ORIGINAL RESEARCH PAPER

Volume-7 | Issue-8 | August-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# DETECTION OF QUINOLONE RESISTANCE GENES (*QNR A, QNR B AND QNR S*) IN *KLEBSIELLA SPP.*, ISOLATES FROM CLINICAL SAMPLES IN BAYELSA STATE, NIGERIA.

Microbiology

**Tolulope Alade** — Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria

**Abdulrasheed Abdu*** — Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria. *Corresponding Author

**Tattfeng Mirabeau** — Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria

## ABSTRACT

The aim of this study was to investigate plasmid-encoded quinolone resistance determinants *QnrA*, *QnrB* and *QnrS* in quinolone-resistant *Klebsiella* spp. isolates recovered in two tertiary hospitals, in Yenagoa, Nigeria. Fifteen quinolone-resistant, *Klebsiella* spp. isolates were studied. PCR with primers specific for *qnrA, qnrB* and *qnrS* genes were used. 5 (33.33%) were positive for PMQR gene. only *qnrB* genes were present in all (100%) the isolates, while none were detected for *qnrA* and *qnrS* genes. Of 14 Nalidixic acid resistant isolates, 5(35.7%) carried *qnrB* genes. Among 9 Ciprofloxacin and Pefloxacin resistant isolates, 4(44.4%) and 3(33.3%) were positive for *qnrB* respectively. Two (25.0%) of eight ofloxacin resistant isolates could amplify *qnrB*. This study identified the first plasmid-mediated quinolone resistance determinant in *Klebsiella* spp. in Yenagoa, Bayelsa, Nigeria.

## KEYWORDS

Plasmid-mediated quinolone resistance genes; *qnrA; qnrB; qnrS; Klebsiella* spp., Yenagoa.

### Introduction

*Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative non motile, enteric rod and a member of the family *Enterobacteriaceae* that produces capsule (Heiat *et al*., 2014; Podschun & Ullmann, 1998). *K. pneumoniae* is among the body micro flora (Heiat *et al*., 2014) and it is recognized as an opportunistic pathogen being one of the most important species involved in healthcare associated infections (Ko, *et al*., 2002; Podschun & Ullmann, 1998). The mortality rate related to it is high and is associated with a variety of diseases which includes: urinary tract infection, septicemia, pneumonia and intra-abdominal infections in hospitalized patients (CDC, 2012); however infections associated to *K. pneumoniae* in the community is less observed (Padilla *et al*., 2010; Paterson *et al*., 2003). From clinical aspect, *K. pneumoniae* strains colonize vastly and seen in hospitalized patients with deficiency in immune system such as diabetic individuals, patients with acquired immune deficiency, elderly patients and children (Moghadasi *et al*., 2016). Severe epidemics due to Klebsiella usually occur in children and endemic infections are mostly in patients with renal disease. Although pneumonia by Klebsiella include a small part of total pneumonia, it is highly fatal and encounters approximately 90% (Yousefi *et al*., 2014).

In search for newer efficacious drugs, the quinolone class of antibiotics was introduced into clinical use in the 1960s (Lescher *et al*., 1962) and has since been an important drug for the treatment of bacterial infections (Yang *et al*., 2014). In the late 1980s, more systemically active drugs (eg, fluoroquinolone) became clinically available (Andersson & MacGowan, 2003). Over the decades since the introduction of fluoroquinolones, resistance to these agents in *Enterobacteriaceae* has become common and widespread (Paterson, 2006; Hooper, 2001). Previous studies have demonstrated that in this group of organisms, the main mechanisms of quinolone resistance arise from chromosomal mutations in genes encoding DNA gyrase and topoisomerase IV (Martínez-Martínez, *et al*., 2006; Ruiz 2003; Hooper 2001; Drlica & Zhao, 1997). Upregulation of efflux pumps and/or decreased expression of outer membrane porins are also classically described mechanisms resulting from chromosomal mutations (Robicsek, *et al*., 2006; Ruiz 2003). Recently, however, plasmid mediated quinolone resistance (PMQR) genes have been detected in *Enterobacteriaceae* (Martinez-Martinez *et al*., 1998). Since the first PMQR determinant, termed *Qnr* (now known as *QnrA1*), was discovered in a clinical isolate of *K. pneumoniae* from the United States in 1998 (Martinez-Martinez *et al*., 2008), several plasmid-mediated quinolone resistance (PMQR) genes (Jacoby, *et al*., 2008) have been reported from clinical isolates, while two mechanisms of PMQR have been reported including the quinolone modification with a piperazinyl substituent by the acetyltransferase *AAC(6')-Ib-cr* and active efflux by *QepA* and *OqxAB*, which are pumps related to major facilitator superfamily transporters (Wang, *et al*., 2008; Yamane *et al*., 2007; Machado, *et al*., 2006; Robicsek *et al*., 2006; Hansen *et al*., 2004; Sørensen *et al*., 2003). The PMQR genes confer low-level quinolone resistance and supplement the level of resistance caused by other resistance mechanisms.

