among human and avian *E. coli* isolates.

Materials and methods

Bacterial strains

A total of 378 non-duplicated *E. coli* strains were used in this study: 277 strains were isolated from humans with extraintestinal infections and 101 from healthy avian sources. Out of 277 human ExPEC strains, 187 strains were recovered from individuals with UTI (87 ciprofloxacin-resistant and 100 ciprofloxacin-susceptible strains) and 90 strains from individuals with sepsis (48 ciprofloxacin-resistant and 42 ciprofloxacin-susceptible strains). Fifty UTI patients (50/187, 27%) were children ≤15 years old, while no isolates from blood were from children. ExPEC strains were collected from outpatients ($n=132$, all with UTI) and/or inpatients ($n=145$), regardless of gender, admitted to four different hospitals (General Hospital in Bergamo, 'Careggi' Hospital in Florence, 'Bambino Gesù' Paediatric Hospital in Rome and 'P. Giaccone' Hospital in Palermo) located in the north (Bergamo), centre (Florence and Rome) and south (Palermo) of Italy, during the period January–December 2009. Each participant hospital laboratory was asked to send: (i) all consecutive ciprofloxacin-resistant *E. coli* isolates from sepsis; (ii) one in every two consecutive ciprofloxacin-resistant isolates from UTI; and (iii) one strain susceptible to ciprofloxacin for each ciprofloxacin-resistant *E. coli* isolate from either sepsis or UTI randomly collected during the same period. Although the overall study period was 1 year in length, in each laboratory the sampling period changed, ranging from 2 weeks to 6 months, depending on the number of urine and/or blood cultures therein processed. Overall, 766 cases of ExPEC infections (662 UTI and 104 sepsis) were diagnosed; of these, 230 cases (181 UTI and 49 sepsis) were caused by ciprofloxacin-resistant isolates.

The 101 avian *E. coli* strains (68 ciprofloxacin susceptible and 33 ciprofloxacin resistant) included 91 strains recovered from chickens and 10 strains from turkeys. All strains were isolated from faeces of healthy animals and were collected during the *Salmonella* surveillance activities performed at poultry farms by three different regional Institutes of Animal Health (Istituto Zooprofilattico delle Venezie, Legnaro, Padova; Istituto Zooprofilattico della Lombardia e Emilia Romagna, Forlì; and Istituto Zooprofilattico dell'Umbria e Marche, Macerata) by including additional tests for *E. coli* detection, during the period January–December 2009. Only one *E. coli* isolate for each farm was included in this study.

Antimicrobial susceptibility testing

Microbial identification, antimicrobial susceptibility testing and screening for extended-spectrum β-lactamase (ESBL) production were each performed according to standard laboratory procedures by either conventional biochemical reactions and agar diffusion susceptibility tests or automated methods (Vitek 2, bioMérieux Italia Spa, Florence, Italy). The interpretative breakpoints were based on CLSI susceptibility criteria.[18] Both human and avian strains were tested for susceptibility to ampicillin, cefotaxime, ceftazidime, ciprofloxacin, trimethoprim/sulfamethoxazole and gentamicin. For each strain, ciprofloxacin susceptibility was confirmed by using the Etest (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar plates (Oxoid Ltd, Hampshire, UK). Suspected ESBL-producing strains were confirmed by Etest ESBL strips (AB Biodisk). An isolate was defined as MDR when it was resistant to at least three antimicrobial agents of different classes (ampicillin for penicillins, cefotaxime and/or ceftazidime for third-generation cephalosporins, ciprofloxacin for fluoroquinolones, trimethoprim/sulfamethoxazole for folate pathway inhibitors, and gentamicin for aminoglycosides).

Phylogenetic typing

E. coli strains were assigned to one of the four major *E. coli* phylogenetic groups (A, B1, B2 and D) by the multiplex PCR-based method described by Clermont *et al.*[19] Total DNA extracts were prepared using the rapid boiling method.

