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Plasmid-mediated quinolone resistance and β-lactamases in *Escherichia coli* from healthy animals from Nigeria

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Objectives: The animal reservoir of plasmid-mediated quinolone resistance (PMQR) and β-lactamases is still controversial and little information is available on the prevalence of these resistance determinants in developing countries. The aim of this study was to identify and characterize PMQR and β-lactamases in a collection of commensal ampicillin-resistant *Escherichia coli* isolated from healthy chickens and pigs at slaughter, collected in November–December 2006, in Ibadan, Nigeria.

Methods: One hundred and sixty-two ampicillin-resistant *E. coli* were obtained from healthy chickens and pigs at slaughter in Ibadan, Nigeria. Strains were tested for antimicrobial susceptibility by disc diffusion assay. MICs of ciprofloxacin were determined by Etest. Resistance genes were screened by PCR and DNA sequencing. Clonal relatedness of the isolates was determined by enterobacterial repetitive intergenic consensus–PCR. Plasmids were transferred by conjugation and transformation and characterized by PCR-based replicon typing and plasmid multilocus sequence typing.

Results: PMQR genes were detected in 18 *E. coli* strains; 11 of them showed reduced susceptibility to ciprofloxacin. Twelve strains carried *qnrS1*, three strains carried *qnrB19*, one strain carried *qnrB10* and three strains carried *qepA*; one strain carried both *qepA* and *qnrB10*. All strains carried the bla_{TEM} gene; one strain was positive for the CTX-M-15 extended-spectrum β-lactamase.

Conclusions: Our findings suggest that food animals could represent an important reservoir of PMQR in this region of Africa. Previous studies reported high prevalence of *qnr* genes in clinical isolates from humans in Nigeria, suggesting that the spread of these resistance determinants in this country could be particularly relevant.

Keywords: food-producing animals, commensal *Escherichia coli*, plasmid-mediated resistance

Introduction

Rapid dissemination of plasmid-mediated quinolone resistance (PMQR) in Enterobacteriaceae has been described in recent years.[1] Three major PMQR mechanisms have been described so far: Qnr proteins (QnrA, QnrB, QnrS, QnrC, QnrVC and QnrD) capable of protecting DNA gyrase and topoisomerase IV from quinolones; Aac(6′)-Ib-cr aminoglycoside acetyltransferase, which modifies the quinolones with a piperazinyl substituent; and efflux pump proteins QepA and OqxAB, which increase the MICs of ciprofloxacin.[1] These mechanisms have been identified in both clinically resistant and susceptible isolates, contributing to an increase in MICs of nalidixic acid and ciprofloxacin of 2- to 8-fold and 8- to 32-fold for resistant and susceptible isolates, respectively.[1]

Information on the prevalence of these resistance determinants in developing countries is scarce. A recent study reported high rates of resistance to quinolones and β-lactam antibiotics among Gram-negative enteric isolates from different hospitals in Nigeria and a surveillance study on zoonotic pathogens identified salmonella strains carrying the *qnrS1* gene.[2–4] In this study we characterized PMQR determinants, β-lactamases, plasmids and strains among commensal *Escherichia coli* isolated from healthy chickens and pigs at slaughter in the city of Ibadan, Nigeria.

Materials and methods

Isolation of bacteria

From November to December 2006, a total of 200 faecal samples were randomly collected from 100 chickens and 100 pigs originating from different farms at slaughter in Ibadan, Nigeria, plated on eosin/methylene blue agar containing 100 mg/L ampicillin and incubated

aerobically at 37°C for 18–24 h. Presumptive *E. coli* colonies were identified by standard methods. Three *qnrS1*-positive *Salmonella enterica* Derby strains from chickens from Nigeria[3] were included in this study for plasmid comparison.

Antimicrobial susceptibility

E. coli strains were tested for antimicrobial susceptibility by a disc diffusion method following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org). MICs of ciprofloxacin were determined by Etest (AB Biodisk, Solna, Sweden) and interpreted in accordance with the EUCAST guidelines.