PMQR genes have been found worldwide in multiple species of *K. pneumoniae* (El-Badawy *et al*., 2017; Moghadasi *et al*., 2016; Cruz *et al*., 2013; Jacoby *et al*., 2003; Lu *et al*., 2007; Pe´richon *et al*., 2007; Robicsek *et al*., 2005, 2006; Strahilevitz *et al*., 2007; Wang *et al*., 2004). Interestingly, on some occasions more than one PMQR gene has been identified in the same organism (Martínez-Martínez *et al*., 2008; .Robicsek *et al*., 2006).

The study aimed to investigate the prevalence of PMQR genes (*qnrA*, -*B*, and -*S*) in quinolone-resistant *K. pneumoniae* recovered from clinical samples in two tertiary hospitals in Bayelsa State, Nigeria.

### Materials and Methods:

#### Bacteria isolates

Fifteen (15) isolates of *K. pneumoniae* recovered from several samples [Urine (8), endocervical swab (1), sputum (2), high vaginal swab (2), throat swab (1)] of hospitalized patients with different clinical infections and the hospital environment (1) were evaluated in two tertiary hospitals of Bayelsa. All isolates were identified by conventional bacteriological tests (Farmer, 2003; Holt *et al*., 1994; Ewing, 1986). Isolates were further characterised biochemically using API 20E. Data interpretation was performed using the Analytical profile index (API) database (V4.1) with the apiwebTM identification software in accordance to protocols of Nyenje *et al*. (2012). The bacterial isolates were kept frozen at -84 ºC before tested.

#### Antimicrobial susceptibility test

For confirmation of isolates with resistance to quinolones, phenotypic method using antibiotic disks of Ciprofloxacin (5 µg), Ofloxacin (5 µg), Perfloxacin (5 µg) and nalidixic acid (30 µg) (Oxoid, England) was conducted by preparation of half McFarland and culture on Muller Hinton agar based on disc diffusion method in accordance to guidelines of Clinical and Laboratory Standards Institute (CLSI, 2017). *Escherichia coli* ATCC25922 was used as the quality control

for antibiotic disks.

**PCR amplification for Molecular detection of *qnr* genes**

All 15 isolates were screened by PMQR multiplex PCR assay in accordance to standardized protocols described by Cattoir *et al*. (2007) and Moghadasi *et al*. (2016). Firstly, we extracted the total DNA from quinolone resistant isolates with the boiling method. The polymerase chain reaction (PCR) method was performed for amplification of genes with specific primers shown in table 1.

**Table 1: Primers for *qnr A, qnrB* and *qnrS* used in this study**

| Target Gene | Primer | Sequence (5′-3′) | Length of product (bp) |
|---|---|---|---|
| *qnrA* | *qnrA* | F:5′GATAAAGTTTTTCAGCAAGAGG<br>R: 5′ATCCAGATCCGCAAAGGTTA | 823 |
| *qnrB* | *qnrB* | F:5′ATGACGCCATTACTGTATAA<br>R: 5′GATCGCAATGTGTGAAGTTT | 408 |
| *qnrS* | *qnrS* | F: 5′ATGGAAACCTACAATCATAC<br>R: 5′AAAAACACCTCGACTTAAGT | 428 |

Whichard *et al.*, 2007

The master mix adjusted in 20µl including 12.5 µl 1X Taq Master mix (Amplicon), 1ul forward primer, 1ul reverse primer with 5 picomol/L, 3 µl template DNA and 7.5 µl double distilled sterile water and PCR was performed with thermocycler (Gene Amp PCR System 9700, USA) device.

**Gel electrophoresis**

The amplicons from M-PCR were separated on 1.5% agarose gels using a suitable DNA ladder; 100 bp DNA ladder. Gels were visualized under a UV transilluminator (Haakebuchler Instruments Inc, USA) and captured using Olympus digital camera, and the DigiDoc-ItProgram (UVP, UK)

**Results:**

Eighty (0.5 isolate per sample) bacteria were isolated from 146 clinical samples collected during the studied period. As shown in table 2, 35(43.7%) were isolated from urine, 11(13.7%) from the Hospitals environment, 10(12.5%) from wound, 8(10.0%) from sputum, 6(7.5%) from endocervical, 7(8.8%) from high vaginal, 2(2.5%) from tracheal aspiration, and 1(1.3%) from urethral. Fifteen (18.8%) of the 80 isolates were *Klebsiella spp*., of which 8(10.03%) were from urine, 2(2.51%) each from sputum and high vaginal swab, while 1(1.25%) was from throat swab, endocervical swab and the hospital environment respectively.

