

**Plasmid-mediated fluoroquinolone-resistance *qnrA* and *qnrB* genes among
Escherichia coli from cattle in Ado-Ekiti, Nigeria**

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ABSTRACT

Objective: This is to investigate the implication of fluoroquinolones usage in veterinary practice and the food chain system.

Subjects and Methods: Five hundred isolates of commensal *E coli* were recovered from the faeces of apparently healthy cattle in Ado-Ekiti, Nigeria. The susceptibility of the bacteria were tested using standard laboratory procedures. The polymerase chain reaction (PCR) was carried out to detect the presence of *qnrA* and *qnrB* genes, which were selected on the basis of their fluoroquinolone-resistant patterns.

Results: The agar disc diffusion technique revealed that the representative isolates showed multiple fluoroquinolone-resistance and this formed the basis for their selection for PCR amplification. The PCR revealed that 9 of the 17 quinolone-resistant representative isolates showed distinct bands which are specific for the *qnrB* gene, in addition, only one strain of the 20 representative isolates of commensal *E coli* carried plasmids on which the *qnrA* gene was detected.

Keywords: Cattle, *Escherichia coli* (*E coli*), food animals, polymerase chain reaction (PCR), *qnr* genes, quinolone resistance

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Doi:10.7727/wimj.2012.101

Conclusion: This study has confirmed that plasmid-mediated quinolone resistance is a possible mechanism among the fluoroquinolone-resistant commensal *E coli* isolated from faeces of apparently healthy cattle in the study location.

INTRODUCTION

The fluoroquinolones are broad-spectrum antibiotics with lethal action against a wide array of gram-positive and gram-negative bacteria and have been used to treat and prevent bacterial infections in clinical and veterinary settings with a view to reducing the mortality and morbidity associated with such bacterial infections (1). It is widely believed that the rapid rate at which fluoroquinolones kill bacteria could minimize the possibility of enteric and other pathogenic bacteria developing resistance to antibiotics. Consequently, due to their potency and broad-spectrum of activity, the fluoroquinolones are frequently used as antibiotics of last resort for the treatment of acute and chronic bacterial infections in humans and animals whenever other options or combinations of antibiotics have failed (2). However, several independent reports have confirmed the emergence and increasing prevalence of fluoroquinolone-resistance among pathogenic bacteria due to over-dependence on fluoroquinolones for treatment of infections in humans and their prophylactic uses in food animals (3–5). In developed countries, such evidence has raised questions whether the use of fluoroquinolones in animals should be banned (2). Despite the report on the increased prevalence of fluoroquinolone-resistance among bacteria in developed countries, it appears that few reports exist for fluoroquinolone-resistance in veterinary settings in low resource countries including Nigeria.

The most prominent of the several mechanisms of fluoroquinolone-resistance that has been described is through chromosomal mutations and plasmid-mediated quinolone-resistance (6). More specifically, fluoroquinolone-resistance among enteric and pathogenic bacteria is caused by chromosomal mutations that reduce membrane permeability and restricts antibiotic concentration within the bacterium (7–9).

The molecular mechanism for resistance against fluoroquinolones involves the alterations in genes that encode subunits of the quinolone targets DNA gyrase (in the *gyrA* and *gyrB* genes), topoisomerase IV (in the *parC* and *parE* genes) and plasmid mediated quinolone resistance. The mutational alterations in these genes lead to the synthesis of proteins with reduced affinity during the synthesis of the DNA and DNA replication (10, 11). To the best of our knowledge, there is no report on the molecular mechanism of resistance to quinolones among enteric bacteria isolated from animals in the study location.

The objective of this study was to determine the molecular basis of resistance to quinolones and specifically amplify plasmid-borne genes located in some representative isolates of commensal *E coli* recovered from the faeces of apparently healthy cattle in Ado-Ekiti, southwest Nigeria.

SUBJECTS AND METHODS

Five hundred isolates of commensal *E coli* were recovered from the faeces of apparently healthy cattle in Ado-Ekiti, Nigeria. The samples were cultured on eosine methylene blue (EMB) agar and isolates with characteristic green metallic sheen were selected and identified using standard procedure.

Antibiotic susceptibility tests

The susceptibility of the bacteria was tested against fluoroquinolones using the agar disc diffusion method and interpreted according to the specification of the Clinical Laboratory Science Institute (12, 13). The five different commonly used fluoroquinolones in the study environment included: norfloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg), ofloxacin (5 µg) and ciprofloxacin (5 µg) [Oxoid, Basingstoke, Hampshire, UK]. Furthermore, the isolates were tested for their susceptibility against conventional antibiotics.

