High carriage of plasmid-mediated quinolone resistance (PMQR) genes by cefotaxime-resistant *Escherichia coli* recovered from surface-leaking sanitary sewers

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Abstract

There is a rapid rise in the incidence of quinolone resistant bacteria in Nigeria. Most studies in Nigeria have focused on isolates from the clinical settings, with few focusing on isolates of environmental origin. This study aimed to investigate the antibiogram and carriage of plasmid-mediated quinolone resistance (PMQR) genes by quinolone-resistant isolates obtained from a pool of cefotaxime-resistant *Escherichia coli* (*E. coli*) recovered from sewage leaking out of some surface-leaking sanitary sewers in a University community in Nigeria. Isolation of *E. coli* from the sewage samples was done on CHRO-Magar *E. coli*, after enrichment of the samples was done in Brain Heart Infusion broth amended with 6 µg/mL of cefotaxime. Identification of presumptive *E. coli* was done using molecular methods (detection of *uidA* gene), while susceptibility to antibiotics was carried out using the disc diffusion method. Detection of PMQR genes (*qnrA*, *qnrB*, *qnrS*, *aac(6\')-ib-cr*, *qepA* and *oqxAB*) was carried out using primer-specific PCR. A total of 32 non-repetitive cefotaxime-resistant *E. coli* were obtained from the sewage, with 21 being quinolone-resistant. The quinolone-resistant isolates showed varying level of resistance to the tested antibiotics, with imipenem being the only exception with 0% resistance. The PMQR genes: *aac(6\')-lb-cr*, *qnrA*, *qnrB*, *qnrS* and *qepA* and *oqxAB* were detected in 90.5%, 61.9%, 47.6%, 38.1%, 4.8% and 0% respectively of the isolates. The findings of this study showed a high level of resistance to antibiotics and carriage of PMQR genes by quinolone-resistant *E. coli* obtained from the leaking sanitary sewers, suggesting a potential environmental and public health concern.

Keywords  
Cefotaxime-resistant bacteria · *Escherichia coli* · Sewage · Sanitary sewers · Antibiotic resistance · Plasmid-mediated quinolone resistance

Introduction

The wastewater environment, specifically composed of high concentrations of bacteria, nutrients, and suspended solids, is a known potential hub for the inception of antibiotic resistance and the stimulation of horizontal gene transfer (Lorenz and Wackernagel 1994). The high levels of antibiotics typically discharged in human wastes into the environment, is known to sufficiently inhibit susceptible bacteria and provide a selective advantage to the resistant ones (Kummerer 2001), making sewage and other wastewaters a favorable environment for bacterial growth and the likely spread of antibiotic resistance (Mezrioui and Baleux 1994). The typical end products (effluent or bio-solids), or non-typical routes like seepage through cracks in tanks, flooding or animal contact, and also aerosols, are channels through which bacteria in wastewater may enter into the environment (Brandi et al. 2000).
The use of quinolone antibiotics in human and veterinary medicine is on the rise due to their efficacy as important weapons against pathogenic bacteria via their ability to selectively inhibit the synthesis of DNA. This group of antibiotics constitutes about 17% of the global consumption of antibiotics (Van Doorslaer et al. 2014), and are frequently encountered in domestic and hospital sewage, because they are largely excreted unchanged in urine and faecal materials. (Kaplan et al. 2013). Quinolones are not easily degraded, and are adsorbed to sewage sludge during biological treatment (Kümmerer 2009). In a study carried out by Lindberg et al. (2006) and Fink et al. (2012), they found out that fluoroquinolones are hardly removed in effluent treatment plants, with approximately 70% remaining attached to sludge. In addition to this, the low effectiveness of treatment plants cannot be overlooked in the continuous spread to sludge. In addition to this, the low effectiveness of treatment plants cannot be overlooked in the continuous spread of multidrug resistant (MDR) bacteria into water aquifers (Korzeniewska and Harnisz 2013).

