First case of qnr B6 and qnr B7 genes in enterobacteriaceae producing extended-spectrum beta-lactamases in Abidjan, Côte d'Ivoire

ABSTRACT

Aims: The aim of this study was to characterize fluoroquinolone resistance genes in enterobacteriaceae that produce extented-spectrum β-lactamases, isolated in Abidjan.
Place and Duration of study: Pasteur Institute of Côte d'Ivoire and research unit on emerging tropical infectious diseases of Aix-Marseille University from January 2016 at July 2017.
Methodology: The study included 90 enterobacteriaceae producing extented-spectrum β-lactamases isolated from biological products from various hospital services in Abidjan. These strains have been pre-identified and stored at the Center for Biological Resources (CeReB) of Pasteur Institute of Côte d'Ivoire. The identification of the strains was confirmed using the mass spectrometry MALDI-TOF (MS) and the antibiotic sensitivity test was performed using Müeller Hinton's agar diffusion method. The fluoroquinolone resistant genes were detected by conventional PCR and then, sequenced.
Results: The strains studied were Escherichia coli (44), Klebsiella pneumoniae (31) and Enterobacter cloacae (15). High resistance rates to ceftriaxone (96.7%), cefotaxime (95.6%), aztreonam (95.6%) and cefoxitine (72.2%) were observed in all strains producing broad spectrum β-lactamases. The resistance rate to fluororquinolones represented by ciprofloxacin was 86.7%. The fluoroquinolone resistance genes detected were qnr A (3.3%) and qnr B (42.2%). Sequencing identified the qnr A1 (3.3%), qnr B1 (31.1%), qnr B6 (2.2%) and qnr B7 (1.1%) genes.
Conclusion: This study made it possible to identify fluoroquinolone resistance genes in enterobacteriaceae producing β-lactamases which have an extended spectrum in Abidjan.

Keywords: Enterobacteriaceae, fluoroquinolones, qnr B6, qnr B7, Abidjan.

1. INTRODUCTION

Quinolones are widely used antibiotics in the treatment of various infections [1]. Quinolones are generally characterized by a broad spectrum of activity, a good oral bioavailability and a good tissue penetration [2] while fluoroquinolones, are characterized by the presence of a fluorine atom in position 6 and a nitrogen ring, and most often by the presence of a piperazine in position 7 [3]. Their main targets are DNA gyrase and topoisomerase IV DNA [4].
Fluoroquinolones interact with the DNA-enzyme complex, i.e. with the DNA gyrase which is bound to bacterial DNA or with the topoisomerase IV, bound to bacterial DNA to create conformational changes. The new fluoroquinolone-enzyme-DNA complex blocks the progression of the replication fork, resulting in the inhibition of enzymatic activity and DNA synthesis [5, 6]. Several mechanisms are involved in fluoroquinolone resistance. These are the mutational modifications of target enzymes, the reduction of membrane's permeability, the reduction of intracellular antibiotic concentration by efflux systems and the action of the QNR protein [7]. The *qnr* gene that codes for the QNR protein is the genetic determinant of plasmid resistance to fluoroquinolones [8]. The importance of this genetic support is its transferability and its ability to accelerate the spread of fluoroquinolone resistance. *Qnr* genes have been identified in different strains of enterobacteriaceae and often associated with the production of extended-spectrum beta-lactamases [4]. This situation is at the root of therapeutic failures and the increase in morbidity and mortality rates worldwide [9]. The objective of this study is to characterise fluoroquinolone resistance genes in enterobacteriaceae producing extended-spectrum β-lactamases isolated in Abidjan, Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1. Selection of strains

This study included 90 strains of enterobacteriaceae producing broad spectrum β-lactamases. The 90 strains were distributed as follows: 44 *Escherichia coli*, 31 *Klebsiella pneumoniae* and 15 *Enterobacter cloacae*. They were taken from a collection of 153 enterobacteriaceae isolated from various biological products (urine, blood, suppurations, saliva) from various hospital services in the city of Abidjan. These strains were pre-identified and stored at Biological Resource Center of Pasteur Institute of Côte d'Ivoire from 2012 to 2015.

2.2. Confirmation of the identity of strains by MALDI-TOF

The strains preserved in deep agars were revived using a enrichment broth which were incubated at 37°C for 24 hours in an oven (ThermoFisher). The strains' isolation was performed on Mac-Conkey agar and their re-identification was confirmed by mass spectrometry (MALDI-TOF) at the laboratory of the Emerging Tropical Infectious Diseases Research Unit at Aix-Marseille University in France.

2.3. Strains' sensitivity to antibiotics

The antimicrobial susceptibility test was performed using Müeller-Hinton agar (BioMérieux SA, France) by the standard method of diffusion in agar described by the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2013). The antibiotics tested were: amoxicillin (25 µg), amoxicillin + clavulanic acid (20 µg + 10 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), imipenem (10µg), ertapenem (10 µg), ciprofloxacin (5 µg). The phenotypic detection of extented-spectrum β-lactamases was carried out by the synergy test comprising amoxicillin+clavulanic acid, cefotaxime, ceftriaxone, aztreonam [10].