In the antibiotic susceptibility test, all (100%) of the *Klebsiella spp*., isolates were resistant to at least one of the quinolones (Table 3). Fourteen (93.33%), 9 (60.00%), 9 (60.00%) and 8(53.33%) isolates were resistant to Nalidixic acid, Ciprofloxacin, Pefloxacin and Ofloxacin respectively.

PCR products were observed after electrophoresis on 1.5% gel agarose. Among the 15 examined quinolones resistant isolates, 5 (33.33%) were positive for at least one PMQR gene (Figure 1). As shown in Table 4, of the PMQR genes, only *qnrB* genes were present in all (100%) the isolates, while *qnrA* and *qnrS* gene were not detected in any of the isolates. Of 14 Nalidixic acid resistant isolates, 5(35.7%) carried *qnrB* genes. Among 9 Ciprofloxacin and Pefloxacin resistant isolates, 4(44.4%) and 3(33.3%) were positive for *qnrB* respectively. Moreover, of 8 with resistance to Ofloxacin resistant isolates, 2(25.0%) harboured *qnrB gene*.

**Discussion:**

This study was conducted to evaluate prevalence of PMQR (*QnrA*, *QnrB* and *QnrS*) genes among quinolone-resistant *Klebsiella* spp. isolated from two tertiary hospitals in Yenagoa over a 2-month period.

The resistance of *Klebsiella spp.* isolates to nalidixic acid, ciprofloxacin, pefloxacin, and ofloxacin was 14(93.33%), 9 (60.00%), 9 (60.00%) and 8(53.33%) respectively. These findings are at variance with prior reports made by Soltan Dallal *et al* (2012). In their report, they reported a prevalence of 42.5% to nalidixic acid in Tehran. In addition, Amin *et al*. (2009) and Moghadasi *et al*. (2016) also reported 42.5% resistance each to nalidixic in Pakistan and Iran respectively. On the other hand, in this study, ciprofloxacin, recorded the 60.0% resistance among the *Klebsiella* spp isolates, while Moghadasi *et al* (2016) reported 27.6% resistance to ciprofloxacin. Amin *et al*. (2009) in Pakistan detected 55% ciprofloxacin resistant among 40 *K. pneumonia* isolates. This disparities could be due to geographical differences (Mahmoudjanlou *et al*., 2012; Yousefi Mashour *et al*., 2014), coupled with the high level and indiscriminate use associated with easy access to antibiotics in our environment.