The occurrence of quinolones in the environment has caused some substantial effects on the microbial structure and community, due to the exposure of these flora to the antibiotics at sub-lethal concentration, making them develop resistance to these agents. This eventually makes the environment a repository of antibiotic resistant bacteria carrying several resistance genes and other mobile genetic elements (MGE) (Aminov 2009; Cordova-Kreylos and Scow 2007). The detection of PMQR determinants in the late 1990s has opened the doors of research into the mechanisms of quinolone resistance in bacteria and their transferability. Different resistance mechanisms have so far been described and they range from the qnr gene families which encode proteins that protect DNA gyrase and topoisomerase IV from the destructive action of quinolones; aac(6’)-Ib-cr gene, coding for aminoglycoside acetyltransferase, which are involved in the acetylation of fluoroquinolones such as ciprofloxacin and norfloxacin; and two active efflux pumps, qepA and oqxAB, which confer decreased susceptibility to fluoroquinolones (Poirel et al. 2012; Ruiz et al. 2012).

Most data on quinolone resistance in Nigeria have focused majorly on the occurrence of these genes in clinical isolates, therefore making data related to the environment very few. This study therefore aimed at investigating the carriage of PMQR genes by cefotaxime-resistant Escherichia coli isolated from surface-leaking sanitary sewers within a University community. Sanitary wastes from the students’ hall of residence, staff quarters, other residential areas and the business pools are being channeled to a treatment plant via sanitary sewers located underground all across the University premises. A common challenge with this system of waste transportation is the breakage of conveyor pipes as a result of several factors. This often times leads to the outflow of the content of the pipes into the environment, creating a very horrible sight and foul-smelling odour. Although this situation is remedied by the concerned unit of the University, it takes time before a positive action is taken on such breakage. The horrible sight of such breakage was a major motivating factor that stimulated the execution of this study. Sewage from the burst pipes were collected aseptically at different dripping points and transported to the laboratories on ice for microbiological analysis. A total of eight sewage samples were collected at the earmarked dripping points on the sewers. Samples were analyzed about one hour after collection.

**Isolation of cefotaxime-resistant Escherichia coli from the samples**

Aliquot (2 mL) of each sewage sample was inoculated into 3 mL Brain Heart Infusion broth (Becton, Dickinson and Company, USA), to which a disc of cefotaxime (30 µg), purchased from Oxoid, UK, has been added, giving a final concentration of 6 µg of the antibiotic in each tube. The tubes were incubated overnight at 35 ± 2 °C. Using the streak plate technique, a loopful of each of the cefotaxime-amended samples was inoculated onto the surface of already prepared plates of CHROMagar E. coli (CHROMagar, France) and incubated overnight at 35 ± 2 °C. Blue colonies presumptive of Escherichia coli were picked, purified and stored in glycerol stock (15% glycerol).

**Identification of cefotaxime-resistant Escherichia coli**

The genomic DNA of presumptive Escherichia coli was extracted using the boiling lysis method according to the modified method of Gugliandolo et al. (2010). The identity of the isolates was confirmed by detecting the presence of uidA, a housekeeping gene in Escherichia coli. The method of Janezic et al. (2013) was used for the PCR amplification of the uidA gene. Escherichia coli OG1a possessing the uidA gene as reported by Adekanmbi et al. (2020) was used as the positive control. Isolates possessing the uidA gene were selected for the antibiotic susceptibility
testing and detection of PMQR genes. The details of the uidA primers are shown in Table 1.

### Susceptibility to antibiotics

The confirmed *Escherichia coli* were subjected to a panel of eight antibiotics using the Kirby–Bauer disc diffusion method (Kirby-Bauer 1996). The procedures used for the susceptibility testing, including the choice of medium, inoculum standardization, reading and interpretation of the zone diameter were as described by CLSI (2018). Isolates that were resistant to ciprofloxacin (a fluoroquinolone), were selected for the detection of the PMQR genes which encode quinolone resistance.

### Detection of PMQR genes

The detection of six genes encoding PMQR in confirmed ciprofloxacin-resistant (CIP-resistant) *Escherichia coli* was done using the methods of Wu et al. (2007) and Park et al. (2009). The detection of qnrA, qnrB and qnrS and qepA was carried out using a duplex PCR, while a monoplex PCR was used for the detection of aac(6’)-lb-cr and oqxAB. The PCR reaction volume was 25 μL consisting of 5 x PCR Master Mix (Jena Bioscience, Germany), forward and reverse primers (0.25 μL each), DNA template (1 μL), with PCR quality water making up the volume. The oligonucleotide primers used in this study are highlighted in Table 1.