Screening of ST131

Human and avian *E. coli* strains belonging to phylogenetic group B2 were screened to identify the ST131 clone by a rapid PCR-based method, as previously described.[20] For confirmation, all PCR-positive strains were subjected to DNA sequencing of both *mdh* and *gyrB* housekeeping genes, in accordance with the *E. coli* MLST scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli).

MLST

Within each source (UTI/sepsis/avian), ∼30% of strains belonging to each of the four phylogenetic groups was subjected to MLST. In addition, irrespective of the phylogenetic group, an extra number of ciprofloxacin-resistant and MDR strains was analysed. Overall, MLST was performed on 172 *E. coli* strains [UTI ($n=83$), of these 22 were susceptible and 61 were resistant to ciprofloxacin; blood ($n=46$), of these 7 were susceptible and 39 were resistant to ciprofloxacin; and avian isolates ($n=43$), of these 19 were susceptible and 24 were resistant to ciprofloxacin], following procedures previously described.[21] Gene amplification and sequencing of the internal fragments from seven specific housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) were performed by using the primers specified on the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli). The allelic profiles of the seven gene sequences and the STs were obtained via the electronic database on the *E. coli* MLST web site.

Detection of ESBL-encoding genes

ESBL-producing strains were screened for identification of the β-lactamase-encoding genes (bla_{TEM}, bla_{CTX-M} and bla_{SHV}) by PCR amplification, as previously described.[9] DNA amplicons were sequenced to determine gene types. 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 web site (www.ncbi.nlm.nih.gov/blast/).

Sequence analysis

PCR fragments were purified with the Qiaquick PCR purification kit (Qiagen, Hilden, Germany). Sequencing was performed with both strands by using the fluorescent dideoxy-chain terminator method on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Data analysis and comparison of proportions were performed with Epi Info (version 3.5.1; CDC) by use of the χ^2 test or Fisher's exact test, as appropriate. Statistical significance was defined as a P value of ≤ 0.05.

Results

Antimicrobial susceptibility

Table 1 shows resistance combinations among 168 ciprofloxacin-resistant *E. coli* isolates stratified by source (UTI, $n=87$; sepsis, $n=48$; and avian, $n=33$) and tested against four additional antimicrobials of different classes (ampicillin, third-generation cephalosporins, trimethoprim/sulfamethoxazole and gentamicin). Only a few isolates of both UTI and avian origin, and no blood isolate, showed single ciprofloxacin resistance. MDR strains isolated from both UTI and avian sources more frequently showed triple resistance to ciprofloxacin, ampicillin and trimethoprim/sulfamethoxazole [27/87 (31.0%) for UTI and 19/33 (57.6%) for avian isolates]. This phenotype, with or without additional resistance to third-generation cephalosporins, was also common among isolates from sepsis [28/48 (58.3%) for both phenotypes together, with and without resistance to third-generation cephalosporins]. Resistance to all five antimicrobial agents (ampicillin, trimethoprim/sulfamethoxazole, gentamicin, third-generation cephalosporins and ciprofloxacin) was found in both UTI and blood isolates [5/87 (5.7%) and 5/48 (10.4%), respectively], but not among avian isolates. A different picture was observed among *E. coli* isolates susceptible to ciprofloxacin (data not shown). About half of these isolates [61/100 (61.0%) for UTI, 18/42 (42.9%) for sepsis and 28/68 (41.2%) for avian isolates] were fully susceptible to the additional antimicrobial agents tested. The most frequent phenotype was single resistance to ampicillin [17/100 (17.0%) for UTI, 15/42 (35.7%) for sepsis and 16/68 (23.5%) for avian isolates] followed by dual resistance to ampicillin and trimethoprim/sulfamethoxazole [15/100 (15.0%) for UTI, 8/42 (19.0%) for sepsis and 16/68 (23.5%) for avian isolates]. MDR strains were rarely observed [2/100 (2.0%) for UTI, 1/42 (2.4%) for sepsis and 3/68 (4.4%) for avian isolates].

