American Journal of Microbiological Research, 2023, Vol. 11, No. 3, 79-82
Available online at http://pubs.sciepub.com/ajmr/11/3/3
Published by Science and Education Publishing
DOI:10.12691/ajmr-11-3-3

Detection of *qnr* Genes That Mediate Fluoroquinolone Resistance in Gram-Negative Bacilli in Abidjan, Côte d'Ivoire

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Received September 07, 2023; Revised October 08, 2023; Accepted October 15, 2023

Abstract: The public health threat of antimicrobial resistance remains a major challenge that keeps expanding as a result of the continuous dissemination of mobile genetic elements that mediate resistance among bacteria species. This study aimed to detect genetic determinants (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qnrVC* genes) that mediate fluoroquinolone resistance in Gram-negative bacilli in Côte d'Ivoire. A total of 30 strains were characterized by biochemical tests and their identity was confirmed by MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was performed by the agar diffusion method and the detection of *qnr* genes by polymerase chain reaction (PCR). Fifteen (57.7%) of the Enterobacteriaceae were extended-spectrum beta-lactamase (ESBL) producers. Susceptibility testing revealed resistance rates of 76.7%, 93.3%, 83.3%, and 65.2% for nalidixic acid, norfloxacin, ofloxacin, and levofloxacin respectively. Detection of fluoroquinolone resistance genes showed the presence of *qnrD* (36.7%), *qnrB* (36.7%), *qnrS* (13.3%), and *qnrA* (6.7%). This study is the first to demonstrate the presence of the *qnrD* gene in some Gram-negative bacilli in Côte d'Ivoire.

Keywords: *resistance, qnr, fluoroquinolones, Gram-negative bacilli, Côte d'Ivoire*

Cite This Article: Kouakou Myrielle Fatoumata, Toty Abalé Anatole, Gadou Victoire, M'Bengue Gbonon Valérie, Konan Kouadio Fernique, Guédé Kipré Bertin, Ouattara Mohamed Baguy, Tiécoura Kouakou, Guessennd Nathalie, and Dosso Mireille, "Detection of qnr Genes That Mediate Fluoroquinolone Resistance in Gram-Negative Bacilli in Abidjan, Côte d'Ivoire." American Journal of Microbiological Research, vol. 11, no. 3 (2023): 79-82. doi: 10.12691/ajmr-11-3-3.

1. Introduction

The discovery of antibiotics at the beginning of the twentieth century brought about a drastic revolution in the treatment of infectious diseases of bacterial origin. It was in 1928 that Alexander Fleming discovered penicillin and its excellent bactericidal potential [1]. From the end of the 1940s to the 1970s, many antibiotics of natural and synthetic origin were discovered. Among these antibiotics, were fluoroquinolones which constitute a family of antibiotics widely used in human and veterinary medicine [2]. However, the high prescription and excessive consumption of these antibiotics have favored the selection of resistant bacteria [3].

Modification in the genome of bacteria resulting from mutation or acquisition of new genes harbored on mobile genetic elements such as plasmids and transposomes helps facilitate the emergence and dissemination of antibiotic resistant bacteria [4]. Resistance to fluoroquinolones in *Enterobacteriaceae* is either mediated by plasmids or modification in the chromosome [5]. Chromosomal mediated resistance results from the modification of the enzymatic target of fluoroquinolones, which are DNA-gyrase and topoisomerase IV. On the other hand, the mechanism of plasmid mediated resistance could be via the protection of quinolone targets by qnr proteins encoded by *qnr A, B, C, S,* and *D* genes, efflux pump associated with QepA that expels hydrophobic fluoroquinolones or acetylation of ciprofloxacin, norfloxacin and aminoglycosides by aac (6')-Ib-cr enzyme [6]. A major characteristic of *qnr* genes is that they are carried by a class 1 integron which is extremely mobile between different plasmids [7].

In Côte d'Ivoire, several studies have investigated *qnrA*, *qnrB*, *qnrS* genes and have reported increased resistance of broad-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* to aminoglycosides and fluoroquinolones resulting in limited treatment options [8,9,10,11]. However,

there is a paucity of information on other variants of *qnr* genes including *qnrD, qnrC*, and *qnrVC* in Côte d'Ivoire. This study was therefore aimed at characterizing *qnr* genes mediating fluoroquinolone resistance in some Gram-negative bacilli in Côte d'Ivoire.

2. Material and Methods

2.1. Bacterial Strains

A total of 30 bacterial strains isolated from biological samples of hospitalized or non-hospitalized patients from January 2017 to August 2018 were obtained from the biocollection of the National Antibiotic Reference Center (CNR). The isolates were characterized by standard microbiology method and their identity confirmed by MALDI-TOF.

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out by the disc diffusion method on Müeller Hinton agar according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (EUCAST/CA-SFM, 2020). The antibiotics tested are listed in Table 1.