### Results

Thirty-two non-repetitive cefotaxime-resistant *Escherichia coli* isolates were recovered from the surface-leaking sanitary sewers, with all the eight collected samples showing the presence of the organism. Twenty-one of the isolates representing 65.6% of the total isolates were resistant to ciprofloxacin (CIP), while 34.4% (11/32) were sensitive to the antibiotic.

### Table 1 Oligonucleotide primers and amplicon sizes of uidA and PMQR genes targeted in this study

| Target gene | Primer sequence (5’–3’) | Amplicon size (bp) | Reference |
|-------------|-------------------------|--------------------|-----------|
| uidA        | AAAACGGCAAGAAAAACGAG    | 146                | Janezic et al. (2013) |
|             | ACGCGTGTCAGCTTGTGCAG   |                    |           |
|             | GCCAGCACCATTACTCA       | 608                | Wu et al. (2007) |
|             | GGCAGCACCATTACTTCCAAA  |                    |           |
| qnrA        | TTAGCGAGGCACTGAAATTCTCA| 389                | Wu et al. (2007) |
|             | GTTTGCGTCGCAAGTGCAGA   |                    |           |
| qnrB        | CAATTCATACATACGGGACC   | 621                | Wu et al. (2007) |
|             | TCAGGATAAACAAACATACCC  |                    |           |
| qnrS        | CCAGTTCCGCAACGGGTAG     | 218                | Wu et al. (2007) |
|             | CTCCCTGCCCCGAAGATGCTGT |                    |           |
| qepA        | GCGAGTCCGCAAGCGGTAG    | 392                | Wu et al. (2007) |
|             | CCACCTTCACGGGAGACGA    |                    |           |
| oqxAB       | CTGCGGCGGGGATGCTGCT    | 482                | Park et al. (2009) |
|             | CTCAGCTCTGCGCCCGAGTCTT |                    |           |
| aac(6’)-lb-cr | TTGGGATGCCTCTATGGTGCTGCA |                   |           |
|             | CCTCGAATGCTTTGGCGCGTTT  |                    |           |

### Table 2 Resistance of the ciprofloxacin-resistant *Escherichia coli* to antibiotics (n = 21)

| Antibiotics (concentration) | Sensitivity (%) | Intermediate (%) | Resistance (%) |
|-----------------------------|-----------------|------------------|----------------|
| SXT (25 μg)                 | 2 (9.5)         | 0 (0)            | 19 (90.5)      |
| CN (15 μg)                  | 5 (23.8)        | 6 (28.6)         | 10 (47.6)      |
| IPM (5 μg)                  | 21 (100)        | 0 (0)            | 0 (0)          |
| CTX (30 μg)                 | 0 (0)           | 0 (0)            | 21 (100)       |
| AMC (30 μg)                 | 10 (47.6)       | 5 (23.8)         | 6 (28.6)       |
| CPD (30 μg)                 | 0 (0)           | 0 (0)            | 21 (100)       |
| CAZ (30 μg)                 | 8 (30.1)        | 6 (28.6)         | 7 (33.3)       |
| CXM (30 μg)                 | 0 (0)           | 0 (0)            | 21 (100)       |

n Total number of ciprofloxacin-resistant bacteria obtained; SXT trimethoprim/sulfamethoxazole (1.25/23.75 μg); CN gentamycin (15 μg); IMP imipenem (5 μg); CTX cefotaxime (30 μg); AMC amoxicillin-clavulanate (20/10 μg); CPD cefpodoxime (30 μg); CAZ cefazidime (30 μg); CXM cefuroxime (30 μg)
All the targeted PMQR genes were detected in varying number of isolates, with the notable exception being \(\text{qxAB}\) gene, which was not detected at all in any isolate as shown in Tables 3 and 4. Of the six PMQR genes targeted in this study, \(\text{qnrA}\) was detected in 13 (61.9%) of the isolates, making it the second most prevalent PMQR gene, after \(\text{aac(6')-lb-cr}\) (which was the most prevalent) with 19 (90.5%) of the isolates harbouring it. The frequency of occurrence of \(\text{qnrB}, \text{qnrS}\) and \(\text{qepA}\) was 10 (47.6%), 8 (38.1%) and 1 (4.8%) respectively, \(\text{qepA}\) was not detected in any of the 21 CIP-resistant \(\text{Escherichia coli}\). One of the isolates (\(E.\ coli\ SWS2a\), did not carry any of the six PMQR genes targeted, while six isolates carried one PMQR gene only. Four isolates carried two and three PMQR genes respectively, while four of the six PMQR genes were carried by 6 (28.6%) of the isolates. There was co-occurrence of PMQR genes in 14 (66.7%) of the isolates.