The polymerase chain reaction (PCR) was carried out to detect the presence of *qnrA* and *qnrB* genes on the plasmids extracted from 20 and 17 representative isolates, respectively, which were selected on the basis of their fluoroquinolone-resistant patterns. The plasmids were extracted from isolates confirmed to be resistant to selected fluoroquinolones using the lysis protocol of standard procedure (14). A 50 µl mixture was made up of 2 µl of total plasmid DNA, 1 X PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl], 1.5 mM MgCl₂, 200 µM of each deoxynucleotide phosphate, 20 pmol of each of the primers for (*qnrA* f-agaggatttctcacgccagg and r-tgccaggcacagatcttga) and *qnrB* r-ttctgygyycgccagtcgaa and f-ggmathgaaattcgccactg), mixed with 2.5 units of *Taq* polymerase [Jena Bioscience, Germany] (15). Amplification was performed with the

following cycling conditions: 10 minutes at 95 °C and 35 cycles of amplification consisting of one minute at 95 °C, one minute at 54 °C and one minute at 72 °C and 10 minutes at 72 °C for the final extension. The DNA fragments were analysed on a 2% agarose gel at 100V for one hour in 1X TAE [40mM Tris-HCl (pH 8.3), 2 mM acetate and 1 mM EDTA] containing 0.05 mg/l ethidium bromide. A molecular marker Lambda *hind* III was used to estimate the amplicon for the *qnrB* gene while the 100 bp ladder was used as marker for the *qnrA* amplicon (Jena Bioscience, Germany). After amplification, the amplicons were separated using the 1% agarose gel electrophoresis, stained with ethidium bromide and visualized using UV transillumination (15).

RESULTS

The antibiotic susceptibility tests using the agar disc diffusion technique revealed that the representative isolates showed multiple fluoroquinolone-resistance and this formed the basis for their selection for PCR amplification (Table 1). The representative isolates that were selected also carried specific plasmids which were used as templates for the PCR amplification. The PCR results revealed that nine of the 17 representative isolates showed distinct quinolone resistant bands which are specific for the *qnrB* gene, with estimate of molecular weights at 537 bp using the lambda *hind III* digest as the molecular weight marker. However, two of the representative isolates (Lanes 8 and 11) showed multiple bands with additional bands different from the amplicon of interests (Table 1). In addition, only one strain of twenty representative isolates of commensal *E coli* carried plasmids on which the *qnrA* gene was detected with an amplicon size of 300 bp (Table 2).

DISCUSSION

In this study, fluoroquinolone-resistant genes *qnrA* and *qnrB* were detected among representative fluoroquinolone-resistant isolates of commensal *E coli* that were recovered from the faeces of apparently healthy cattle in the study location. Previously, the susceptibility of five hundred isolates to different fluoroquinolones was determined with a relatively high rate of multiple fluoroquinolone-resistance among some of the isolates and a high rate of carriage of plasmids among representatives of the commensal isolates (13). For the selection of the representative isolates for the molecular studies, we placed emphasis on those isolates with high multiple fluoroquinolone-resistant phenotypes with a view to increasing the possibility of detecting plasmids and quinolone resistant genes on those plasmids.

Considering the relatively high rate of resistance to fluoroquinolones observed in this study, it was imperative to determine the molecular basis for resistance to the fluoroquinolones as this is considered crucial for the prevention of emergence of fluoroquinolone-resistant bacteria and also for the development of novel antibiotics that can be used as alternative clinical and animal prophylactic therapy due to the failing option of fluoroquinolone treatment, not only among humans, but also among animals, particularly cattle and other food animals.

The reduced susceptibility of commensal isolates of *E coli* from faeces of apparently healthy cattle, as obtained in this study, agrees with other studies that have also confirmed the increasing prevalence of fluoroquinolone-resistance among bacteria from animals (16–18). The serious implication is that this could pose serious risk to human and animal health. Fluoroquinolone-resistant bacteria are constantly and faecally shed both by healthy and sick animals. As reported in previous studies, the relatively

high rate of fluoroquinolone-resistance among the commensal *E coli* could be attributed to the heavy reliance on fluoroquinolones for various therapeutic and sub-therapeutic uses in animals, which creates a selection pressure that favours the emergence of fluoroquinolone-resistant bacteria (2, 19).

The fluoroquinolone resistant genes *qnrA* and *qnrB* gene were detected in one and 10 of the 17 representative isolates respectively (Tables 1, 2). The presence of these genes carried by the isolates under study is definitely responsible for the relatively high prevalence of fluoroquinolone-resistance (20). The quinolone resistant genes code for pentapeptide proteins that prevents the quinolones from binding to the DNA gyrase and the DNA topoisomerase which are targets of action of the fluoroquinolones and are necessary for the lethal action of the fluoroquinolones (21). This is quite different from the chromosomally mediated mechanism of fluoroquinolone-resistance which involves a series of mutations on the DNA gyrase and topoisomerase IV genes which change the expression of outer membrane and efflux pumps (22). The results obtained also revealed that *qnrB* gene occurred more frequently than *qnrA* (Tables 1, 2) and further molecular study is required to determine which of the genes is responsible for resistance to fluoroquinolones within the study location.

