High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical Escherichia coli from commercial poultry flocks in Italy

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Abstract

The aim was to investigate occurrence and diversity of plasmid-mediated resistance to third-generation cephalosporins (3GC) and quinolones in clinical Escherichia coli from 200 industrial poultry farms across Italy. E. coli was isolated from colibacillosis lesions in turkeys (n = 109), broilers (n = 98) and layers (n = 22) between 2008 and 2012. 3GC-resistant isolates were screened for extended-spectrum and AmpC β-lactamase (ESBL/AmpC), while all isolates were tested for plasmid-mediated quinolone resistance (PMQR) genes. ESBL/AmpC- and PMQR-positive isolates were typed by pulsed-field gel electrophoresis and antimicrobial susceptibility testing, and their plasmids were characterised by replicon typing, multilocus sequence typing, restriction fragment length polymorphism and conjugation. EBSL/AmpC genes (blaCTX-M-1, blaCTX-M-14, blaCTX-M-2, blaSHV-12 and blaCMY-2) were detected in 7%, 9% and 4% of isolates from turkeys, broilers and layers, respectively. We identified seven ESBL/AmpC-encoding plasmid types, usually conjugative (78%), with a marked prevalence of IncI1/pST3 plasmids carrying blaCTX-M-1. PMQR occurred less frequently among isolates from turkeys (0.9%) compared to those from broilers (5%) and layers (4%). The PMQR genes qnrS, qnrB19 and oqxA/B were located on three plasmid types and two non-typeable plasmids, mostly (85%) conjugative. ESBL/AmpC- and PMQR-positive isolates were genetically unrelated and 64% of them were additionally resistant to aminoglycosides, sulfonamides and tetracyclines. Our data show that 3GC- and quinolone-resistant clinical E. coli in Italian poultry production represent a highly diverse population often resistant to most antimicrobials available for poultry. These findings underline the crucial need to develop new strategies for prevention and control of colibacillosis.

1. Introduction

Colibacillosis is an avian disease caused by Escherichia coli and characterised by high morbidity, high mortality, reduced productivity and carcass condemnation. Antimicrobial treatment of infected flocks is crucial to prevent major economic losses to the poultry industry (Nolan et al., 2013). Thus, the spread of antimicrobial resistance in clinical E. coli from colibacillosis has important economic and animal health implications. Moreover, there is an increasing public health concern about zoonotic transmission of resistance to critically important antimicrobial classes such as third-generation cephalosporins (3GC) and fluoroquinolones (EFSA & ECDC, 2016). Both types of resistance can be mediated by conjugative plasmids that are transferrable across animal and human E. coli lineages (Carattoli, 2013).

Previous studies have evidenced high geographical variability in the occurrence of plasmid-mediated resistance to 3GC and fluoroquinolones in clinical E. coli, mainly broiler isolates (Briñas et al., 2005; Cerquetti et al., 2009; Yuan et al., 2009; Ahmed et al., 2013; Qabajah et al., 2014; Yang et al., 2014; Meguenni et al., 2015; Solà-Ginés et al., 2015; Awad et al., 2016; da Silva et al., 2017). Such variability likely reflects local antimicrobial usage practices as well as methodological differences between the studies. The same studies have also shown geographical differences in the distribution of ESBL/AmpC and plasmid-mediated quinolone resistance (PMQR) genes.

Taken together, these studies indicate the need to study the epidemiology of ESBL/AmpC and PMQR genes locally to gather information relevant for both veterinary therapy and public health. The aim of this study was to investigate occurrence and diversity of plasmid-mediated resistance to 3GC and quinolones in clinical E. coli from broilers, layers and turkeys from industrial poultry farms in Italy.