Studies have demonstrated that the *qnr* genes encode proteins that protect DNA gyrase and topoisomerase IV from inhibition by quinolones (Pan *et al*., 1996; Martinez-Martinez *et al*., 1998; Hata *et al*., 2005; Tran *et al*., 2005a; 2005b), and have recently been identified worldwide (Wang *et al*., 2004; Rodriguez-Martinez *et al*., 2006; Ciesielczuk *et al*., 2013; Yousefi- Mashour *et al*., 2014). Depending on the selection criteria, the period of study, and the type of the bacterial isolates, studies shown that the prevalence of the *qnr* genes in bacterial isolates may range from <1% to >50% (Wang *et al*., 2008; Jeong *et al*., 2005; Shin *et al*., 2008; Kim *et al*., 2009; Yang *et al*., 2014). Among the studies carried out in China, Wang *et al* (2008) reported the incidences of *qnr* as 7.5% and 11.9% among ciprofloxacin-resistant *E coli* and *K pneumoniae* isolates respectively, while, *qnr*A, *qnr*B and *qnr*S were detected either alone or in combination in 3.8%, 4.7% and 3.8% of these isolates respectively. In Korea, Shin *et al* (2008) reported that 5.6% of *E coli* and 55.9% of *K pneumoniae* ciprofloxacin-resistant isolates contained only *qnr*B (*qnr*B2, *qnr*B4 and/or *qnr*B6). Jeong *et al* (2005) reported that the prevalence of *qnr*A in Korea was 0.8% in *E coli* isolates (ciprofloxacin susceptible and resistant) between 2001 and 2003. Kim *et al* (2009) documented that 0.5% of *E coli* and 5.9% of *K pneumoniae* (ciprofloxacin susceptible and resistant) isolates in Korea contained *qnr* (*qnr*B or *qnr*S). In our present study, of the *qnr* variants, we did not detect *qnr*A and *qnr*S, but *qnrB* was the most common. Epidemiological investigations, including our present study, have shown that *qnr*B (especially *qnr*B4) (Seo *et al*., 2010; Yang *et al*., 2014; Moghadasi *et al*., 2016) is common, while *qnr*A and *qnr*S are present in Korea at relatively low prevalences (Jeong *et al*., 2005; Shin *et al*., 2008; Kim *et al*., 2009). Even though our screening gave negative results for the *qnrA* and *qnrS* gene, five (33.3%) of the 15 clinical *Klebsiella* spp. strains analysed were positive for the *qnrB* gene. This prevalence of qnrB (33.3%) falls within the available range (<1% to >50%) documented, however, in contrast, it is lower to the 50% reported in previous investigations (Wang et al., 2008; Shin et al., 2008; Yang et al., 2014), but higher than 5.9%, 11.9% of Kim et al. (2009) and Wang et al. (2008) respectively. PMQR in the family of *Enterobacteriaceae* is one kind that is rapidly transmitted among strains through horizontal means. The horizontal transmission of these plasmids to normal flora should be of concern, particularly in its ability to aid in the dissemination of antibiotic resistances (Robicsek *et al*., 2006; Pallecchi *et al*., 2009; Lamikanra *et al*., 2011). Various reasons have been adduced for this spread, of which the higher level of quinolones use in veterinary medicine have been considered an important factor (Poirel *et al*., 2012). As reported by various authors, another factor of concern associated with the advent of quinolone resistance is their close relation with other agents especially the expression of *extended-spectrum beta-lactamases* (ESBLs) and aminoglycosides (Robicsek *et al*., 2006; Park *et al*., 2006; Deepak *et al*., 2009; Strahilevitz *et al*., 2009; Zhang *et al*., 2012; Andres *et al*., 2013; Liu *et al*., 2013; Shaheen *et a*l., 2013). It is quit unfortunate, that this biologic relationship among these agents has caused a suitable opportunity for dissemination of multidrug-resistant among the *Enterobacteriaceae*, thus resulting to restrictions on treatment choices. Therefore, this should be of concern to the physicians when prescribing quinolones that the resistance to cephalosporins and aminoglycosides and other resistance forms which are associated with PMQRs may occur as well (Robicsek *et al*., 2006).

Contrast to this present study, a study conducted by Kim *et al*. (2009) in Boston, USA, reported a recovery rate of 13 (10%) plasmid mediated quinolone resistance genes among 135 *K. pneumonia*, of which four isolates were positive for *qnrB* and *qnrS* genes, respectively, while none of isolates amplied *qnrA* and *qnrC* genes (Kim *et al*., 2009). In this study, none of the isolates was *qnr A* and *qnr S* positive and all were positive for *qnrB* gene. The higher prevalence of *qnrB* genes as observed in the present study is in consistent with earlier reports made by Minarini *et al* (2008) were none of their clinical isolates were *qnrA* positive, and *qnrB* was the predominant *qnr* genes.

It is worthy to note that in this study, all the *qnrB* genes were detected from nalidixic acid resistant, 5(4 resistant and 1 sensitive) to

ciprofloxacin, 5(3 resistant and 2-senstive) to pefloxacin, while 5(2 resistant and 3-sensitive) to norfloxacin. In an investigation conducted by Azadpour *et al* (2014) in Khorramabad (Iran), among 107 clinical isolates of *K. pneumoniae* isolates 34 were resistant to ciprofloxacin and 66 were susceptible. Eighteen of 107 isolates (16.8%) were *qnr* positive, among which, 16 (88.9%) and 1 (5.55%) were *qnrB* and *qnrS* positive, respectively while one isolate was positive for both genes. These genes were detected in 8 (23.5%) ciprofloxacin resistant and 9(13.6%) from susceptible isolates. no significant relationship was detected between ciprofloxacin resistance and *qnr* genes. Azadpour *et al* (2014) have indicated that higher rate of these genes as reported in this study could be due to other factors such as bacteriophages, transposons and integrons in addition to the plasmids.

**Conclusion:**

We identified PMQR genes in 33.3% (5 of 15) of quinolone non-susceptible *Klebsiella* spp isolated from two tertiary-care hospitals in Bayelsa, Nigeria. The prevalent PMQR gene was *qnrB*. PMQR genes were highly prevalent among quinolones resistant *Klebsiella spp*. isolated from urine cultures in our hospital. It is therefore, necessary to monitor for spread of PMQR genes of clinical isolates and to ensure careful antibiotic use in a hospital setting.

