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Research paper

Identification and characterization of plasmid-mediated quinolone resistance determinants in *Enterobacteriaceae* isolated from healthy poultry in Brazil

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ABSTRACT

The expression of plasmid-mediated quinolone resistance (PMQR) genes confers low-level quinolone and fluoroquinolones resistance alone. However, the association to chromosomal resistance mechanisms determines an expressively higher resistance in *Enterobacteriaceae*. These mechanisms are horizontally disseminated within plasmids and have contributed to the emergence of bacteria with reduced susceptibility or resistant to therapies worldwide. The epidemiological characterization of PMQR dissemination is highly relevant in the scientific and medical context, to investigate the dissemination within enterobacteria, from different populations, including humans and food-producing animals. In the present study, 200 *Enterobacteriaceae* isolates were harvested from poultry with cloacal swabs and identified as *Escherichia coli* (90.5%), *Escherichia fergusonii* (5.5%), *Klebsiella oxytoca* (2.5%) and *Klebsiella pneumoniae* (1.5%). Among isolates evaluated, 46 (23%) harboured PMQR genes including *qnr*B (43/200), *qnr*S (2/200) and *aac(6′)-Ib*-cr (1/200). All isolates carrying PMQR genes showed multidrug-resistance phenotype. The 36 *E. coli* isolates showed 18 different PFGE types. All *E. fergusonii* isolates showed the same PFGE type. The two *Klebsiella oxytoca* belonged to two different PFGE types. The phylogenetic groups A, B1, and D were found among the *E. coli* harboring PMQR genes. Based on the phylogenetic analysis and PFGE, the population structure of *E. coli* isolates was diverse, even within the same farm. All isolates carrying *qnr*B and *qnr*S genes also harboured ColE-like plasmids. The Southern blot hybridization using the *S1*-PFGE revealed that the *qnr*B genes were located on low molecular weight plasmids, smaller than 10Kb. Resistance plasmids were sequenced and showed 100% identity with plasmid pPAB19-3. The association of PMQR genes with mobile genetic elements, such as transferable plasmids, favours the selection and dissemination of (fluoro) quinolones resistant bacteria among food-producing animals, and may play an important role in the current increased prevalence of resistant bacteria in different environments reported worldwide.

1. Introduction

The introduction of fluoroquinolones in clinical therapies occurred in the 1980s and the advantage of oral administration associated with the bactericidal activity against Gram-negative microorganisms contributed to the frequent election of this class of antimicrobials to treat infections caused by *Escherichia coli*, *Salmonella* and other *Enterobacteriaceae* in humans and animals, to date (Dalhoff, 2012; Vanni et al., 2014; Zawack et al., 2016).

Among different fluoroquinolone resistance mechanisms, chromosomal mutations in *gyr*A and/or *par*C genes in quinolone-resistance-determining region (QRDR), may change the antibiotic target site, consequently reducing the therapeutic efficacy. This mechanism associated to the expression of plasmid-mediated quinolone resistance (PMQR) genes increases the resistance level of the bacteria, frequently reaching impracticable minimal inhibitory concentration (MIC) (Harada and Asai, 2010; Gagliotti et al., 2008). The main PMQR determinants reported are the *qnr* genes (*qnr*A, *qnr*B, *qnr*C, *qnr*D and *qnr*S) that mediate resistance to quinolones by protecting the antimicrobial target protein, type II DNA topoisomerases, from the action of these antimicrobials (Rodriguez-Martinez et al., 2011). Moreover, there are other PMQR determinants, for example, *aac*(6′)-*Ib*–cr genes, which

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codify acetyltransferase, an aminoglycoside modifying enzyme able to acetylate fluoroquinolones due to the "–cr variant" and the QepA and OqxAB efflux pump proteins, that decrease susceptibility to hydrophilic fluoroquinolones. The expression of these genes associated with other resistance mechanisms, such as alteration of the target site, porin alterations, and overexpression of chromosomal efflux systems have been contributing to the increased level of quinolone resistance as well as the selection of resistant clones in enterobacteria (Strahilevitz et al., 2009; Tamang et al., 2011).