### Discussion

The fluoroquinolone antibiotics have been used extensively in the treatment of human and animal infections, mainly because of their selective inhibition of the synthesis of DNA in bacterial cells. They account for close to one-fifth of the global antibiotic consumption (Van Doorslaer et al. 2014). Ciprofloxacin, a second-generation fluoroquinolone has been widely used in the treatment of bacterial infections of human origin and is one of the most abundant antibiotics detected in municipal biosolids, owing to their widespread use and the fact that it is excreted mostly unchanged in urine and wastes of faecal origin, making them available at high concentration in wastewater from sewage and clinical settings (Kaplan et al. 2013; Van Doorslaer et al. 2014; Xiong et al. 2015). The major difference between the quinolones of the first and second generations is the possession of a fluoroine atom at the sixth carbon atom of the quinolone moiety by the latter, and they exhibit a high level of potency against gram-negative bacteria and several gram-positive bacterial genera. In addition to this, however, ciprofloxacin shows a high level of activity against \(\text{Pseudomonas aeruginosa}\),
making it a drug of choice in the treatment of a variety of infections (Panton and Reeves 1988; Van Bambeke et al. 2005).

Resistance to fluoroquinolones has increased worldwide over the past few years and this has limited available treatment options, resulting in several cases of resistance and treatment failures (Briales et al. 2012; Okade et al. 2014; Piekarska et al. 2015). The rising tide of antimicrobial resistance has been majorly linked to the widespread and continuous use of antibiotics in agriculture, human medicine and veterinary practices. This has placed a lot of selective pressure on bacteria, making it difficult to curtail the geometric rise in antibiotic resistance over the years (Baquero et al. 2008; Harnisz et al. 2015; Kotlarska et al. 2015). Sewage and wastewater on the other hand have been taunted as ideal environment for the acquisition of antibiotic resistance genes (ARG) and this has been largely linked to influence from anthropogenic activities (Marti et al. 2014; Osińska et al. 2016).

In our study, a total of 21 CIP-resistant E. coli was obtained from the pool of 32 cefotaxime-resistant isolates recovered from the broken sanitary sewers. Bacteria showing resistance to quinolones, especially E. coli have been well reported in several studies. Osińska et al. (2016) reported the isolation of fluoroquinolone-resistant bacteria from sewage and surface water samples in Poland, while studies carried out by Guillard et al. (2014), Yang et al. (2014) and Ranjbar et al. (2019), pointed out the fact that Escherichia coli ranked as the most frequently occurring fluoroquinolone-resistant bacteria from water, wastewater, animals, food and the clinical environment. A study carried out in Nigeria by Ehwarie and his colleagues reported the occurrence of fluoroquinolone-resistant enteric bacteria from samples of human and animal origin, with Escherichia coli being the predominant organism obtained (Ehwarie et al. 2021).

In this study, the target PMQR genes with the exception of oqxAB, were detected in 20 of the 21 CIP-resistant isolates obtained, with one isolate devoid of any of the six PMQR genes. The qnr determinants, which have been reported to occur on chromosomal DNA in many environmental strains (Poirel et al. 2005) are considered exclusively plasmid-borne in the Enterobacteriaceae (Rodriguez-Martinez et al. 2011). These genes are proteins of the pentapeptide repeat family and are concerned with the protection of DNA gyrase and topoisomerase (IV) from the deleterious effect of the quinolone antibiotics. They are a very diverse group of PMQR determinants and have been found resident in bacteria of animal, human and environmental origin. In this current study, three variants of the qnr gene were targeted, with varying degree of detection observed in the CIP-resistant isolates. Of the qnr determinants, qnrA was the most prevalent in this study, with 13 of the isolates (61.9%) harbouring it, followed by qnrB with 10 isolates (47.6%) and qnrS 8 (38.1%). The relative occurrence of the qnr genes in this study is higher than the frequency of occurrence in a study carried out by Ranjbar et al. (2019), where they reported the detection of qnrA, qnrB and qnrS in 1%, 9% and 28% of the total isolates obtained from water sources in Iran, while a study on hospital wastewater in the same country by Ranjbar et al. (2017), reported the detection of qnrB and qnrS in eleven (45.8%) and seven (29.2%) respectively of the E. coli isolates obtained. However, none of the isolates in their study harboured qnrA gene, which is contrary to this study.