ESBL production and characterization of ESBL-encoding genes

Of the 277 human ExPEC isolates, 48 (17.3%) were ESBL producers. A higher percentage of ESBL-positive strains was found among blood isolates compared with UTI isolates [27/187 (14.4%) for UTI and 21/90 (23.3%) for sepsis]. Notably, ESBL production was found to be associated with ciprofloxacin

Table 1. Resistance combinations among ciprofloxacin-resistant *E. coli* strains stratified by source (UTI, sepsis and avian) and tested against four additional antimicrobials of different classes

Resistance pattern	Human ExPEC strains ($N=135$)		Avian strains ($N=33$), *n* (%)
	UTI ($n=87$), *n* (%)	sepsis ($n=48$), *n* (%)	
Single resistance			
ciprofloxacin	6 (6.9)	0	2 (6.1)
Resistance to two antimicrobials of different classes			
ampicillin + ciprofloxacin	13 (14.9)	7 (14.6)	3 (9.1)
third-generation cephalosporins + ciprofloxacin	1 (1.1)	0	0
trimethoprim/sulfamethoxazole + ciprofloxacin	2 (2.3)	0	0
Resistance to three antimicrobials of different classes			
ampicillin + trimethoprim/sulfamethoxazole + ciprofloxacin	27 (31.0)	14 (29.2)	19 (57.6)
ampicillin + gentamicin + ciprofloxacin	4 (4.6)	3 (6.3)	1 (3.0)
ampicillin + third-generation cephalosporins + ciprofloxacin	7 (8.0)	1 (2.1)	3 (9.1)
gentamicin + trimethoprim/sulfamethoxazole + ciprofloxacin	2 (2.3)	0	0
third-generation cephalosporins + trimethoprim/sulfamethoxazole + ciprofloxacin	1 (1.1)	0	0
Resistance to four antimicrobials of different classes			
ampicillin + trimethoprim/sulfamethoxazole + gentamicin + ciprofloxacin	10 (11.5)	4 (8.3)	0
ampicillin + trimethoprim/sulfamethoxazole + third-generation cephalosporins + ciprofloxacin	6 (6.9)	14 (29.2)	5 (15.2)
ampicillin + gentamicin + third-generation cephalosporins + ciprofloxacin	3 (3.4)	0	0
Resistance to five antimicrobials of different classes			
ampicillin + trimethoprim/sulfamethoxazole + gentamicin + third-generation cephalosporins + ciprofloxacin	5 (5.7)	5 (10.4)	0

Table 2. Distribution of ESBL types among *E. coli* strains isolated from human extraintestinal infections (UTI and sepsis) and avian species

ESBL type	UTI strains ($N=27$), *n* (%)	PhG, ST, CC (*n*)	CIP^R, *n*	Sepsis strains ($N=21$), *n* (%)	PhG, ST, CC (*n*)	CIP^R, *n*	Avian strains ($N=8$), *n* (%)	PhG, ST, CC (*n*)	CIP^R, *n*
CTX-M-15 (group 1)	20 (74.1)	B2, 131, —[a] (16)	16	19 (90.5)	B2, 131, — (13)	13	0	0	0
		A, 617, 10 (1)	1		A, 617, 10 (1)	1			
		D, 648, — (1)	1		A, 559, 10 (2)	2			
		D, 405, 405 (2)	2		A, 410, 23 (2)	2			
					D, 648, — (1)	1			
CTX-M-15+SHV-12	1 (3.7)	B2, 131, — (1)	1	0	0	0	0	0	0
CTX-M-1 (group 1)	2 (7.4)	A, 167, 10 (1)	1	1 (4.8)	B2, 131, — (1)	1	3 (37.5)	A, 617, 10 (1)	1
		A, 410, 23 (1)	1					A, 1818, — (1)	0
								B1, 683, — (1)	0
CTX-M-2 (group 2)	0	0	0	0	0	0	2 (25.0)	D, 95, 95 (1)	0
								B2, 117, — (1)	1
CTX-M-14 (group 9)	0	0	0	0	0	0	2 (25.0)	B1, 602, 446 (2)	2
CTX-M-27 (group 9)	0	0	0	1 (4.8)	B2, 131, — (1)	1	0	0	0
TEM 20	1 (3.7)	B1, 1086, — (1)	0	0	0	0	0	0	0
TEM 52	1 (3.7)	D, 405, 405 (1)	1	0	0	0	0	0	0
SHV-12	1 (3.7)	A, 23, 23 (1)	0	0	0	0	1 (12.5)	D, 1011, — (1)	1
Unclassified	1 (3.7)	B1, 448, 448 (1)	1	0	0	0	0	0	0