The prevalence of *qnr*B genes is higher than other *qnr* genes in *Enterobacteriaceae* isolates from human, animal, and environment (Poirel et al., 2012) with about 80 different alleles assigned in the Lahey website (http://www.lahey.org/qnrStudies/), currently. The *qnr*B19 gene has been reported worldwide in *Enterobacteriaceae* isolated from healthy humans, clinical infections and food-producing animals (Dionisi et al., 2009; Karczmarczyk et al., 2010; Pallecchi et al., 2011). This gene has been reported in plasmids IncN, IncX and small rolling-circle-replication (RCR) plasmids (Carattoli, 2013). The Xer-mediated site-specific recombination is often identified in RCR plasmids, especially ColE1, and has been suggested as the source of acquisition of the resistance gene (Tran et al., 2012). Most of the *qnr*-carrying plasmids share high similarity with ColE1 replicon (Carattoli, 2013). This study was conducted to evaluate the prevalence of PMQR determinants and characterize plasmids harboring these genes in *Enterobacteriaceae* isolated from poultry in Brazil.

2. Materials and methods

2.1. Isolation of bacteria

From 2011 to 2012, a total of 200 isolates were screened from 200 healthy chickens from two different poultry Farms in Sao Paulo State, Brazil. The procedure for sampling was previously approved by the Institutional Animal Use and Ethics Committee (Proc. n. 12.1.248.53.7). The cloacal swab samples were seeded onto MacConkey agar plates containing ciprofloxacin (2 μg/mL) and incubated for 24 h/37 °C. Colonies were identified by biochemical standard methods, confirmed by API 20E test (BioMérieux, France) and, in some cases, the identification was performed by VITEK®2 (BioMérieux, France).

2.2. Screening of PMQR genes by multiplex PCR

The total genomic DNA of the isolates was extracted as described previously (Bolano et al., 2001), and used in the PCR for the identification of the PMQR genes in all isolates. The genes *qnr*A, *qnr*B, *qnr*S (Cattoir et al., 2007), *qnr*C, *qnr*D (Wang et al., 2009), *acc*(6′)-Ib-cr, *qep*A and *oqx*AB (Minarini et al., 2008) were searched as previously described. Both strands of the amplicons were sequenced using the ABI 3730 sequencer (Applied Biosystems, USA) with the same primers used for PCR.

2.3. Antimicrobial susceptibility

The antimicrobial susceptibility of *E. coli* isolates with PMQR genes were assessed by agar disk diffusion method following the recommendations by Clinical Laboratory Standards Institute (CLSI, 2002), using breakpoints recommended by CLSI (2013). Fifteen antimicrobial drugs (Oxoid, UK) were tested: amoxillin/clavulanic acid (20/10 μg), piperacillin/tazobactam (100/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), cefoxitin (30 μg), aztreonam (30 μg), ertapenem (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), enrofloxacin (5 μg), tetracycline (30 μg), gentamicin (10 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg) and chloramphenicol (30 μg). Multidrug-resistance (MDR) phenotype was characterized by non-susceptibility or resistance to at least one agent in three or more antimicrobial classes (Canton and Ruiz-Garbajosa, 2011; Magiorakos et al., 2012).

2.4. Pulsed-field gel electrophoresis (PFGE)

Genomic DNA of *qnr* positive *E. coli* isolates were digested with restriction enzyme *Xba*I (Fermentas, USA) and the macrorestriction separation was carried out using pulsed-field gel electrophoresis (PFGE) in the CHEF-DRIII System (Bio-Rad Laboratories, USA). The assay was performed in 1,2% agarose PFGE gels at 6.0 V/cm with an initial/final switch time of 10/40 s and an angle of 120° at 14 °C for 24 h. Macrorestriction patterns were analysed using the BioNumerics 5.0 software (Applied Maths, USA). Relationship among PFGE-types was analysed by the Dice similarity index to obtain a dendrogram with clusters. The dendrogram was constructed using the unweighted-pair group method using average linkage algorithm (UPGMA). The minimum threshold value for similar PFGE patterns was defined at 85%.

2.5. Phylogenetic analysis

The phylogenetic groups were determined according to the previously described method (Clermont et al., 2000), classifying isolates to one of the four phylogenetic groups (A, B1, B2, or D) based on the presence of *chu*A, *yja*A genes and *Tsp*E4C2 DNA fragment.

2.6. Plasmid replicon typing

Plasmids were investigated by PCR-based Replicon Typing (PBRT) method using primers for 18 major incompatibility (Inc) groups described (FIA, FIB, FIC, HI2, I1-I, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, FIIA, HI1) among *Enterobacteriaceae* as described previously (Carattoli et al., 2005). ColE plasmids were also searched by previously described method (Garcia-Fernandez et al., 2009). The Inc groups were determined in parental strains to characterize all plasmids, including the non-conjugative.