The quinolone efflux pump (qepA) which was first described in a study by Yamane et al. (2007), confers on the bacterial cell, the ability to reduce quinolone accumulation in the cell. It has been detected widely in many gram-negative genera in most countries of the world. In our study, one isolate (4.8%) of the entire 21 CIP-resistant bacteria, harboured this particular gene. A low frequency of occurrence of the gene has been reported in several studies, and this could be linked to its limited host spectrum. Chen et al. (2007) reported a percentage occurrence of 2.6% for qepA in 1022 E. coli isolates obtained in their study, while Cattoir et al. (2007) and Yamane et al. (2008) detected qepA in 0.8% and 0.3% of the total isolates obtained in their respective studies.

There is limited data on oqxAB, an efflux pump-mediating gene, which was discovered in the late 2000s. Its role as a PMQR determinant was not identified until then, thereby making data available on the gene quite limited. It confers resistance to the veterinary growth-promoting quinoxaline-di-N-oxide olaquindox, and was first discovered in Escherichia coli from swine (Hansen et al. 2004; Sørensen et al. 2003; Strahilevitz et al. 2013). The frequency of occurrence of the gene in most studies is very low in comparison with other PMQR determinants and this could explain the absence of the gene in all the CIP-resistant isolates obtained in this study.

Aminoglycoside acetyltransferase (aac(6′)-lb-cr) acts by causing the acetylation of quinolones, causing the modification of the antibiotics, which eventually leads to their inactivation. On its own, the resistance conferred on bacteria by aminoglycoside acetyltransferase is typically low, but it becomes elevated with the presence of qnr gene in the cell. In most reports on quinolone resistance in bacteria, aac(6′)-lb-cr appears to be the commonest PMQR determinant (De Jong et al. 2018; Strahilevitz et al. 2009; Shaheen et al. 2013), and this corroborates the observation in this study, where 19 of 21 (90%) of the CIP-resistant bacteria carried the gene. This observation is in agreement with several other reports where the occurrence of the gene and its high prevalence in clinical samples and wastewater of hospital origin have been widely reported. In a study on hospital wastewater carried out by Chandran et al. (2014), they detected aac(6′)-lb-cr in 89% of their
isolates, while Guillard et al. (2014) and Piekarska et al. (2015) detected the gene in 96.7% (118/122) and 85.7% of isolates obtained from samples and wastewater of clinical origin, respectively in their studies. In a different twist to the aforementioned, Su et al. (2013) reported the detection of \( aac(6')-lb-cr \) in 5.1% of the total 452 quinolone-resistant isolates from a municipal treatment facility in China, making it the least occurring PMQR determinant in their study.

**Limitations of the study**

Despite the abundance of PMQR genes in the isolates obtained in this study, the limitations stem from the fact that the sampling process stopped as soon as the repair works on the burst sewers commenced, making it difficult to get more samples for the isolation of more resistant isolates. Despite this limitation, other studies could venture into the detection of PMQR determinants in other bacterial genera from the environment and the genomic characterization of such isolates using advanced molecular biology tools. This will enrich the few available reports on PMQR determinants in isolates of environmental origin.

**Conclusion**

This present study has provided some information on the occurrence of cefotaxime-resistant *Escherichia coli* carrying PMQR genes in sewage samples, with \( aac(6')-lb-cr \) being the most predominant PMQR gene, while \( oqxAB \) was not detected. The resistance shown to the tested antibiotics by the CIP-resistant isolates varied, with imipenem (a carbapenem) being the only antibiotic, to which resistance was not observed to. This suggests that the receiving environment of these untreated leaky wastewater is a potential repository of these resistant bacteria and PMQR genes, which could expose the University community to a potential public health challenge. This calls for a holistic approach to the concept of antibiotic resistance, which is majorly being looked at from the clinical perspective, as the environment is not spared of this menace. There is also a need for more proactive and urgent mechanisms to be put in place to address the issues of broken sewers within communities.

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**Declarations**

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