PhG, phylogenetic group; CIP^R, resistant to ciprofloxacin.
[a]CC not defined in MLST database.

Table 3. Phylogenetic group distribution for 378 *E. coli* strains stratified by source (UTI, sepsis and avian) and by ciprofloxacin susceptibility status

	Ciprofloxacin-susceptible strains, $N=210$			Ciprofloxacin-resistant strains, $N=168$			P[a] (susceptible versus resistant)		
Phylogenetic group	UTI ($n=100$), *n* (%)	sepsis ($n=42$), *n* (%)	avian ($n=68$), *n* (%)	UTI ($n=87$), *n* (%)	sepsis, ($n=48$), *n* (%)	avian ($n=33$), *n* (%)	UTI	sepsis	avian
A	8 (8.0)	5 (11.9)	22 (32.4)	17 (19.5)	15 (31.3)	13 (39.4)	0.021	0.028	—
B1	9 (9.0)	8 (19.0)	18 (26.5)	5 (5.7)	4 (8.3)	12 (36.4)	—	—	—
B2	66 (66.0)	22 (52.4)	7 (10.3)	43 (49.4)	26 (54.2)	1 (3.0)	0.022	—	—
D	17 (17.0)	7 (16.7)	21 (30.9)	22 (25.3)	3 (6.3)	7 (21.2)	—	—	—

[a]Only significant *P* values ($P \leq 0.05$) are shown.

resistance in both UTI and sepsis isolates ($P<0.01$). Regarding avian isolates, 8 of 101 (7.9%) strains were ESBL producers.

Characterization of the ESBL genes is shown in Table 2. Among both UTI and blood isolates, most ESBL-producing strains [23/27 (85.2%) for UTI and 21/21 (100%) for sepsis] were CTX-M positive; all were also ciprofloxacin resistant. In particular, 40 human strains (21 UTI and 19 sepsis) contained $bla_{\text{CTX-M-15}}$, 3 strains (2 UTI and 1 sepsis) $bla_{\text{CTX-M-1}}$ and 1 strain from sepsis $bla_{\text{CTX-M-27}}$. One of the CTX-M-15-producing strains also contained the $bla_{\text{SHV-12}}$ gene. Of eight ESBL-producing strains isolated from avian species, seven (87.5%) contained a $bla_{\text{CTX-M}}$ gene. In particular, three strains carried $bla_{\text{CTX-M-1}}$, two strains $bla_{\text{CTX-M-2}}$ and two strains $bla_{\text{CTX-M-14}}$.

Phylogenetic analysis

Overall, human ExPEC strains mostly fell into the phylogenetic group B2, irrespective of the isolation site [109/187 (58.3%) for UTI and 48/90 (53.3%) for sepsis], whereas avian strains mainly belonged to group A [35/101 (34.7%)] closely followed by groups B1 [30/101 (29.7%)] and D [28/101 (27.7%)]. Table 3 shows the phylogenetic group distribution for the 378 *E. coli* isolates stratified by source and by ciprofloxacin susceptibility status. A significant increase in the proportion of group A strains associated with ciprofloxacin resistance was observed among both UTI and sepsis isolates [8/100 (8.0%) versus 17/87 (19.5%), $P=0.021$ for UTI and 5/42 (11.9%) versus 15/48 (31.3%), $P=0.028$ for sepsis]. Moreover, ciprofloxacin-resistant

isolates from UTI were found to belong significantly less frequently to group B2 in comparison with ciprofloxacin-susceptible isolates from the same source [66/100 (66%) versus 43/87 (49.4%), $P=0.022$].