**Table 2: Distribution of bacteria isolates per specimen during the studied period**

| Specimen | E. coli | Klebsiella spp. | Proteus spp. | P. aeruginosa | S. aureus | Total (%) |
|---|---|---|---|---|---|---|
| Urine | 12(34.3) | 8(22.9) | 3(8.5) | 4(11.4) | 8(22.9) | 35(43.7) |
| Endocervical swab | - | 1(16.7) | - | - | 5(71.4) | 6(7.5) |
| Sputum | - | 2(25.0) | 2(25.0) | - | 4(50.0) | 8(10.0) |
| High vaginal swab | 2(33.3) | 2(28.5) | 3(50.0) | - | - | 7(8.8) |
| Wound swab | - | - | - | 4(40.0) | 6(60.0) | 10(12.5) |
| Throat swab | - | 1(50.0) | - | - | 1(50.0) | 2(2.5) |
| Urethral swab | - | - | - | - | 1(100.0) | 1(1.3) |
| Hospital environment | 2(18.2) | 1(9.1) | 2(18.2) | 6(54.5) | - | 11(13.7) |
| Total | 16(20.0) | 15(18.8) | 10(12.5) | 14(17.5) | 25(31.2) | 80(100.0) |

**Table 3: Quinolone Susceptibility Profiles of Clinical isolates from Klebsiella spp., during the period of study.**

| Antibiotics | Susceptible (%) | Resistant (%) |
|---|---|---|
| Ofloxacin | 7(46.7) | 8(53.3) |
| Pefloxacin | 6 (40.0) | 9(60.0) |
| Ciprofloxacin | 6(40.0) | 9(60.0) |
| Nalidixic acid | 1(6.7) | 14(93.3) |

**Table 4: Distribution and Prevalence of plasmid-mediated quinolone resistance genes in Klebsiella spp., isolates**

| Isolate / Specimen type | Antibiotics | | | | *Qnr* gene |
|---|---|---|---|---|---|
| | OFX | PEF | CIP | NAL | |
| EC17 | - | - | - | - | *nil* |
| L16 | + | + | - | - | *qnrB* |
| SP20 | + | + | + | - | *nil* |
| SP9 | + | - | + | - | *nil* |
| TS2 | - | + | + | - | *nil* |
| UR10 | - | - | - | - | *qnrB* |
| UR16 | + | + | + | - | *qnrB* |
| UR30 | + | - | - | - | *qnrB* |
| UR35 | + | + | + | + | *nil* |
| UR42 | - | - | - | - | *nil* |
| UR48 | - | - | - | - | *qnrB* |
| UR55 | - | - | - | - | *nil* |
| UR56 | - | - | - | - | *nil* |
| VS17 | - | - | - | - | *nil* |
| VS18 | + | + | + | - | *nil* |

**Keys:** EC, Endocervical; L, Hospital environment; SP, Sputum; TS, Throat swab; UR, Urine; VS, Vaginal swab; NAL, nalidixic acid; CIP, ciprofloxacin; OFX, Ofloxacin; PEF, Pefloxacin; +, sensitive, -, resistant; nil, non-detected