In addition, this study was primarily aimed at detecting the presence of quinolone-resistance genes on plasmids of representative isolates. The first reported case of plasmid-mediated fluoroquinolone-resistance was reported between 1994 and 1998 and since then, many reports have independently confirmed the emergence and spread of plasmid-mediated quinolone resistance among bacteria from clinical and veterinary sources (15, 23). Recent studies have also shown that plasmid-mediated resistance mechanisms also play a major role in fluoroquinolone resistance, and its

prevalence is increasing worldwide, not only in clinical medicine but also in veterinary medicine (22). According to previous reports (23, 24), the *qnrB* gene is a novel gene located on plasmids among enteric bacteria and most plasmids confirmed to carry the *qnrB* gene also code for resistance against other antibiotics. Another report (25), described the presence of plasmid-mediated fluoroquinolone-resistance determinants among enteric bacteria isolated from companion and food-producing animals. The quinolone-resistance protein, which is a group of proteins coded by the quinolone resistance (*qnr*) genes, has been identified worldwide with a high prevalence among enteric isolates (26). Such fluoroquinolone-resistance genes have been detected among bacteria isolated from poultry and other food animals (11, 27, 28). To the best of our knowledge, this study reports the presence of quinolone resistance gene *qnrB* among enteric bacteria isolated from cattle and it is the first of its kind within the study location.

It should be noted that apart from the usual faecal shedding of fluoroquinolone resistant bacteria into the immediate environment, the presence of the quinolone resistance genes *qnrA* and *qnrB* on plasmids carried by commensal *E coli* recovered from cattle will definitely increase the prospect of dissemination of the fluoroquinolone-resistance genes into the immediate environment and ultimately get to the food chain, where it will imperil human health (29). These extrachromosomal entities with high frequency of conjugation may spread to other bacteria by conjugative transfer within the same environment thereby conferring quinolone resistance on other bacteria of the same or related species. This widespread acquisition of quinolone resistance through the plasmids may limit the prophylactic or sub-therapeutic use of the fluoroquinolones.

In summary, the observations in this study have confirmed that plasmid-mediated quinolone resistance is a possible mechanism among fluoroquinolone-resistant commensal *E coli* isolated from faeces of apparently healthy cattle in the study location, which poses grave clinical and public health implications. There should be proactive measures to strictly regulate the use of the fluoroquinolones and other related antibiotics used in food animals.

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Table 1: Plasmid polymerase chain reaction on fluoroquinolone-resistant isolates for *qnrB*

| Lanes | Isolate code | Mol. Wt of anplicon (approx) | Antibiotic resistance phenotypes | Fluoroquinolone-resistance phenotypes |
|-------|----------------------------------|------------------------------|----------------------------------|---------------------------------------|
| M | Lambda Hind <i>III</i> digest | - | - | - |
| 1 | E80 | 573bp | Amp-Gen-Nit-Col-Tet | Ofx |
| 2 | E277 | 573bp | Amp-Gen-Col | Pef-Cip |
| 4 | E404 | 573bp | Amp-Cot-Gen-Nal-Nit-Col-Str-Tet | Pef |
| 5 | E406 | 573bp | Amp-Cot-Gen-Nal-Nit-Col-Str-Tet | Pef |
| 6 | E440 | 573bp | Amp-Gen-Col-Str-Tet | Pef |
| 8 | E467 | 573bp | Amp-Gen-Str-Tet | Ofx-Nor-Lev |
| 11 | E466 | 573bp | Amp-Gen-Str-Tet | Pef |
| 13 | E580 | 573bp | Amp-Cot-Gen-Nal-Nit-Col-Str-Tet | Pef-Ofx-Cip-Lev |
| 15 | E625 | 573bp | Amp-Gen-Nit-Col-Str-Tet | Pef-Ofx-Nor |
| 16 | E646 | 573bp | Amp-Gen-Nal-Nit-Col-Str-Tet | Pef-Cip |

Amp – ampicillin, Col – colistin, Cot – cotrimoxazole, Gen – gentamicin, Nal – nalidixic-acid, Nit – nitrofurantoin, Tet – tetracycline, Strep – streptomycin, Pef – pefloxacin, Ofx – ofloxacin, Cip – ciprofloxacin, Lev – levofloxacin, Nor – norfloxacin.

Table 2: Polymerase chain reaction on plasmids fluoroquinolone-resistant isolates for *qnrA*

| <i>Lane</i> | <i>Isolate code</i> | <i>Mol Wt of amplicon (approx)</i> | <i>Antibiotic resistance phenotypes</i> | <i>Fluoroquinolone-resistance phenotypes</i> |
|-------------|---------------------|------------------------------------|---|--|
| 7 | E467 | 300bp | Amp-Gen-Str-Tet | Ofx-Nor-Lev |

Amp – ampicillin, Col – colistin, Cot – cotrimoxazole, Gen – gentamicin, Nal – nalidixic-acid, Nit – nitrofurantoin, Tet – tetracycline, Strep – streptomycin, Pef – pefloxacin, Ofx – ofloxacin, Cip – ciprofloxacin, Lev – levofloxacin, Nor – norfloxacin.