In avian strains, no significant difference was observed between ciprofloxacin-susceptible and ciprofloxacin-resistant strains with respect to phylogenetic group distribution.

MLST analysis

All group B2 strains from the three sources were subjected to PCR screening methods for ST131 and those that were positive were confirmed by DNA sequencing. Overall, the 129 human ExPEC strains (83 strains from UTI and 46 from sepsis) analysed by MLST were distributed into 37 STs, irrespective of the ciprofloxacin susceptibility status, but only 6 of these STs comprised ≥3 strains. The 37 STs were grouped into 15 CCs, defined as groups of closely related STs differing by only one allele. As shown in Table 4, the predominant ST was ST131, comprising 37/109 (33.9%) phylogenetic group B2 strains isolated from UTI and 26/48 (54.2%) B2 strains from sepsis (37/109 versus 26/48, $P=0.01$). ST131 accounted for 39.1% (34/87) and for 30.8% (20/65) of all ciprofloxacin-resistant and MDR strains isolated from UTI, respectively, and for 50.0% (24/48) and 46.3% (19/41) of all ciprofloxacin-resistant and MDR strains isolated from sepsis, respectively. About half of the ST131 isolates [17/37 (46.0%) for UTI and 15/26 (57.7%) for sepsis isolates] were ESBL positive, with 32 CTX-M-1-group-producing strains and 1 CTX-M-27-producing isolate; the majority of them [20/37 (54.1%) for UTI and 19/26 (73.1%) for sepsis isolates] were MDR strains. The second most frequent ST associated with humans was ST410 (CC23), encompassing 7 isolates (3/83 for UTI and 4/46 for sepsis) that were predominantly ciprofloxacin-resistant and MDR strains. The remaining STs (including at least three human strains) linked to multidrug resistance and common to both UTI and sepsis isolates were ST559, ST617 (both belonging to CC10) and ST38. Conversely, ST405 only included UTI strains (all ciprofloxacin resistant and 2/4 MDR). Finally, ST638 (CC73) mainly comprised ciprofloxacin-susceptible strains isolated from UTI.

The 43 avian *E. coli* strains were widely distributed in 30 different STs and grouped into 10 CCs (Table 4). The prevalent STs (including at least three strains) were ST23 and ST156, both including ciprofloxacin-resistant and MDR strains. Only one strain belonged to ST131 [1/8 (12.5%) of total B2 strains]; it was susceptible to ciprofloxacin. Finally, we found a novel ST that was submitted to the MLST web site as ST2200 (only one ciprofloxacin-susceptible strain). Comparing UTI, sepsis and avian strains, and considering both STs and CCs, we observed that the majority of group A strains from all three sources belonged to the same CC10 and CC23 [altogether including 11/13 (84.6%) group A strains from UTI, 10/10 (100.0%) group A strains from sepsis and 15/21 (71.4%) group A strains of avian origin], with some of them also sharing the same ST (ST410, ST23, ST10 and ST617). Taking into consideration the number of strains analysed by MLST and stratified by source, CC10 and CC23 together accounted for 13.3% (11/83) of all UTI strains, for 21.7% (10/46) of all sepsis strains and for 34.9% (15/43) of all avian strains.