**Reference**


1. Amin A, Ghumro P, Hussain S, Hameed A. Prevalence of antibiotic resistance among clinical isolates of Klebsiella pneumoniae isolated from a Tertiary Care Hospital in Pakistan. Malays J Microbiol 2009; 5(2): 81-6.
2. Andersson MI, MacGowan AP. Development of the quinolones. J Antimicrob Chemother 2003; 51(Suppl 1):1-11.
3. Andres, P., Lucero, C., Soler-Bistue´ , A., Guerriero, L., Albornoz, E., Tran, T., Zorreguieta, A., Galas, M., and Corso, A. (2013). Differential distribution of plasmid-mediated quinolone resistance genes in clinical enterobacteria with unusual phenotypes of quinolone susceptibility from Argentina. Antimicrob Agents Chemother 57, 2467–2475.
4. Azadpour M, Soleimani Y, Rezaie F. Prevalence of qnr Genes and Antibiotic Susceptibility Patterns among Clinical Isolates of Klebsiella pneumoniae in West of Iran. J Bacteriol Parasitol 2014; 5(5): 1-4.
5. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother 2007;60:394-7.
6. Cavaco, L. M., H. Hasman, S. Xia, and F. M. Aarestrup. 2009. qnrD, a novel gene conferring transferable quinolone resistance in Salmonella enterica serovar Kentucky and Bovismorbificans strains of human origin. Antimicrob. Agents Chemother. 53:603–608.
7. Centre for Disease Control and Prevention (CDC). (2012). Klebsiella pneumoniae in Healthcare settings
8. Ciesielczuk H, M. Hornsey M, Choi V, Woodford N, Wareham DW. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. Journal of Medical Microbiology 2013, 62, 1823–1827.
9. CLSI (Clinical and Laboratory Standards Institute) (2017). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-seventh Information Supplement, Wayne, PA, 37(1), M100-S26:1-249.
10. Cruz, G. R., Radice M, Sennati S, Pallecchi L, Rossolini GM, Gutkind G, Alejandro Di Conza, J. 2013. Prevalence of plasmid-mediated quinolone resistance determinants among oxyiminocephalosporin-resistant Enterobacteriaceae in Argentina. Mem Inst Oswaldo Cruz, Rio de Janeiro, 108(7): 924-927.
11. Deepak, R. N., Koh, T. H. & Chan, K. S. (2009). Plasmid-mediated quinolone resistance determinants in urinary isolates of Escherichia coli and Klebsiella pneumoniae in a large Singapore hospital. Ann Acad Med Singapore 38, 1070–1073.
12. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev 1997;61:377-92.
13. El-Badawy, M.F., Tawakol, W.M., El-Far, S.W., Maghrabi, I.A., Al-Ghamdi, S.A., Mansy, M.S., Ashour, M.S., and Shohayeb, M.M. 2017. Molecular Identification of Aminoglycoside-Modifying Enzymes and Plasmid-Mediated Quinolone Resistance Genes among Klebsiella pneumoniae Clinical Isolates Recovered from Egyptian Patients. Hindawi International Journal of Microbiology 2017, Article ID 8050432, 12 pages https://doi.org/10.1155/2017/8050432
14. Ewing, W. H. (1986): The genus Shigella. In Identification of Enterobacteriaceae, 4th edition, pp. 135–172. Edited by W. H. Ewing. New York: Elsevier Science Publishing.
15. Farmer, J. J. (2003): Enterobacteriaceae: introduction and identification. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. ASM Press, Washington, D.C. pp. 636–653.
16. Hansen LH JE, Burmølle M, Sørensen AH, Sørensen SJ. Plasmidencoded multidrug efflux pump conferring resistance to olaquindox in Escherichia coli. Antimicrob Agents Chemother 2004;48:6.
17. Hata, M., M. Suzuki, M. Matsumoto, M. Takahashi, K. Sato, S. Ibe, and K. Sakae. 2005. Cloning of a novel gene for quinolone resistance from a transferable plasmid in Shigella flexneri 2b. Antimicrob. Agents Chemother. 49:801–803.
18. Heiat M, Rezaeimehr M, Moghaddam M, et al. Molecular genetic analysis of quinolone resistance-determining region of DNA Gyrase-A in fluoroquinolones resistant Klebsiella pneumoniae based on GenBank data and reported studies. Mol Genet Microbiol Virol 2014; 29(4): 211-5.
19. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (editors) (1994): Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore, MD: Williams and Wilkins.
20. Hooper, D. C. 2001. Emerging mechanisms of fluoroquinolone resistance. Emerg. Infect. Dis. 7:337–341.
21. Jacoby, G. A., N. Chow, and K. B. Waites. 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. 47:559–562.
22. Jacoby, G., V. Cattoir, D. Hooper, L. Martínez-Martínez, P. Nordmann, A. Pascual, L. Poirel, and M. Wang. 2008. qnr gene nomenclature. Antimicrob. Agents Chemother. 52:2297–2299.
23. Jeong JY, Yoon HJ, Kim ES, et al. Detection of qnr in clinical isolates of Escherichia coli from Korea. Antimicrob Agents Chemother 2005;49:2522-4.
24. Kim ES, Jeong JY, Jun JB, et al Prevalence of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme among Enterobacteriaceae blood isolates in Korea. Antimicrob Agents Chemother 2009;53:2643-5.
25. Kim HB, Park CH, Kim CJ, et al. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob Agents Chemother 2009; 53(2): 639 -45.
26. Ko W-C, Paterson DL, Sagnimeni AJ, et al. Community-acquired Klebsiella pneumoniae bacteremia: global differences in clinical patterns. Emerg Infect Diseases 2002; 8(2): 160-6.
27. Lamikanra A, Crowe JL, Lijek RS, et al. Rapid evolution of fluoroquinolone-resistant Escherichia coli in Nigeria is temporally associated with fluoroquinolone use. BMC Infect Dis 2011; 11(1): 1-10.
28. Lescher GY, Froelich EJ, Gruett MD, Bailey JH, Brundage RP. 1,8-naphthyridine derivatives. A new class of chemotherapeutic agents. J Med Pharm Chem 1962;91:1063-5.
29. Liu, B. T., Liao, X. P., Yue, L., Chen, X. Y., Li, L., Yang, S. S., Sun, J., Zhang, S., Liao, S. D. & Liu, Y. H. (2013). Prevalence of b-lactamase and 16S rRNA methylase genes among clinical Escherichia coli isolates carrying plasmid-mediated quinolone resistance genes from animals. Microb Drug Resist 19, 237–245.
30. Lu, X., X. Ma, and R. Yu. 2007. Study of qnrB among Cephalosporin- Resistant Enterobacteriaceae species in West China. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., Chicago, IL.
31. Machado, E., T. M. Coque, R. Canto´n, F. Baquero, J. C. Sousa, L. Peixe, et al. 2006. Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1- producing Enterobacteriaceae strains containing the aac(6 )-Ib-cr gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Antimicrob. Agents Chemother. 50:3220–3221
32. Mahmoudjanlou H, Moradi A, Shakeri F, et al. Minimum Inhibitory Concentration of