Table 1. Antibiotics used in this study (EUCAST-CASFM, 2020)

Antibiotics	Charge of disc (µg)	Critical diameter (mm)		References
		S	R	
Amoxicillin+acid	10-20	≥ 16	< 16	Bio-Rad, France
clavulanic	10	≥ 22	< 19	Bio-Rad, France
Ceftazidime	5	≥ 20	< 17	Bio-Rad, France
Cefotaxime	30	≥ 27	< 21	Bio-Rad, France
Cefepime	10	≥ 22	< 16	Bio-Rad, France
Imipenem	5	≥ 24	< 22	Bio-Rad, France
Ofloxacin	10	≥ 22	< 19	Bio-Rad, France
Norfloxacin	30	≥ 19	< 14	Bio-Rad, France
Nalidixic Acid	5	≥ 26	< 24	Bio-Rad, France
Ciprofloxacin	5	≥ 23	< 19	Bio-Rad, France

2.3. Detection of ESBL

Detection of ESBL in *Enterobacteriaceae* was done by the classical method based on the detection of the synergy between an amoxicillin–clavulanic acid disc and three third generation cephalosporin discs [12].

2.4. DNA Extraction

The extraction of the DNA was carried out by alkaline lysis using tris-Hcl, buffer (pH 8), sodium hydroxide (NaOH), sodium acetate (pH 5.2), 90% and 70% ethanol and sterile EDTA glycerol.

2.5. Detection of *Qnr* Genes

The detection of *qnr* A, B, C, D, VC and S genes was done by PCR using primers listed in Table 2. PCR amplification was done in a thermocycler (Applied Biosystem 9700, Luxembourg) under the following conditions: an initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 45 s, and elongation at 72°C for 30 s. A final elongation step at 72°C for 5 min was necessary to terminate the reaction. Amplified products were electrophoresed on 2% agarose gel at 100 V for 1 hour.

Table 2. Primers used for the detection of fluoroquinolone resistance genes

Genes	Primers	Sequences 5'-3'	Amplicon size (bp)	References
qnrD	qnrD- F	AGGTGTAGCATGTATGGAAAAGC	691	[13]
	qnrD- R	ACATTGGGGCATTAGGCGTT		
qnrA	qnrA- F	AGAGGATTTCTCACGCCAGG	580	[14]
	qnrA- R	TGCCAGGCACAGATCTTGAC		
qnrS	qnrS- F	GCAAGTTCATTGAACAGGGT	428	[14]
	qnrS- R	TCTAAACCGTCGAGTTCGGCG		
qnrB	qnrB- F	GGMATHGAAATTCGCCACTG	264	[14]
	qnrB -R	TTTGCYGYYCGCCAGTCGAA		
qnrC	qnrC- F	GCGAATTTCCAAGGGGCAAA	135	[13]
	qnrC- R	ACCCGTAATGTAAGCAGAGCAA		
qnrVC	qnrVC -F	GAGYTKTATGGTTTAGAYCCTCG	71	[13]
	qnrVC R	TGTTCYTGYTGCCACGARCA		

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3. Results

3.1. Identification of Strains

Of the 30 bacterial strains studied, the identification tests showed that the strains belonged to 5 main bacterial species. These are *Enterobacter cloacae complex* (6 strains), *Enterobacter aerogenes* (8 strains), *Klebsiella pneumoniae* (10 strains), *Proteus mirabilis* (2 strains) and *Acinetobacter baumannii* (4 strains) (Table 3).

Table 3. Distribution of strains according to the bacterial species

Strains	Effective	Rate (%)	Identification Score
A. baumannii	4	13,3%	99,9
K. pneumoniae	10	33,3%	99,9
E. aerogenes	8	26,7%	99,9
E. cloacae	6	20%	99,9
P. mirabilis	2	6,7%	99,9

Table 4. Susceptibility profile of strains to antibiotics

Strains	AMC n (%)	CAZ n (%)	CTX n (%)	FEP n (%)	IPM n (%)	NAL n (%)	NOR n (%)	OFX n (%)	LVX n (%)	CIP n (%)	ESBL n (%)
E. cloacae (N=6)	6(100)	3(50)	5(83,3)	4(66,6)	0(0)	4(66,6)	5(83,3)	3(50)	6(100)	6(100)	2(33,3)
E. aerogenes (N=8)	6(75)	6(75)	5(62,5)	4(50)	0(0)	5(62,5)	7(87,5)	6(75)	4(50)	4(50)	4(50)
K. pneumoniae (N=10)	10(100)	6(60)	9(90)	10(100)	1(11)	10(100)	10(100)	10(100)	5(50)	6(60)	8(80)
P. mirabilis (N=2)	2(100)	0(0)	2(100)	0(0)	0(0)	0(0)	2(100)	2(100)	NT	2(100)	1(50)
A.baumanii (N=4)	4(100)	4(100)	4(100)	4(100)	1(25)	4(100)	4(100)	4(100)	NT	4(100)	NT