2.7. Determination of the PMQR

To determine the plasmids harboring the PMQR genes, PFGE was performed, after digestion with *S1* nuclease (*S1*-PFGE). The Lambda Ladder PFG Marker and Low Range PFG Marker (Biolabs, USA) were used as size standards. The Southern blot and hybridization were performed using DNA probes specific for quinolone resistance genes prepared with AlkPhos Direct Labeling and Detection System with CDP-Star (GE Healthcare Life Sciences, USA) (Sambrook et al., 1989).

2.8. Characterization of ColE-like plasmids

The plasmids were extracted using PureYield™ Plasmid Miniprep System (Promega, USA), according to manufacturer instructions. The ColE-like plasmids were characterized using the PCR-based method described elsewhere (Pallecchi et al., 2010). The complete sequence of these plasmids was obtained by Sanger sequencing method (ABI 3730 sequencer, Applied Biosystems, USA) and analysed using Chromas Pro (Technelysium, Australia) and BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) softwares.

3. Results and discussion

Antimicrobial resistance has been exponentially noticed in *Enterobacteriaceae* isolated from infected (Dalhoff, 2012) and colonized humans (Woerther et al., 2013) and also from food and food-producing animals (Bardon et al., 2013; Clemente et al., 2015; Liebana et al., 2013; Szmolka et al., 2011; Tamang et al., 2011). Two hundred ciprofloxacin resistant Enterobacteriaceae isolates were isolated from 200 cloacal swabs streaked on MacConkey agar plates, selecting one colony

Table 1
Phenotypic and genotypic characteristics of PMQR-harboring *E. coli* isolates from poultry.

Isolates	Species	Farm	Resistance	PMQR gene	Plasmid family	Phylogenetic group	PFGE-type
157a	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, GEN, TET, SXT	*qnr*B19	ColE, HI1, I1, F	A	A
167	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, GEN, TET, SXT	*qnr*B19	ColE, FIB, F	A	A
166a	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, GEN, TET, SXT	*qnr*B19	ColE, FIB, F	A	B
109	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, TET, SXT	*qnr*B19	ColE, K	A	C
107	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, TET, SXT, CHL	*qnr*B19	ColE, FIB, F	D	D
110	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, TET, SXT, CHL	*qnr*B19	ColE, F	D	E
112	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
115	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
125a	*E. coli*	2	AMC, CTX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
127	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
135	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
138	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
141b	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
148	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
149	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
114b	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	D	E
122	*E. coli*	2	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE FIB, F	D	E
176	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, GEN, TET, SXT	*qnr*B19	ColE, FIA, FIB, F	D	F
39a	*E. coli*	1	AMC, CTX, CAZ, FOX, NAL, CIP, LEV	*qnr*B19	ColE, K, B/O	A	G
74	*E. coli*	1	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET	*qnr*B19	ColE, K	A	G
91	*E. coli*	1	AMC, CTX, CAZ, FOX, NAL, CIP, LEV	*qnr*B5	ColE, K	A	G
123b	*E. coli*	2	AMC, CTX, NAL, CIP, LEV, GEN, TET	*qnr*B19	ColE, K	A	H
136	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, GEN, TET	*qnr*B19	ColE, K	A	H
146	*E. coli*	2	AMC, CTX, NAL, CIP, LEV, GEN, TET	*qnr*B19	ColE, K	A	H
105	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, GEN, TET	*qnr*B19	ColE, FIA	A	H
147	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, GEN, TET	*qnr*B19	ColE, K	A	H
86	*E. coli*	1	AMC, CTX, ATM, NAL, CIP, LEV, GEN, TET	*qnr*B5	ColE, FIB, FIC, F	A	N
106	*E. coli*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, GEN, TET, SXT	*qnr*B19	ColE, FIA, F	D	I
143	*E. coli*	2	AMC, CTX, FOX, NAL, CIP, LEV, SXT	*qnr*B19	ColE, K	A	J
12	*E. coli*	1	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, CHL	*qnr*B19	ColE, FIC, K, F	A	K
37	*E. coli*	1	AMC, CTX, CAZ, ATM, FOX, NAL, CIP, LEV, TET	*qnr*B19	ColE, K, B/O	A	L
89	*E. coli*	1	AMC, CTX, CAZ, ATM, FEP, NAL, CIP, LEV, TET, CHL	*qnr*B5	ColE, F	B1	M
82	*E. coli*	1	AMC, CTX, FEP, NAL, CIP, TET	*qnr*B5	ColE, I1, FIB, F	A	O
49a	*E. coli*	1	AMC, CTX, CAZ, ATM, FEP, NAL, CIP, LEV, TET, SXT, CHL	*aac(6′)-Ib*-cr	FIB, F,Y	B1	P
01	*E. coli*	1	AMC, CTX, CAZ, ATM, FEP, NAL, CIP, TET	*qnr*S1	ColE, I1, FIB, F, A/C	B1	Q
93	*E. coli*	1	AMC, CTX, ATM, FEP, NAL, CIP, LEV, TET, SXT, CHL	*qnr*S1	ColE, HI1, I1, FIB, F	A	R

AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, FOX: cefoxitin, ATM: aztreonam, NAL: nalidixic acid, CIP: ciprofloxacin, LEV: levofloxacin, TET: tetracycline, CHL: chloramphenicol, SXT: trimethoprim-sulfamethoxazole, GEN: gentamicin. The plasmids that carried PMQR genes are underlined.

per plate after incubation. Among the identified isolates, 90.5% were *E. coli* (181/200), 5.5% *Escherichia fergusonii* (11/200), 2.5% *Klebsiella oxytoca* (5/200) and 1.5% *Klebsiella pneumoniae* (3/200).

Out of 200 *Enterobacteriaceae* isolates examined in this study, 23% (46/200) carried PMQR genes including *qnr*B (43/200), *qnr*S (2/200) and *aac(6′)-Ib*-cr (1/200). Among Qnr-producing isolates carrying *qnr*B, 33 were *E. coli* isolates (*qnr*B19, n = 29; *qnr*B5, n = 4), 7 *E. fergusonii* (*qnr*B19), 2 *K. oxytoca* (*qnr*B19) and 1 *K. pneumoniae* (*qnr*B19). The *qnr*S1 was found in two *E. coli* isolates and *aac(6′)-Ib*-cr gene was found in one *E. coli* isolate (Table 1). None isolate was positive for *qnr*A, *qnr*C, *qnr*D, *qep*A and *oqx*AB genes. In our work, the most prevalent PMQR determinant was *qnrB* (n = 43), found in different bacterial species. Other research groups have also reported this gene as the most frequent PMQR determinant, followed by *qnr*S (Cattoir and Nordmann, 2009; Rodriguez-Martinez et al., 2011; Strahilevitz et al., 2009). The *qnrB*19 (n = 39) was the most prevalent gene detected in our study, followed by *qnrB*5 (n = 4). The gene *qnr*B19 was reported in other countries, in *E. coli* from chickens, however, in most cases, in lower prevalence (Ben Sallem et al., 2014; Fortini et al., 2011; Literak et al., 2013; Oh et al., 2016) than described in the present study. Our results show a high prevalence of *qnr*B19 in poultry produced for meat consumption. There is few data about PMQR determinants either in humans or in animals in Brazil, therefore the prevalence of these genes is poorly understood. Another study in Brazil, also reported the gene *qnr*B19 carried by ColE-like plasmids in four ExPEC isolates from poultry (Cunha et al., 2017).

In this study, all isolates carrying PMQR genes (46/46) showed MDR phenotype. Among these isolates, the resistance to antibiotics tested was: amoxicillin-clavulanic acid (100%), cefotaxime (100%), ceftazidime (56%), cefepime (15%), cefoxitin (63%), aztreonam (41%), nalidixic acid (NAL 100%), ciprofloxacin (CIP 91%), levofloxacin (LEV 82%), enrofloxacin (ENR 100%), tetracycline (89%), gentamicin (24%), trimethoprim-sulfamethoxazole (63%), and chloramphenicol (46%). None isolate showed resistance to piperacillin/tazobactam and ertapenem (Tables 1 and 2). NAL, CIP and LEV are mainly used to treat urinary tract infections and respiratory infections in human patients. However, ENR is a fluoroquinolone exclusively developed for use in veterinary medicine (Lopez-Cadenas et al., 2013) and has been extensively used as a prophylactic measure in poultry farms, to reduce intestinal infections by *Salmonella* and other pathogens, which may have contributed for the selection of fluoroquinolone resistance among these animals. Furthermore, the oral use of ENR also contributes for selection of resistance to non-quinolone antimicrobials in commensal *E. coli* isolated from chickens (Jurado et al., 2015). The use of other antibiotic classes, such as beta-lactam antibiotics, could advantage co-selection of fluoroquinolones non-susceptible *E. coli* from retail poultry (Ingram et al., 2013), when mobile genetic elements (MGE), carrying multiple antimicrobial determinants are involved, as explained elsewhere (Canton and Ruiz-Garbajosa, 2011).