2.4. Research of fluoroquinolone resistance genes by PCR

The strains' DNA was extracted using the EZ1 extraction kit (Qiagen) as recommended by the manufacturer. The search for the *qnr* A and *qnr* B genes was carried out by conventional PCR. The amplification reaction was performed in a reaction volume of 25 µL composed of 12.5 µL Master Mix (Quantitect Probe PCR Master mix, Qiagen), 1 µL sense and anti-sense primer (Eurogentec), 5 µL total DNA and 6.5 µL ultra-pure water (Invitrogen). The primers of the fluoroquinolone resistance genes used in this work have been summarized in **Table 1.**

Table 1. Primers used for the detection of fluoroquinolone resistance genes

Gene name	Primer name	Primer sequence (5' → 3')	Amplicon size (bp)
qnr A	QnrA_F QnrA_R	GATAAAGTTTTTCAGCAAGAGG ATCCAGATCGGCAAAGGTTA	542
qnr B	QnrB_F QnrB_R	GACAGAAACAGGTTCACCGGT CAAGACGTTCCAGGAGCAACG	594

The amplification of genes by conventional PCR consisted of an initial DNA denaturation step at 95°C for15 min. This step was followed by 35 amplification cycles including a denaturation at 94°C for 1 min, a hybridization at 55°C for 50 s, an elongation at 72°C for 2 min and a final elongation step of 7 min at 72°C. The amplification products were analyzed by 1.5% agarose gel electrophoresis prepared with 0.5% Tris-Borate-EDTA (TBE) and 3.75% SYBR SAFE. The DNA bands of the amplicons were visualized on a transilluminator.

2.5. DNA sequencing

The amplicons were purified and sequenced using the BigDye® kit (Life technologies) as recommended the manufacturer in an automate ABI PRISM 3730xl Genetic Analyser PLC. In addition, genes' identification was carried out in the ARG-ANNOT (Antibiotic Resistance Gene Annotation) database of the IHU-Marseille in France.

3. RESULTS

3.1. Antibiotics susceptibility

A high resistance rates to ceftriaxone (96.7%), cefotaxime (95.6%), aztreonam (95.6%) and cefoxitine (72.2%) were observed in all strains producing broad spectrum β-lactamases. The resistance rate to fluoroquinolones represented by ciprofloxacin was 86.7%.

The analysis of the results of the susceptibility testing of *Escherichia coli* strains to antibiotics showed that for antibiotics of the β-lactam family, 100% of the strains were resistant for amoxicillin and for amoxicillin-clavulanic acid. The cephalosporin resistance rate was 98% and 100% for cefotaxime and ceftriaxone respectively. All strains were susceptible to imipenem, however 27.3% of strains were resistant to ertapenem. The ciprofloxacin resistance rate was 95.4%.

In *Klebsiella pneumoniae* strains, cephalosporins resistance rate was 71% for cefoxitin, 96.8% for cefotaxime and ceftriaxone respectively. In this species too, all strains were sensitive to imipenem, however 35.5% of strains were resistant to ertapenem. The resistance rate of K. pneumoniae strains was 100% to amoxicillin clavulanic acid. In addition, the ciprofloxacin resistance rate was 74.2%.

For *Enterobacter cloacae* strains, the resistance rate to amoxicillin clavulanic acid and cefoxitin was 100% while 86.7% of strains were resistant to cefotaxime and ceftriaxone.

All strains of *E. cloacae* were susceptible to imipenem, however 33.3% of the strains were resistant to ertapenem. **Table 2** summarizes the antibiotic resistance rates in the different species studied.

Table 2. Antibiotic resistance rate

Antibiotics	Strains producing ESBL (%)		
	E. coli **n= 44**	***K. pneumoniae*** **n= 31**	***E. cloacae*** **n= 15**
Amoxicillin	44 (100)	31 (100)	15 (100)
Amoxicillin-clavulanic acid	44 (100)	31 (100)	15 (100)
Aztreonam	44 (100)	29 (93.5)	13 (86.7)
Cefotaxime	43 (98)	30(96.8)	13 (86.7)
Cefoxitin	28 (63.6)	22 (71)	15 (100)

Ceftriaxone	44 (100)	30 (96.8)	13 (86.7)
Ciprofloxacin	42(95.4)	23 (74.2)	13 (86.7)
Ertapenem	12 (27.3)	11 (35.5)	5 (33.3)
Imipenem	0	0	0

** ESBL: extended-spectrum beta-lactamase, n: number*

3.2. Resistance genes identified

The search for fluoroquinolone resistance genes showed the presence of *qnr* B genes in 38 strains, thus, representing a rate of 42.2% and *qnr* A in 3 strains, representing a rate of 3.3%.
The distribution of fluoroquinolone resistance genes by species showed that 3 strains of *E. coli* (6.8%) hosted the *qnr* A gene and 7 strains (15.9%) the *qnr* B gene.
The *qnr* A gene was not detected in any of the *K. pneumoniae* and *E. cloacae* strains. However, 19 strains of K. *pneumoniae*, i.e. about 61.3%, and 12 strains of E. *cloacae* (80%) hosted the *qnr* B gene.