Discussion

The steady increase in the prevalence of FQ-resistant ExPEC isolates is particularly concerning and suggests a need to identify their origins, reservoirs and transmission pathways. In this study, comparison of the resistance phenotypes of the human ExPEC strains isolated from UTI and sepsis with those of the *E. coli* isolates from avian species revealed that: (i) most ciprofloxacin-resistant isolates from the three sources (74.7%, 85.4% and 84.8% for UTI, sepsis and avian isolates, respectively) were MDR strains; and (ii) human and avian isolates shared the major resistance patterns. The presence of overlapping resistance phenotypes between *E. coli* isolates from human and avian sources has been previously described.[22,23] In this study, evaluation of the potential zoonotic risk of the *E. coli* isolates from poultry relies on both changes in the phylogenetic group distribution associated with ciprofloxacin resistance and the identification of drug-resistant clones shared among human and avian strains. Regarding phylogenetic groups, overall, human ExPEC strains mainly belonged to group B2, but, when they were stratified by ciprofloxacin susceptibility, the proportion of resistant group A strains significantly increased. Considering that more than one-third of the avian *E. coli* isolates we tested belonged to group A and that chicken meat has previously been found dominated by A and B1 isolates,[22] this finding suggests the hypothesis that poultry might be a source for at least some human ciprofloxacin-resistant group A strains. The shift away from group B2 in drug-resistant ExPEC has been previously observed;[24,25] however, the recent emergence and diffusion of the ciprofloxacin-resistant B2 ST131 clone seemed to contradict these observations.[6–9] Here, by applying MLST, we confirmed the strong predominance of the ST131 clone among ExPEC strains carrying multiple antimicrobial resistances, especially those isolated from sepsis. However, besides this successful clone, two complexes (CC10 and CC23) including closely related STs (ST410, ST559, ST617 and others, each with a few strains) were associated with ciprofloxacin resistance and multiple resistances among UTI and sepsis strains. Notably, almost all phylogenetic group A human strains we analysed by MLST were distributed between these complexes (CC10 and CC23). Among the other minor STs, we would like to mention ST405 and ST38 (both belonging to phylogenetic group D), which were mainly detected among ciprofloxacin-resistant UTI strains. Reviewing the literature, we noticed that the ST clones herein detected were different from both those commonly found associated with UTI and/or extraintestinal infections in humans such as ST14, ST73 and ST95 complex (all belonging to group B2) and ST59 and ST69 (both belonging to D)[26–29] and those previously associated with trimethoprim/sulfamethoxazole and ciprofloxacin resistance such as ST95, CC31, CC69 and ST393 (all belonging to group D).[10,11,30] An exception was a Spanish study that, in agreement with us, found both CC10 and CC23 to be quite common among clinical ESBL-producing *E. coli*.[31]

Looking at avian strains, our findings demonstrated that the main ST clones associated with multidrug resistance were ST156 (belonging to the phylogenetic group B1) and ST23 (group A). ST156 was previously detected among avian isolates carrying ESBL CTX-M-15,[32] but no strains harbouring such an ESBL were found in the present study, while ST23 belonged to CC23. Similar to that observed in ExPEC, most ciprofloxacin-resistant phylogenetic group A strains from an avian source belonged to either CC10 or CC23, suggesting that these ST complexes might constitute a

Table 4. Distribution of MLST STs and CCs among *E. coli* strains from human extraintestinal infections (UTI and sepsis) and avian species, according to both phylogenetic group and antimicrobial susceptibility status