As shown in Table 1, the 36 *E. coli* isolates carrying PMQR genes exhibited 18 different PFGE types. The *qnr*B resistance genes (*qnr*B19 and *qnr*B5) were present in different *E. coli* isolates classified in showed 15 PFGE types (A to O), demonstrating the dissemination of the resistance gene within a diverse bacterial population. Among the different PFGE types the major cluster “E” included 12 isolates producing *qnr*B19. Two different PFGE types were found in the two *E. coli* isolates carrying *qnr*S1 (Q and R). The *E. coli* carrying *aac(6′)-Ib*-cr belonged to

Table 2
Phenotypic and genotypic characteristics of PMQR-harboring *K. oxytoca*, *K. pneumoniae* and *E. fergusonii* isolates from poultry.

Isolates	Species identified	Farms	Resistance	PMQR gene	Plasmid family	PFGE
121a	*K. oxytoca*	2	AMC, CTX, CAZ, FOX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, FIB, F	Ko A
137a	*K. oxytoca*	2	AMC, CTX, CAZ, ATM, FOX, FEP, NAL, CIP, LEV, TET, SXT, CHL	*qnr*B19	ColE, I1	Ko B
108a	*K. pneumoniae*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT, CHL	*qnr*B19	ColE	Kp A
117c	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT	*qnr*B19	ColE, I1, F	Ef A
123a	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, CHL	*qnr*B19	ColE, HI1, I1, F	Ef A
151c	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT, CHL	*qnr*B19	ColE, HI1, I1, F	Ef A
156b	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT	*qnr*B19	ColE, HI1, I1, F	Ef A
157b	*E. fergusonii*	2	AMC, CTX, CAZ, NAL, CIP, LEV, TET	*qnr*B19	ColE, HI1, I1, F	Ef A
169b	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT	*qnr*B19	ColE, I1, F	Ef A
171b	*E. fergusonii*	2	AMC, CTX, NAL, CIP, LEV, TET, SXT	*qnr*B19	ColE, I1, F	Ef A

AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, FOX: cefoxitin, ATM: aztreonam, NAL: nalidixic acid, CIP ciprofloxacin, LEV: levofloxacin, TET: tetracycline, CHL: chloramphenicol, SXT: trimethoprim-sulfamethoxazole, GEN: gentamicin. The plasmids that carried PMQR genes are underlined.

a different PFGE type (P). Results in Table 2, show that all *E. fergusonii* carrying *qnr*B19 belonged to the same PFGE type (Ef A). Although, the two *Klebsiella oxytoca* isolates carrying *qnr*B19 were from different PFGE types (Table 2). The evaluation of the resistant *E. coli* population from farm 1 and farm 2 shows nine different PFGE types in each farm, carrying the resistance genes, without any common PFGE type shared between farms. These findings suggest that the dissemination of the resistance genes is most likely associated to MGE (*e.g.* plasmids), exchanged among different isolates, rather than with a unique resistant isolate prevailing. The phylogenetic groups A, B1, and D were found among the *E. coli* harboring PMQR genes. Most isolates were identified in the phylogenetic group A (50%), other 42% of the isolates were classified in the phylogenetic group D and 8% of the isolates were identified phylogenetic group B1 (Table 1). None of the isolates belonged phylogenetic group B2. In addition, 14 *qnr*B19-producing belonged to phylogenetic group D. Based on the phylogenetic analysis and PFGE, the population structure of *E. coli* isolates was diverse, even within the same poultry farm, characterizing a non-clonal dissemination and demonstrating the potential of these genes and MGEs to be maintained by different *E. coli* populations. Isolates within phylogenetic group B2 reported, as the most virulent, frequently causing infections were not detected. Nevertheless, among the phylogenetic groups identified in the present work, group D has also been involved in extra-intestinal infections, causing concerns in clinical settings. The phylogenetic groups A and B1 are considered less virulent, however, were also described in human extraintestinal infections (Pitout, 2012).