2. Materials and methods

2.1. Bacterial isolates

Bacterial isolates were obtained from the private diagnostic laboratories of the 200 poultry farms involved in the study. The farms were located in northern Italy which is the most densely populated poultry area (DPPA) in the country and were representative of the main poultry production in this country. Swab samples from lesions consistent with colisepticemia (e.g. pericarditis, perihepatitis or airsacculitis) were collected from diseased birds between 2008 and 2012. The strain collection consisted of 229 clinical E. coli from individual birds, of which 109 were from 89 turkey farms, 98 were from 91 broiler farms, and 22 were from 20 layer hen farms. The majority of isolates derived from flocks raised at different farms. In few cases, isolates were collected from different flocks raised at the same farm at different times (2 isolates/10 farms and 3/3 for turkeys, and 2/3 for broilers) and from the same flocks sampled at different times (2 isolates/5 flocks for turkeys, 2/2 and 3/1 for broilers, and 4/2 for layers). All the isolates were stored in 20% glycerol at -80 °C prior to phenotypic and genotypic characterization.

2.2. Detection and identification of ESBL/AmpC genes

All isolates were screened for 3GC resistance by disk diffusion using cefpodoxime (10 µg), ceftazidime (30 µg) and cefotaxime (30 µg) (CLSI, 2013). All disks were purchased from DID (Italy). Isolates resistant to at least one 3GC were subjected to PCR for blaTEM, blaCTX-M, blaSHV and blaCMY-2 using primers and conditions as previously described (Dierikx et al., 2012). blaCTX-M-positive isolates were further tested with the group- and variant-specific primers for blaCTX-M-1, blaCTX-M-2 (Dierikx et al., 2012), blaCTX-M-8 (Eller et al., 2013), blaCTX-M-9 (Bouallègue-Godet et al., 2005), blaCTX-M-14/17 (Dierikx et al., 2012) and blaCTX-M-25 (Chmelnitsky et al., 2005). Amplicons were sequenced (Macrogen Europe, The Netherlands). Sequence data were analysed using CLC Main Workbench 6.8.4 (CLC Bio, Denmark). Nucleotide sequences and derived amino acid sequences were compared with publicly available sequences (www.ncbi.nlm.nih.gov/ and www.lahey.org/studies/webt.html).

2.3. Detection and identification of PMQR genes

All isolates were screened by PCR with primers and conditions used in a previous study (Dotto et al., 2014) for qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxA, oqxB and those suggested by Kim et al. (2009) for aac(6')-Ib-cr. Amplicons were sequenced and analysed as mentioned above.

2.4. Plasmid characterisation

Plasmid DNA was isolated from isolates positive for ESBL/AmpC and PMQR genes by the alkaline extraction method (Birnboim and Doly, 1979) and transformed into electrocompetent Genehog E. coli (Invitrogen, Denmark). Transformants were selected on Brain Heart Infusion agar (Oxoid, Denmark) supplemented with 1 µg/mL of cefotaxime (CTX) for selection of ESBL/AmpC-positive transformants or 0.06 µg/mL of ciprofloxacin (CIP) for selection of PMQR-positive transformants. The presence of the relevant ESBL/AmpC

and PMQR genes was confirmed by PCR using the primers described above. S1 nuclease-digested genomic DNA from the transformants was used to determine plasmid number and size by pulsed field gel electrophoresis (PFGE) (Barton et al., 1995).

Plasmids from transformants were typed by PCR-based replicon typing (PBRT) using a commercial kit (Diatheva, IT) and, if typeable, by plasmid multi-locus sequence typing (pMLST) (https://pubmlst.org/plasmid/). The same approach was used to analyse plasmid content of the clinical E. coli isolates that were used as donors for the transformants. Furthermore, plasmid DNA from transformants was typed by restriction fragment length polymorphism (RFLP). Plasmid DNA was isolated using PureLink® HiPure Plasmid Midiprep Kit (Invitrogen, Denmark) and digested (on separate digestions) with BglII and PstI for IncI1-Iγ plasmids, EcorV and PstI for IncK plasmids, PstI, SalI and EcorI for IncX1 plasmids. All restriction enzymes were purchased from ThermoScientific (Sweden). Restriction profiles were visualised on 0.8% agarose gels and band patterns were compared by visual inspection. Plasmids showing indistinguishable profiles were designated with the same capital letter (e.g. 'A') and a number was added to indicate closely related subtypes (e.g. A1, A2, A3) differing by one or two bands.