Cefotaxime by E-test Method on Klebsiella in Gorgan. Medical Lab J 2012; 6(2): 16 -20.
33. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet 1998;351:797-9.
34. Martinez-Martinez, L., A. Pascual, and G.A. Jacoby. 1998. Quinolone resistance from a transferable plasmid. Lancet 351:797–799
35. Martínez-Martínez, L., M. E. Cano, J. M. Rodríguez-Martínez, J. Calvo, and A. Pascual. 2008. Plasmid-mediated quinolone resistance. Expert. Rev. Anti-Infect. Ther. 6:685–711.
36. Minarini LA, Poirel L, Cattoir V, et al. Plasmid-mediated quinolone resistance determinants among enterobacterial isolates from outpatients in Brazil. J Antimicrob Chemoth 2008; 62(3): 474-8.
37. Moghadasi M, Mirzaee M, Mehrabi MR. Frequency of Quinolone Resistance and qnrB and qnrC Genes in Clinical Isolates of Klebsiella pneumoniae. J Med Bacteriol. 2016; 5(5, 6): 39-45
38. Nyenje, E., F. Tanih. (2012). "Current Status of Antibiograms of Listeria ivanovii and Enterobacter cloacae Isolated from Ready-To-Eat Foods in Alice, South Africa." Int. J. Environ. Res. Public Health 9: 3102-3144.
39. Padilla E, Llobet E, omenech-Sanchez A. Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother 2010; 54(1): 177-83.
40. Pallecchi L, Riccobono E, Mantella A. High prevalence of qnr genes in commensal enterobacteria from healthy children in Peru and Bolivia. Antimicrob Agents Chemother 2009; 53(6): 2632-5.
41. Pan, X. S., Ambler, J., Mehtar, S. & Fisher, L. M. (1996). Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in Streptococcus pneumoniae. Antimicrob Agents Chemother 40, 2321–2326.
42. Park, C. H., Robicsek, A., Jacoby, G. A., Sahm, D. & Hooper, D. C. (2006). Prevalence in the United States of aac(69)-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 50, 3953–3955.
43. Paterson DL, Hujer KM, Hujer AM, . Extended-spectrum β-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV-and CTXM- type β-lactamases. Antimicrob Agents Chemother 2003; 47(11): 3554-60.
44. Paterson, D. L. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. Med. 119(Suppl. 1):S20–S28.
45. Pe´richon, B., P. Courvalin, and M. Galimand. 2007. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in Escherichia coli. Antimicrob. Agents Chemother. 51:2464–2469.
46. Podschun R, Ullmann U. Klebsiella spp. As nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11(4): 589-603.
47. Poirel L, Cattoir V, Nordmann P. Plasmidmediated quinolone resistance; interactions between human, animal, and environmental ecologies. Front Microbiol 2012; 3(24): 1-7.
48. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis 2006;6:629-40.
49. Robicsek A, Strahilevitz J, Jacoby GA, et al. Fluoroquinolone modifying enzyme: A new adaptation of a common aminoglycoside acetyltransferase. Nat Med 2006;12:83-8.
50. Robicsek, A., D. F. Sahm, J. Strahilevitz, G. A. Jacoby, and D. C. Hooper. 2005. Broader distribution of plasmid-mediated quinolone resistance in the United States. Antimicrob. Agents Chemother. 49:3001–3003.
51. Robicsek, A., J. Strahilevitz, D. F. Sahm, G. A. Jacoby, and D. C. Hooper. 2006. qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob. Agents Chemother. 50:2872–2874.
52. Rodriguez-Martinez, JM, Poirel L, Pascual A, Nordmann P. Plasmid-Mediated Quinolone Resistance in Australia. Microbial Drug Resistance 2006; 12(2): 99-102.
53. Ruiz, J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J. Antimicrob. Chemother. 51:1109–1117.
54. Seo MR, Park YS, Pai H. Characteristics of plasmid-mediated quinolone resistance genes in extended-spectrum cephalosporinresistant isolates of Klebsiella pneumoniae and Escherichia coli in Korea. Chemotherapy 2010;56:46-53.