Strain typing, PMQR and β-lactamase gene identification

The genetic relatedness of the strains was determined by enterobacterial repetitive intergenic consensus–PCR (ERIC–PCR); strains differing for more than two bands were classified in different ERIC types, strains differing for two bands were classified in the same ERIC type, but differentiated by letters (i.e. 7a–7b, 6a–6b–6c).[5]

The *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac(6′)-Ib-cr*, *qepA*, *oqxAB*, bla_{TEM} and bla_{LAP} genes were screened using previously described primers and conditions.[6] The bla_{CTX-M} and bla_{SHV} genes were searched in strain Z3.[6] PCR products were fully sequenced.

Plasmids

Plasmid DNA was purified by Qiagen Plasmid Midi kit (Qiagen, Inc., Milan, Italy) and used to transform Top10 *E. coli* chemically competent cells (Invitrogen, Milan, Italy). Since the MIC of ciprofloxacin for the *E. coli* recipient was 0.03 mg/L, transformants were selected on Luria–Bertani (LB) agar plates containing 0.06 mg/L ciprofloxacin or 50 mg/L ampicillin. Conjugation experiments were performed at 25°C by liquid mating assay using rifampicin-resistant *E. coli* CSH26 as recipient and selecting transconjugants on LB agar plates supplemented with 100 mg/L rifampicin and 0.06 mg/L ciprofloxacin. Plasmids were assigned to incompatibility groups by PCR-based replicon typing (PBRT).[7]

IncHI2 and IncI1 plasmids were further typed by plasmid multilocus sequence typing (pMLST; www.pubmlst.org/plasmid/). A HindIII library was obtained from a prototypic IncX2 plasmid. Several recombinant clones were randomly selected and fully sequenced.

Results

One hundred and sixty-two ampicillin-resistant *E. coli* strains, 96 from chickens and 66 from pigs, were isolated from 200 faecal samples obtained from healthy animals at slaughter in the city of Ibadan, Nigeria. Chickens and pigs were produced locally for the Nigerian market and for meat exportation to neighbouring west African countries. Eighty-nine ampicillin-resistant strains (55%) showed resistance or reduced susceptibility to fluoroquinolones; 69/96 (72%) strains from chickens and 2/66 (3%) from pigs showed ciprofloxacin MICs > 4.0 mg/L and 13/96 (13.5%) and 5/66 (7.5%) strains from chickens and pigs, respectively, showed reduced susceptibility to ciprofloxacin with MICs ranging from 0.125 to 0.5 mg/L. Strain Z3 was unique in showing resistance to cephalosporins. PMQR genes were searched for among these 89 strains.

Eighteen isolates (20%) were positive for one or more PMQR genes and 12 of them were from the group showing reduced susceptibility to ciprofloxacin: 15 strains were from chickens; and three strains were from pigs. Higher levels of resistance to fluoroquinolones were probably associated with mutations in the chromosomal target genes and were not further investigated.

Among the PMQR determinants, 12 strains carried *qnrS1*, 3 strains carried *qnrB19*, 1 strain carried *qnrB10* and 3 strains carried *qepA1*; 1 carried both *qepA1* and *qnrB10*. None of the isolates carried *qnrA*, *qnrC*, *qnrD*, *aac(6′)-1b-cr* or *oqxAB* genes. Ampicillin resistance was associated with the presence of the bla_{TEM-1} gene in all the strains; strains were negative for bla_{LAP}. Strain Z3 was positive for the $bla_{CTX-M-15}$ gene (Table 1).

PMQR strains were typed by ERIC–PCR and identical profiles (profile no. 1 in Table 1) were obtained for the three strains of swine origin and two strains from chickens. The remaining 13 isolates were discriminated in seven different ERIC profiles.