NT = not tested
n = number of resistant strains
N = total number of strains
AMC: Amoxicillin/clavulanic acid; CAZ: Ceftazidim; CTX: Cefotaxim; FEP: Cefepim; IPM: Imipenem; NAL: Nalidixic acid; NOR: Norfloxacin; OFX: Ofloxacin; LVX: Levofloxacin; CIP: Ciprofloxacin; ESBL: Extended spectrum beta-lactamase

3.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing showed a high resistance rate to all tested molecules. The rate of resistance to nalidixic acid was 76.7%, 93.3% for norfloxacin, 83.3% for ofloxacin, 65.2% for levofloxacin and 73% for ciprofloxacin. *A. baumanii* a non-fermentative Gram-negative bacillus, was resistant to all (100%) the fluoroquinolones tested. Rate of resistance observed among enterobacteria ranged from 50% to 100% (Table 4). *E. cloacae* displayed the highest rate of resistance to levofloxacin (100%) and ciprofloxacin (100%) and the lowest to ofloxacin (50%). While *E. aerogenes*, had the highest rate of resistance to norfloxacin (87.5%) and the lowest to levofloxacin and ciprofloxacin (50%). As for *K. pneumoniae*, the highest level resistance was to nalidixic acid, norfloxacin and ofloxacin while the lowest to ciprofloxacin and levofloxacin. *P. mirabilis* displayed 100% resistance to all the fluoroquinolones tested.

3.3. Detection of ESBL

The phenotypic detection of ESBL showed that of the 26 enterobacteria tested, 15 were producers of β-lactamases with an extended spectrum, which represented a rate of 57.7%.

3.4. Detection and Amplification of *Qnr* Genes

PCR amplification of fluoroquinolone resistance genes revealed that the strains resistant to fluoroquinolones harbored *qnrA*, *qnrB*, *qnrD* and *qnrS* genes. However, none of the strains harbored *qnrC* and *qnrVC* genes. *qnrD* and *qnrB* genes predominated with respective rates of 36.7%. *qnrS* gene was present at a rate of 13.3% in 4 strains and *qnrA* gene was found in 2 (6.7%) strains.

4. Discussion

In this study, the prevalence of bacteria strains harboring *qnr* genes with ESBL-producing capability was high (57.7%). This high rate of ESBL-producing *qnr* strains could be explained by the fact that ESBL genes and fluoroquinolone resistance genes such as *qnr* are often carried on the same plasmids [15].

Furthermore, the resistance rate of strains in this study to cefotaxime (83.3%), cefepime (73.3%) and ceftazidime (63.3%) was high. The observed high rate of resistance could be due to the frequent use of cephalosporins in the treatment of bacterial infections [16]. Similar results have been reported by [9] in Côte d'Ivoire. In their study, the resistance rates of enterobacteria producing β-lactamases with an expanded spectrum to cefotaxime, cefepime and ceftazidime were respectively 90.2%, 73.2% and 75.6%. Imipenem in this study was the most active antibiotic. This justifies its place as first choice in the treatment of severe infections with multidrug-resistant bacteria [17].

Regarding fluoroquinolones, the resistance rate was high for all the molecules tested. This high resistance rate could be explained by the fact that fluoroquinolones are the most prescribed antibiotic after β-lactams in Africa and particularly in Côte d'Ivoire [18]. In this study, resistance rate to nalidixic acid was 76.7%. This result is higher than that reported by [19] who had 50% of *E. cloacae* resistant to nalidixic acid in Algeria. On the other hand, ciprofloxacin resistance rate in this work was 73%. This rate is lower than that obtained by [20] who, during his work in Côte d'Ivoire, reported that 86.7% of enterobacteria were resistant to ciprofloxacin. This result is also lower than those obtained by some authors in Africa. Indeed, in the Central African Republic, the work of [21] showed that 84.8% of the ESBL-producing strains tested were resistant to ciprofloxacin. In Burkina Faso, [22] reported that 80% of ESBL-producing strains were resistant to ciprofloxacin.The disparity in results may be attributed to fewer isolates used in this study.

In this study, *qnr* genes, including *qnrA* (6.7%), *qnrB* (36.7%), *qnrS* (13.3%) and *qnrD* (36.7%) genes were detected in the isolates with *qnrD* and *qnrB* genes predominating. These results are lower than those obtained by [23] who reported respective rates of (47.74%) *qnrB*, (47.10%) *qnrS* and (2.58%) *qnrA* in ESBL-producing enterobacteria. These results suggest that *qnrD* and *qnrB* genes are the main genes that mediate quinolone resistance in Gram-negative bacilli in Côte d'Ivoire.

5. Conclusion

The results of this study revealed Gram-negative bacilli that displayed resistance to fluoroquinolones and β-lactam antibiotics. The isolates harbored diverse *qnr* genes including *qnrA*, *qnrB*, *qnrD* and *qnrS* which are of public health significance since they mediate resistance to

fluoroquinolones. To our knowledge, this study is the first to report *qnrD* gene in strains isolated in Côte d'Ivoire.

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