To detect co-transfer of resistance to antimicrobials other than β-lactams and/or quinolones, transformants were tested by disk diffusion for susceptibility to chloramphenicol (30 μg), florfenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), sulfamethoxazole-trimethoprim (25 μg), tetracycline (30 μg), streptomycin (10 μg) and sulfonamides (30 μg) according to CLSI guidelines (2013). Human CLSI breakpoints were used for compounds without established veterinary breakpoints (2013).

Plasmid conjugative transfer was tested by filter-mating experiments using a rifampicin-resistant, lactose-negative E. coli J62-2 strain as recipient. Transconjugants were selected on MacConkey agar (Merck, Denmark) supplemented with 2 μg/mL CTX and 25 μg/mL rifampicin (for selection of ESBL/AmpC-positive transconjugants) or 0.06 μg/mL CIP and 25 μg/mL rifampicin (for selection of PMQR-positive transconjugants). Transfer of the relevant genes was confirmed by PCR as detailed above.

2.5. Strain typing

ESBL/AmpC- and PMQR-positive E. coli were characterised by XbaI-PFGE as previously described (Ribot et al., 2006). Salmonella enterica serovar Braenderup H9812 was used as molecular size marker. PFGE profiles were analysed with GelCompar II version 6.6.11 (Applied Maths, Belgium) using the Dice similarity coefficient and clustered by the unweighted pair group method with arithmetic averages. Band optimisation and position tolerance were set at 1%. Isolates were considered related if the Dice similarity index was ≥ 80%.

The same isolates were also tested by disk diffusion according to the CLSI (2013) guidelines. The following disks (Oxoid, UK) were used: ampicillin (10 μg), cefotaxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), florfenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), sulfamethoxazole-trimethoprim (25 μg), streptomycin (10 μg), sulfonamides (30 μg) and tetracycline (30 μg).

3. Results

3.1. Occurrence of ESBL/AmpC and PMQR genes

ESBL/AmpC genes were detected in 18 (8%) isolates (Table 1) with a prevalence of 7%, 9% and 4% among isolates from turkeys, broilers and layers, respectively. While blaCTX-M-1 was found in 12 isolates from all types of poultry production, blaCTX-M-14, blaCTX-M-2, blaSHV-12 and blaCMY-2 were sporadically detected (Table 1). More than half (n = 11) of the ESBL/AmpC-positive isolates additionally harboured blaTEM-1b (Table 1).

Table 1. Occurrence of β-lactam and plasmid-mediated quinolone resistance genes in clinical Escherichia coli from Italian poultry flocks.

Animal category (N)a	β-lactamase-encoding genes (n b; %)	PMQR genes (n b; %)
Turkeys (109)	blaCTX-M-1 (1; 0.9%)	qnrB19 (1; 0.9%)
	blaTEM-1b and blaCTX-M-1 (5; 4.5%)	
	blaCTX-M-2 (1; 0.9%)	
	blaTEM-1b and blaCTX-M-14 (1; 0.9%)	
Broilers (98)	blaCTX-M-1 (3; 3%)	qnrS1 (4; 4%)
	blaTEM-1b and blaCTX-M-1 (2; 2%)	oqxA/B (1; 1%)
	blaCTX-M-14 (1; 1%)	
	blaSHV-12 (1; 1%)	
	blaTEM-1b and blaCMY-2 (2; 2%)	
Layers (22)	blaTEM-1b and blaCTX-M-1 (1; 4.5%)	qnrS1 (1; 4.5%)

a

N, number of isolates examined.

b

n, number of isolates positive for the specific genes.

PMQR genes were identified in seven (3%) isolates with a prevalence of 0.9%, 5% and 4% among isolates from turkeys, broilers and layers, respectively (Table 1). qnrS1 was detected in four and one broiler and layer isolates, respectively. qnrB19 gene was identified in one turkey isolate, and oqxA/B was detected in one broiler isolate (Table 1). No isolates were found positive for qnrA, qnrC, qnrD, qepA and aac(6')-Ib-cr genes.