PMQR-positive transformants were successfully obtained from 13/18 parental strains by transformation of the recipient *E. coli*, on agar plates containing 0.06 mg/L ciprofloxacin. The Z46, Z39 and Z41 strains positively transferred PMQR-positive plasmids by conjugation. PMQR determinants were not transferred from strains Z14 and Z31, but ampicillin-resistant transformants were obtained from strain Z14 on agar plates containing 100 mg/L ampicillin.

All the strains transferred the PMQR genes in association with bla_{TEM-1} (Table 1).

The *qnrS1* genes were located on three different plasmid types: IncI1; IncN; and IncX2. The IncI1 plasmids from *E. coli* and those from *Salmonella* carrying *qnrS1*/bla_{TEM-1} (Z13, S88, S121 and S266) or the bla_{TEM-1} gene only (Z14) were all assigned to the same ST12 sequence type by pMLST, suggesting a common plasmid circulating among commensal and pathogenic bacteria.

The *qepA1* gene was located on IncHI2 plasmids that were further typed by pMLST and assigned to the ST2 sequence type. The *qnrB19* gene was located on ColE-like plasmids that were similar to the pTPqnrS-1a plasmid previously described in *Salmonella* Typhimurium (AM46977).

Since pMLST was not available for the IncX2 plasmids, recombinant clones of a random library obtained from the prototypic IncX2 plasmid (Z10) were selected and fully sequenced. Approximately 10 kb were sequenced demonstrating that this plasmid showed 97%–99% nucleotide identity with the partially sequenced IncX2 reference plasmid R6K (EMBL accession numbers Y10906.1, X95535.1, J01779.1, J01776.1 and M11000).

Discussion

The results obtained in this study showed that PMQR determinants were highly prevalent in commensal strains from healthy animals in Nigeria. In particular, a multifocal distribution of strains was observed, identifying four PMQR gene variants (*qnrB10*, *qnrB19*, *qnrS1* and *qepA1*), located on five different plasmid types (IncHI2, ColE, IncI1, IncN and IncX2). This impressively high variability of genes, plasmids and strains suggests wide circulation of resistance determinants among the faecal flora of healthy food-producing animals in this country. Our study suggests that the dissemination of these PMQR determinants is mostly due to the transmission of successful plasmids by horizontal exchange rather than to the spread of specific bacterial clones. The most frequently

Table 1. Characteristics of PMQR-positive *E. coli* isolated from pigs and chickens and their relative transformants/transconjugants

Strain	Resistance	Ciprofloxacin MIC (mg/L)	ERIC	PMQR and β-lactamase genes	Transferred genes	Inc (pMLST)
Z46	NAL, CIP, SUL, STR, TET, CHL, KAN, GEN, AMP	>32	8	*qepA1*, bla_{TEM-1}	*qepA1*	HI2 (ST2)
Z39	NAL, CIP, SUL, STR, TET, CHL, KAN, GEN, AMP	>32	5	*qepA1*, bla_{TEM-1}	*qepA1*	HI2 (ST2)
Z41	NAL, CIP, SUL, STR, TET, CHL, KAN, GEN, AMP	>32	6a	*qnrB10*, *qepA1*, bla_{TEM-1}	*qnrB10*, bla_{TEM-1}	UT
Z53	NAL, SUL, STR, TET, CHL, KAN, AMP	0.5	3	*qnrB19*, bla_{TEM-1}	*qnrB19*, bla_{TEM-1}	$ColE_{Tp}$
Z55	NAL, SUL, STR, CHL, AMP	0.5	6b	*qnrB19*, bla_{TEM-1}	*qnrB19*, bla_{TEM-1}	$ColE_{Tp}$
Z37	NAL, SUL, STR, TET, AMP	0.25	8	*qnrB19*, bla_{TEM-1}	*qnrB19*, bla_{TEM-1}	$ColE_{Tp}$
Z14	NAL, CIP, SUL, STR, TET, CHL, KAN, AMP	>32	4	*qnrS1*, bla_{TEM-1}	bla_{TEM-1}	I1 (ST12)
Z31	NAL, SUL, STR, TET, KAN, GEN, AMP	0.25	7a	*qnrS1*, bla_{TEM-1}	negative	ND
Z13	NAL, CIP, SUL, TET, STR, KAN, AMP	0.75	5	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	I1 (ST12)
Z10	NAL, CIP, SUL, STR, TET, KAN, AMP	>32	6c	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	X2
Z87	NAL, SUL, STR, TET, KAN, AMP	1	1	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	X2
Z1	NAL, SUL, TET, KAN, AMP	3	1	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	X2
Z66	NAL, SUL, STR, TET, CHL, KAN, GEN, AMP	0.25	2	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	X2
Z62	NAL, SUL, TET, STR KAN, AMP	0.25	2	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	X2
Z3	NAL, CIP, SUL, TET, CHL, KAN, GEN, AMP, CTX	>32	7b	*qnrS1*, bla_{TEM-1}, $bla_{CTX-M-15}$	*qnrS1*, bla_{TEM-1}	N
P14	NAL, SUL, AMP	0.125	1	*qnrS1*	*qnrS1*	UT
P30	NAL, SUL, AMP	0.25	1	*qnrS1*, bla_{TEM-1}	*qnrS1*	N
P42	NAL, TET, AMP	0.25	1	*qnrS1*, bla_{TEM-1}	*qnrS1*	N
S88	AMP, TET	0.25	ND	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	I1 (ST12)
S121	AMP, TET	0.25	ND	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	I1 (ST12)
S266	AMP, TET	0.25	ND	*qnrS1*, bla_{TEM-1}	*qnrS1*, bla_{TEM-1}	I1 (ST12)