As shown in Table 1, in all isolates, the *qnr*B and *qnr*S genes were harboured on ColE-like plasmids. Moreover, 43 *E. coli* isolates carrying *qnr*B in ColE-like plasmids, also carried other replicon types, including IncHI1 (n = 1), IncI1 (n = 2), IncFIA (n = 3), IncFIB (n = 17), IncFIC (n = 2), IncF (n = 22), IncK (n = 12) and IncB/O (n = 2). *E. coli* isolates harboring *qnr*S1 showed the presence of IncHI1 (n = 1), IncI1 (n = 2), IncFIB (n = 2) IncF (n = 2) and IncA/C (n = 1). The *E. coli* isolate carrying *aac(6′)-Ib*-cr showed IncFIB, IncF, and IncY, however it was not possible to determine which plasmid harboured this gene. As shown in Table 2, *E. fergusonii* isolates carrying *qnr*B gene in ColE-like plasmids, also harboured IncHI1 (n = 4), IncI1 (n = 7) and IncF (n = 8). *K. oxytoca* isolates harboring *qnr*B also showed the replicon types IncFIB (n = 2), IncI1 (n = 7) and IncF (n = 8).

The Southern blot hybridization using the *S1*-PFGE revealed that the *qnr*B19 and *qnr*B5 genes were located on low-molecular weight plasmids, smaller than 10Kb.The plasmids sequences from isolates carrying *qnr*B19 gene were analysed using GenBank database tools, and 100% identity with plasmid pPAB19-3 was found (GenBank accession number JN985534). The sequenced plasmids were determined as ColE-like plasmids and the size was 2989 bp. The ColE-like backbone found included regions for plasmid replication and mobilization. The *qnr* region was located near the Xer specific recombination site. The IS*Ecp1* was found in these plasmids downstream from *psp* gene (activator of the stress-inducible) and from *qnr* gene. Moreover, the presence of this MGE in different poultry Farms, bacterial species and *E. coli* isolates of different PFGE-types and phylogenetic groups, suggests the evidence of lateral gene transfer.

The ColE plasmids identified in all our isolates shared the same genetic sequence as those reported in *E. coli* from hospital, in Argentina, which has been circulating since 2007 in that country (Tran et al., 2012). However, now we report this replicon associated to PMQR determinants in isolates from food-producing poultry in Brazil, suggesting the capacity to disseminate. Moreover, it has been proposed that this RCR plasmid arrangement could play a role in the evolution of plasmids and present a model for DNA swapping between plasmid DNA mediated by site-specific recombination events at *oriT* and a Xer target site (Tran et al., 2012). The plasmids pPAB19-1, pPAB19-2, pPAB19-3 (pPAB19-3 also detected here), and pPAB19-4 share extensive homology among themselves and with other previously described small *qnr*B19-harboring plasmids (Tran et al., 2012).

Overall, as summarized in Tables 1 and 2, a high prevalence of *Enterobacteriaceae*, including *E. coli*, *E. fergusonii* and *K. oxytoca*, were found carrying PMQR determinant genes harboured in ColE-like plasmids. Moreover, these isolates also harboured other important replicon types. The increased resistance to quinolones and fluoroquinolones, determined by PMQR genes, was noticed in isolates that also showed resistance to other important antibiotics such as AMC, CTX, CAZ, ATM, GEN, CHL, TET. These characteristics were present in a diverse population of *E. coli*, as shown by PFGE and phylogeny, demonstrating that this dissemination of resistance has a non-clonal nature. The concern about the dissemination of PMQR genes is that these genes may facilitate the selection of higher levels of quinolone resistance (Jacoby et al., 2014). It is difficult to determine the incidence and prevalence of this plasmid carrying these genes, however, silent dissemination may be occurring in many ecological settings and in different countries in South America.

4. Conclusion

A high prevalence of PMQR determinants was found in *Enterobacteriaceae* isolated from healthy chickens. Different *Enterobacteriaceae* harboured these genes and the genes were found in two poultry Farms, showing the dissemination of resistant bacteria in food-producing animals, which may play an important role in the current increased prevalence of MDR bacteria in different environments reported worldwide. Therefore, the surveillance of resistant genes in the food chain is considered a key point to assist in the control of antimicrobial resistance.

Conflict of interest statement

None to declare.

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