Co-existence of ESBL/AmpC and PMQR genes was not observed in any isolate and each ESBL/AmpC- or PMQR-positive isolate originated from a different farm.

3.2. Diversity of ESBL/AmpC-positive plasmids

Transformants were obtained from all the 18 ESBL/AmpC-positive donors, indicating that all ESBL/AmpC genes were plasmid-borne (Table 2). Plasmid size ranged from approximately 40 kb to 216 kb. The following incompatibility groups were identified: IncI1-Iγ (n = 12), IncI1-Iγ and IncP (n = 2), IncK (n = 2), IncFIB (n = 1) and IncN (n = 1). IncI1-Iγ plasmids were from all three bird categories and carried blaCTX-M-1 in isolates from broilers, turkeys and layers, and blaSHV-12 or blaCMY-2 in single broiler isolates. Despite having a similar size of approximately 104 kb, the IncI1-Iγ plasmids carrying blaCTX-M-1 exhibited diversity when subtyped by MLST and RFLP, and co-transferred resistance to different antimicrobials depending on plasmid sequence type (pST3 vs pST36) and bird species (Table 2). The one carrying blaSHV-12 was larger (approximately 138 kb) and belonged to pST26, as the one carrying blaCMY-2 (Table 2)

Table 2. Genetic and phenotypic traits of clinical Escherichia coli harbouring ESBL/AmpC and PMQR plasmids in Italian poultry.

Host species	Isolate	Year of isolation	Detected genes	Plasmid characterisation				Genetic background	
PBRT	pMLST or FAB	pRFLPa	Plasmid size (kb)b	Transferability c	PBRT of wild-type		Antimicrobial resistance d		
Tukeys	E146	2008	blaCTX-M-2	I1-Iγ, P	ST26	B	~138	positive	I1-Iγ, P, FIB, FII	AMP, CIP, CTX, NAL, SSS, SXT, TET
	E145	2010	blaCTX-M-1	I1-Iγ	ST3	E2	~104,5	positive	I1-Iγ, FIB, FII	AMP, CTX, NAL, SSS, STR, SXT, TET
	E170	2010	blaCTX-M-1	I1-Iγ	ST3	E2	~104,5	negative	I1-Iγ, FIB, FII, K	AMP, CTX, NAL, SSS, STR, SXT, TET
	E148	2010	blaCTX-M-1	I1-Iγ	ST3	E3	~104,5	positive	I1-Iγ, FIB, FII	AMP, CTX, NAL, SSS, STR, SXT, TET
	E158	2010	blaCTX-M-1	I1-Iγ	ST3	E3	~104,5	positive	I1-Iγ, FIB, FII, HI1, HI2, N, P, X1	AMP, CTX, GEN, NAL, SSS, STR, SXT, TET
	E159	2010	blaCTX-M-1	I1-Iγ	ST3	E4	~104,5	positive	I1-Iγ, FIB, FII	AMP, CHL, CIP, CTX, KAN, NAL, SSS, STR, SXT, TET
	E172	2010	blaCTX-M-1	N	ST1	–	~40	positive	N, FIB, FII, I1-Iγ,	AMP, CHL, CTX, GEN, NAL, SSS, STR, SXT, TET
	E177	2010	blaCTX-M-14	K	–	B	~104,5	negative	K, FIB, FII, Y	AMP, CIP, CTX, NAL, SSS, STR, SXT, TET
	E310	2012	qnrB19	NT	–	–	ND	positive	FIB, FII, HI2, P	AMP, CHL, NAL, SSS, STR, SXT, TET