AMP, ampicillin; NAL, nalidixic acid; CHL, chloramphenicol; KAN, kanamycin; GEN, gentamicin; SUL, sulphonamides, STR, streptomycin; CIP, ciprofloxacin; TET, tetracycline; CTX, cefotaxime; ND, not determined; UT, untypeable; P, *E. coli* from pigs; Z, *E. coli* from chickens; S, *Salmonella* isolates described in Fashae *et al.*[4]

encountered plasmid type in *E. coli* analysed in this study belonged to the IncX2 family, which is a very rarely reported plasmid family.[8] In contrast, the other two plasmid types, IncI1 and IncN, are frequently identified worldwide in enterobacteria, being among the most prevalent plasmid families in commensal avian strains from the USA in collections not biased for antimicrobial resistance.[8] Interestingly, we demonstrated that the same plasmid type (IncI1 sequence type ST12) identified in commensal *E. coli* was also detected in *Salmonella* strains collected in independent surveillance studies performed in this country in two different time periods. Small ColE-like plasmids harbouring *qnrB19* or *qnrS1* have been described worldwide in commensal *E. coli* and in *Salmonella* from the Netherlands, the UK, Germany, Colombia, Bolivia, Peru and in this study from Nigeria, suggesting that they could be very common in Enterobacteriaceae, being disseminated in different species of enterobacteria in different countries from both human and animal sources.[8] The identification of the *qepA* gene was unexpected since this resistance determinant is not commonly identified in strains from food-producing animals but was recently identified in Enterobacteriaceae of human origin in Nigeria.[2]

Antibiotics are used in animal production in Nigeria because they are available over the counter without prescription, but the use of fluoroquinolones for growth promotion, prophylaxis and treatment of food animals still seems rare in this country, because these drugs are relatively expensive.[9,10] However, PMQR-positive isolates could be positively selected by other drugs thanks to the association of multiple resistance determinants and transmitted to humans through the food chain. Antibiotic-resistant bacteria carried by animals can enter the human food chain through the consumption of meat or by direct contact. In Africa PMQR determinants have already been demonstrated to be prevalent in Enterobacteriaceae of human origin from Nigeria.[2] The prevalence observed could therefore derive from dissemination of these resistance determinants in food-producing animals.

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Transparency declarations

All authors have no conflicts of interest to declare relevant to this study.

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