55. Shaheen, B. W., Nayak, R., Foley, S. L. & Boothe, D. M. (2013). Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant Escherichia coli isolates recovered from companion animals in the USA. J Antimicrob Chemother 68, 1019–1024.
56. Shin JH, Jung HJ, Lee JY, Kim HR, Lee JN, Chang CL. High rates of plasmid-mediated quinolone resistance QnrB variants among ciprofloxacin-resistant Escherichia coli and Klebsiella pneumoniae from urinary tract infections in Korea. Microb Drug Resist 2008;14:221-6.
57. Soltan-Dallal MM, Miremadi SA, Sharify Yazdi MK, et al. Antimicrobial resistance trends of Klebsiella spp. isolated from patients in Imam Khomeini hospital. Payavard Salamat 2012; 6(4): 275-81.
58. Sørensen AH, Hansen LH, Johannesen E, Sørensen SJ. Conjugative plasmid conferring resistance to olaquindox. Antimicrob Agents Chemother 2003;47:798-9.
59. Strahilevitz, J., D. Engelstein, A. Adler, V. Temper, A. E. Moses, C. Block, and A. Robicsek. 2007. Changes in qnr prevalence and fluoroquinolone resistance in clinical isolates of Klebsiella pneumoniae and Enterobacter spp. collected from 1990 to 2005. Antimicrob. Agents Chemother. 51:3001–3003.
60. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmidencoded quinolone resistance protein Qnr with Escherichia coli DNA gyrase. Antimicrob Agents Chemother 2005a;49:118-25.
61. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmidencoded quinolone resistance protein QnrA with Escherichia coli topoisomerase IV. Antimicrob Agents Chemother 2005b;49:3050-2.
62. Wang A, Yang Y, Lu Q, et al. Presence of qnr gene in Escherichia coli and Klebsiella pneumoniae resistant to ciprofloxacin isolated from pediatric patients in China. BMC Infect Dis 2008;8:68.
63. Wang, M. H., X. Xu, S. Wu, D. Zhu, and M. Wang. 2008. A new plasmidmediated gene for quinolone resistance, qnrC, abstr. O207. Abstr. 18th Eur. Cong. Clin. Microbiol. Infect. Dis., Barcelona, Spain.
64. Wang, M., D. F. Sahm, G. A. Jacoby, and D. C. Hooper. 2004. Emerging plasmid-mediated quinolone resistance associated with the qnr gene in Klebsiella pneumoniae clinical isolates in the United States. Antimicrob. Agents Chemother. 48:1295–1299
65. Whichard JM, Gay K, Stevenson JE, Joyce KJ, Cooper KL, Omondi M, et al. Human Salmonella and Concurrent Decreased Susceptibility to Quinolones and Extended-Spectrum Cephalosporins. Emerg Infect Dis. 2007;13(11):1681-1688. https://dx.doi.org/10.3201/eid1311.061438
66. Yamane K, Wachino J, Suzuki S, et al. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an Escherichia coli clinical isolate. Antimicrob Agents Chemother 2007;51:3354-60.
67. Yang HY, Nam YS, Lee HJ. Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-nonsusceptible Escherichia coli and Klebsiella pneumoniae isolated from blood cultures in Korea. Can J Infect Dis Med Microbiol 2014;25(3):163-169.
68. Yousefi Mashour R, Alijani P, Saidijam M, et al. Study of antibiotic resistance pattern and phenotypic detection of ESBLs in Klebsiella pneumoniae strains isolated from clinical samples and determination of minimum inhibitory concentrations of imipenem and ceftazidim antibiotics. Scientific Journal of Hamadan University of Medical Sciences 2014; 20(4): 295-302.
69. Zhang, Y., Yang, J., Ye, L., Luo, Y., Wang, W., Zhou, W., Cui, Z. & Han, L. (2012). Characterization of clinical multidrug-resistant Escherichia coli and Klebsiella pneumoniae isolates, 2007-2009, China. Microb Drug Resist 18, 465–470.