Broilers	E321	2012	blaCTX-M-14	FIB	(18 : - : 1)	–	~180	negative	FIB, FII, HI1, HI2, I1-Iγ, X1	AMP, CHL, CIP, CTX, NAL, SSS, STR, TET
	E309	2011	blaCTX-M-1	I1-Iγ	ST3	E1	~104,5	positive	I1-Iγ, FIB, FII, P, U	AMP, CTX, GEN, NAL, SSS, STR, TET
	E115	2012	blaCTX-M-1	I1-Iγ	ST3	E1	~104,5	positive	I1-Iγ, FIB, FII	AMP, CTX, NAL, SSS, TET
	E223	2012	blaCTX-M-1	I1-Iγ	ST36	D	~104,5	negative	I1-Iγ, FIB, FII, X1	AMP, CHL, CTX, FFN, SSS
	E235	2012	blaCTX-M-1	I1-Iγ	ST36	D	~104,5	positive	I1-Iγ, FIB, FII	AMP, CHL, CTX, NAL, SSS, STR, SXT
	E86	2011	blaCTX-M-1	I1-Iγ, P	ST26	A	~120	positive	I1-Iγ, P, FIB, FII, I2, X1, Y	AMP, CHL, CIP, CTX, NAL, SSS, STR, SXT, TET
	E149	2009	blaSHV-12	I1-Iγ	ST26	C	~138	positive	I1-Iγ, FIB, FII	AMP, CHL, CTX, NAL, SSS, STR, TET
	E106	2011	blaCMY-2	I1-Iγ	ST26	F	~216	positive	I1-Iγ, FIB, FII, I2, X1, Y	AMP, CHL, CIP, CTX, KAN, NAL, SSS, STR, SXT, TET
	E102	2011	blaCMY-2	K	–	A	~104,5	positive	K, B/O, FIB, FII, I1-Iγ, U	AMP, CHL, CTX, NAL, SSS, STR, SXT
	E315	2010	qnrS1	X1	–	B	ND	positive	X1, FIA, FIB, N, Y	AMP, CIP, GEN, KAN, NAL, SSS, STR, SXT, TET
	E56	2010	qnrS1	X2	–	–	~33,3	positive	X2, FIB, FII, I1-Iγ, X1	AMP, CHL, NAL, SSS, STR, SXT, TET
	E297	2011	qnrS1	NT	–	–	~65	positive	FII, HI1, Y	AMP, CHL, CIP, GEN, KAN, NAL, SSS, STR, SXT, TET
	E245	2012	qnrS1	B/O	–	–	~90	positive	B/O, FIB, I2	AMP, CIP, NAL, STR, SXT
	E332	2010	oqxA/B	–	–	–	–	negative	L, R	AMP, CHL, CIP, KAN, NAL, SSS, STR, SXT, TET
Layers	E323	2011	blaCTX-M-1	I1-Iγ	ST3	E5	~104,5	positive	I1-Iγ, FIB, FII, FIC, FIIS	AMP, CTX, NAL, SSS, TET
	E232	2012	qnrS1	X1	–	A	ND	positive	X1, FIB, FII	AMP, SSS

NT: non-typeable; ND: not determined; -, not performed.

AMP, ampicillin, CHL, chloramphenicol, CIP, ciprofloxacin, CTX, cefotaxime, FFN, florfenicol, GEN, gentamicin, KAN, kanamycin, NAL, nalidixic acid, SSS, sulfonamides, STR, streptomycin, SXT, sulfamethoxazole-trimethoprim, TET, tetracycline

Underlined: intermediate resistance.

In bold: resistance transferred by the ESBL/AmpC/PMQR plasmid (transfer of AMP, CTX and low-level CIP resistance was determined by growth of transformants/transconjugants on selective plates).

a

Different restriction enzymes were used for plasmids belonging to different Inc groups.

b

Plasmid size was deduced by comparing the migration of the transformant band with the closest corresponding marker band.

c

as tested by conjugation.

d

as tested by disk diffusion.

Two multi-replicon IncI1-Iγ and IncP plasmids were isolated from turkeys and broilers. These plasmids belonged to pST26 but differed between the turkey and broiler isolates with regard to ESBL gene content, plasmid size, RFLP profile and co-transferred resistance (Table 2). The ESBL/AmpC content, size and origin of the remaining plasmids are described in Table 2.