Human ExPEC strains ($N=129$)											
UTI ($n=83$)				sepsis ($n=46$)				Avian strains ($N=43$)			
CC (n)	ST (n)	CIP^R, n	MDR, n	CC (n)	ST (n)	CIP^R, n	MDR, n	CC (n)	ST (n)	CIP^R, n	MDR, n
Phylogenetic group A											
23 (5)	410 (3)	2	2	23 (6)	410 (4)	4	3	23 (11)	23 (8)	5	5
	23 (1)	0	0		23 (1)	1	1		410 (2)	2	1
	88 (1)	1	1		88 (1)	1	1		367 (1)	0	0
10 (6)	559 (2)	2	2	10 (4)	559 (2)	2	2	10 (4)	617 (1)	1	1
	10 (1)	0	0		10 (1)	1	1		744 (1)	1	1
	617 (2)	2	2		617 (1)	1	1		48 (1)	1	0
	167 (1)	1	1						10 (1)	1	1
—[a]	216 (1)	0	0					165 (1)	165 (1)	0	0
—	746 (1)	1	1					398 (1)	398 (1)	1	1
								—	347 (1)	0	0
								—	1626 (1)	0	0
								—	1251 (1)	0	0
								—	1818 (1)	0	0
Phylogenetic group B1											
469 (1)	162 (1)	1	1	469 (1)	162 (1)	1	1	156 (4)	156 (4)	3	2
448 (1)	448 (1)	1	1	448 (1)	448 (1)	1	1	155 (3)	155 (2)	1	1
155 (1)	58 (1)	1	1	—	1126 (1)	0	0		572 (1)	0	0
—	154 (1)	0	0	—	1434 (1)	0	0	446 (2)	602 (2)	2	0
—	1086 (1)	0	0					—	683 (1)	0	0
								—	424 (1)	1	1
Phylogenetic group B2											
—	131 (37)	34	20	—	131 (26)	24	19	—	131 (1)	0	0
73 (6)	638 (5)	1	0	12 (1)	12 (1)	1	1	—	117 (1)	1	1
	73 (1)	0	0					—	1638 (1)	0	0
95 (2)	95 (2)	0	0								
14 (1)	537 (1)	0	0								
—	141 (1)	1	1								
—	372 (1)	0	0								
—	707 (1)	1	0								
—	805 (1)	0	0								
—	952 (1)	1	1								
—	1282 (1)	0	0								
Phylogenetic group D											
405 (4)	405 (4)	4	2	31 (1)	393 (1)	1	1	350 (2)	350 (1)	0	1
38 (2)	38 (2)	2	2	38 (1)	38 (1)	0	0		57 (1)	1	1
69 (1)	69 (1)	0	1	69 (2)	69 (2)	0	0	95 (1)	95 (1)	0	0
354 (2)	354 (2)	2	2					354 (1)	354 (1)	1	0
394 (1)	394 (1)	0	0					—	362 (1)	0	0
—	648 (1)	1	1	—	648 (1)	1	1	—	648 (1)	1	1
—	854 (1)	1	1					—	1011 (1)	1	1
—	1011 (1)	1	0					—	2200 (1)	0	0

CIP^R, resistant to ciprofloxacin.
[a]CC not defined in MLST database.

potential zoonotic risk. Interestingly, we didn't find strong evidence for an ST131 reservoir in avian species, since only one avian ST131 strain was detected, in agreement with previous observations of other authors that only sporadically reported ST131 among poultry.[30,33] Based on our results, the ST131 clone remains human associated and predominates among ciprofloxacin-resistant strains, but can also be present among susceptible isolates, although in a low number.

Even though this study was not focused on 'antibiotic resistance genes', some consideration of the principal ESBL types circulating in human and avian strains is of interest, since poultry has been considered a potential reservoir for ESBL-producing Gram-negative bacteria.[34–36] Overall, ESBL genes differed between human and avian strains. In agreement with several previous studies, CTX-M-15 was the most common ESBL among human ExPEC strains isolated from both UTI and sepsis,[37,38] but it was not detected among our avian isolates. CTX-M-1 only was shared by UTI, sepsis and avian isolates, but the majority of strains carrying them appeared genetically diverse (with regard to both STs and CCs) among the sources, with the exception of two ciprofloxacin-resistant isolates (one from UTI and one from an avian source) belonging to two genetically related ST clones (ST167 and ST617), both included in CC10. Obviously, since ESBL genes are generally located on plasmids, the horizontal transfer of these genes from chicken to humans could not be ruled out when the same ESBL genes were present, but this investigation was not carried out in the present study.

In conclusion, this investigation allows us to identify the major ST clones associated with ciprofloxacin resistance/multiresistance in both human and avian *E. coli* strains in Italy. Overall, heterogeneity of ST clones was observed, with ST131 strongly predominant in human strains, but not in avian strains. However, two major ST complexes (CC10 and CC23, both belonging to group A) were shared among strains isolated from all sources (UTI/sepsis/avian). This finding, together with the shift in phylogenetic distribution towards group A associated with ciprofloxacin resistance and observed only among human strains, supports the hypothesis that poultry might constitute a reservoir at least for a subset of well-defined ciprofloxacin-resistant/multiresistant clonal groups. However, further studies are required to determine their true zoonotic potential.

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Transparency declarations

None to declare.

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