Transfer by conjugation was observed for most (78%) ESBL/AmpC-positive plasmids. The non-self-transferable plasmids belonged to different lineages (IncI1-Iγ, IncK and IncFIB), carried different ESBL types (CTX-M-1 and CTX-M-14) and originated from different hosts (turkeys and broilers) (Table 2).

3.3. Diversity of PMQR-positive plasmids

Five transformants were obtained but only plasmids from two qnrS1-positive transformants could be visualised on S1 PFGE gels. These plasmids derived from broiler isolates and had different size (33 and 65 kb) and incompatibility group (IncX2 and non-typeable) (Table 2). The remaining plasmids were distinct IncX1 plasmids carrying qnrS1 from a broiler and a layer isolate, and a non-typeable plasmid carrying qnrB19 from a turkey isolate. Transconjugants were obtained for six (86%) of the plasmids harbouring PMQR genes, which also allowed characterisation of an additional plasmid carrying qnrS1. This plasmid was found in a broiler isolate, measured approximately 90 kb and belonged to IncB/O (Table 2). No transformant and transconjugant were obtained from the oqxA/B-positive isolate. None of the PMQR plasmids transferred resistance to additional antimicrobials.

3.4. Strain typing

The 25 E. coli harbouring ESBLAmpC- or PMQR-encoding plasmids showed unique PFGE profiles with less than 65% similarity (data not shown). The wild-type E. coli showed resistance to multiple antimicrobials (Table 2). Sulfonamide resistance was the most frequent (96% of isolates), followed by resistance to nalidixic acid (92%), streptomycin and tetracycline (80%), and sulfamethoxazole-trimethoprim resistance (72%). Chloramphenicol and ciprofloxacin resistance occurred in approximately half (52% and 40%, respectively) of the isolates. Finally, gentamicin and kanamycin resistance were observed in 20% of strains, whereas florfenicol resistance was observed in one strain only (Table 2).

The plasmid replicons detected in the transformants were confirmed in the isolates used as donors, which yielded two to seven additional replicons (Table 2). F replicons were present in all but one strain. The other most common replicons were IncX1 (24% of the isolates), IncY (20%), IncI1-Iγ (16%). No specific pattern of plasmid replicons was identified in association with animal category and with ESBL, AmpC and PMQR plasmid occurrence.

4. Discussion

This study showed a prevalence of 3GC resistance of 8% and a high diversity of resistance genes and associated plasmids in clinical E. coli from turkey, broiler and layer flocks in Italy. No overlap of gene and plasmid types in E. coli across poultry categories was observed, with the exception of related (though distinguishable) IncI1-Iγ/pST3 plasmids harbouring blaCTX-M-1 that occurred in isolates from turkeys, broilers and layers. This level of genetic diversity suggests multiple introductions of ESBL/AmpC genes in E. coli populations in poultry, which is compatible with the presence of selective pressure favouring 3GC-resistant strains. Notably, the clinical isolates analysed in this study were collected when a 3GC (i.e. ceftiofur) was still used for prophylaxis in day-old chicks. Prior to this study, information on ESBL/AmpC occurrence in avian E. coli in Italy was limited to commensal isolates in broilers and turkeys. Giufrè et al. (2012) identified ESBL genes in 8% of 101 E. coli isolated from broiler and turkey farms across Italy in 2009. In the Italian monitoring of antimicrobial resistance in 2014 (Commission Implementing Decision 2013/652/EU), the ESBL/AmpC phenotype were detected in 6.5% and 1.2% of indicator E. coli collected from broilers and turkeys, respectively (EFSA & ECDC, 2016). All ESBL/AmpC genes detected in our study were previously reported among broiler and turkey commensal isolates in Italy (Bortolaia et al., 2010; Giufrè et al., 2012; EFSA & ECDC, 2016), with the exception of blaCXT-M-32 reported only in commensal E. coli from broilers in 2007 (Bortolaia et al., 2010). Also the associations between ESBL/AmpC genes and plasmid types were similar in avian pathogenic and commensal E. coli in Italy and across Europe, with a marked prevalence of IncI1-Iγ/pST3/blaCXT-M-1 plasmids (Accogli et al., 2013; Smith et al., 2015). Interestingly, the predominant plasmid lineage was IncI1/ST26, which has previously been described in E. coli isolated from poultry in Italy (Bortolaia et al., 2011; Accogli et al., 2013). This plasmid backbone was associated with four ESBL/AmpC genes (blaSHV-12, blaCTX-M-1, blaCTX-M-2 and blaCMY-2) and, in two isolates, with the IncP replicon, suggesting that it is widespread and undergoes frequent genetic rearrangements in the E. coli population of Italian poultry.

The prevalence of PMQR occurrence was 3% in our E. coli collection and a high degree of gene and plasmid diversity was detected. qnrS1 was the predominant PMQR gene detected in E. coli from turkeys, broilers and layers, and was associated with different plasmid types across the three poultry categories. The

occurrence of qnrS1 on IncX1 and IncX2 plasmids was previously described in poultry isolates in Italy (Cerquetti et al., 2009) and in other countries (Fortini et al., 2011; Veldman et al., 2012), whereas to the best of our knowledge the presence of this PMQR gene on IncB/O plasmids was not described in poultry prior to this study. The plasmids harbouring qnrB19 and oqxA/B could not be typed, which indicates that there are still some unknown plasmids in Enterobacteriaceae. However, in the case of oqxA/B any attempt of mobilisation by transformation and conjugation failed, which might indicate that the gene was located on the chromosome. Although PMQR genes usually confer low-level resistance to fluoroquinolones, they contribute to high level resistance in combination with mutations in the target topoisomerase genes (Jacoby et al., 2014). Thus, their occurrence in clinical E. coli might complicate antimicrobial treatment of colibacillosis, which often relies on use of enrofloxacin in turkeys and broilers in Italy. To note, high-level resistance associated with gyrA and parC mutations was detected in a previous study carried out on the same E. coli strain collection (Vanni et al., 2014), mainly in isolates harbouring qnrS1 and oqxA/B genes.

As expected, E. coli harbouring plasmid-borne ESBL/AmpC and PMQR genes were genetically diverse. An interesting feature was the occurrence of several plasmid replicons in all strains indicating simultaneous presence of different plasmid types. A previous study reported a significantly higher prevalence of IncB/O, IncFIIA, IncFIB, IncHI2, IncN, IncP1-α in avian pathogenic E. coli (APEC) compared to avian commensal isolates (Johnson et al., 2012). All these replicons were also identified in our strain collection of clinical isolates from colibacillosis. The occurrence of co-resistance to several antimicrobials was remarkable, with the vast majority of the isolates being resistant to most antimicrobials used for treatment of colibacillosis in poultry. Therefore, it appears that there are very limited therapeutic options, if any, to treat infections by a noticeable proportion of E. coli associated with colibacillosis in turkeys and broilers in Italy.

This study has limitations. The collection of isolates is relatively old but nonetheless can constitute valuable information on the landscape of ESBL and PMQR genes and plasmids circulating in clinical E. coli in Italian poultry farms until 2012. Although based on convenience sampling, this collection encompasses a large number of farms across Italy with ordinary farm management and biosecurity levels. It is also important to emphasise that the use of 3GC in poultry was discontinued in Italy in 2012. Thus, the baseline data provided by this study will be useful to evaluate whether that has influenced the prevalence of ESBL/AmpC-producers in clinical isolates.

In conclusion, this study shows the frequency of ESBL/AmpC and PMQR genes in clinical E. coli isolated from poultry flocks in Italy in 2008–2012 prior to the discontinuation of 3GC use. Molecular characterisation of these strains revealed a high diversity of ESBL/AmpC genes and, to a lesser extent, PMQR genes and associated plasmid types, along with widespread resistance to most antimicrobials available for use in poultry. The emergence of E. coli infections for which no or very limited therapeutic options are available represents a challenge for the Italian poultry industry, and alternative approaches to prevent and treat colibacillosis are urgently needed.

Conflict of interest

None to declare.

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