Table 3. Distribution of genes between strains

Detected genes	**Strains producing ESBL (%)**		
	E. coli **n= 44**	***K. pneumoniae*** **n= 31**	***E. cloacae*** **n= 15**
Qnr A	3 (6.8)	0	0
Qnr B	7 (15.9)	19 (61.3)	12 (80)

** ESBL: extended-spectrum beta-lactamase, n: number*

The sequencing technique helped to identify the *qnr* A1 genes in 3 strains of *E. coli* at a rate of 3.3%. *qnr* B1 was identified in 28 strains (31.1%) including 13 strains of *K. pneumoniae* (14.4%), 11 strains of *E. cloacae* (12.2%) and 4 strains of *E. coli* (4.4%). The *qnr* B6 gene was identified in 2 strains of *K. pneumoniae* (2.2%) and the *qnr* B7 gene in 1 strain of *K. pneumoniae* (1.1%).

4. DISCUSSION

Fluoroquinolones act at the time of DNA replication. Their targets are DNA gyrase and topoisomerase IV, which regulate the topology of DNA to allow replication [11]. The resistance to fluoroquinolones in enterobacteriaceae is generally the result of a chromosomal mutation causing the alteration of bacterial target enzymes [4]. However, resistance caused by plasmids has also been reported as a result of the acquisition of resistance genes qnr, qepA, and aac(6')-Ib-cr [12, 13]. Plasmids carrying the qnr A and qnr B genes frequently carry resistance genes to β-lactamines, aminosides, and tetracycline [4].
The objective of this study was to characterise fluoroquinolones resistance genes in enterobacteriaceae producing extented spectrum β-lactamases.
In this work, the fluoroquinolones resistance rate represented by ciprofloxacin in enterobacteriaceae producing extented spectrum β-lactamases was 86.7%. This rate is higher than that reported by Guessennd et al (2008b) [14] who, in their work, showed that 70.2% of the strains producing extented spectrum β-lactamases were resistant to ciprofloxacin. Ouattara et al. (2014) [15] reported a 93.2% ciprofloxacin resistance rate in strains producing broad spectrum β-lactamases.

The high resistance rates could be explained by the fact that fluoroquinolones are the most prescribed molecules after β-lactamines in Africa and particularly in Côte d'Ivoire [16]. These results are agree with those obtained by some authors in Africa. Indeed, in the Central African Republic, the results of the work of Rafai et al (2015) [17] showed that 84.8% of the broad spectrum strains, producing β-lactamases tested were resistant to ciprofloxacin. Similarly, in Burkina Faso, Ouedraogo et al (2016) [18] reported that 80% of the strains producing broad spectrum β-lactamases were resistant to ciprofloxacin. In Algeria, Mathlouthi et al (2016) [19] reported that 80% of strains producing extented spectrum β-lactamases tested in their work were resistant to ciprofloxacin.

The qnr genes detected included qnr B gene which was detected at a rate of 42.2% followed by the qnr A gene (3.3%) in strains producing extented spectrum β-lactamases. The sequencing carried out made it possible to identify in addition to the qnr A1 (3.3%) and qnr B1 (31.1%) genes, the qnrB6 (2.2%) and qnr B7 (1.1%) genes which are involved in the resistance to fuoroquinolone. Moreover, the qnr A1 and qnr B1 genes were reported in 2008 in Côte d'Ivoire [14]; however, this study is the first to report the presence of the qnr B6 and qnr B7 genes involved in fluoroquinolone resistance..

Elsewhere in the world, the qnr B6 gene has been detected in South Korea in a strain of Enterobacter aerogenes producing broad spectrum β-lactamases from a collection of 644 enterobacteriaceae from 12 clinical laboratories [20]. Similarly, in Argentina, Cruz et al (2013) [21] reported the presence of qnr B6 in 5% of enterobacteriaceae that produce broad spectrum β-lactamase. Also, in Morocco, the qnr B6 gene was detected in 0.9% of strains producing extented spectrum β-lactamasess tested in the work of Jamali et al (2014)[22].

The qnr B6 and qnr B7 genes were found in South Korea in a study of 347 enterobacteriaceae from two hospitals. These genes were detected respectively in a strain of K. pneumoniae and a strain of Citrobacter freundii [23].

4. CONCLUSION

This study showed a high level of resistance to fluoroquinolones in enterobacteriacea producing extented-spectrum β-lactamases. The qnr B gene was the most detected (42.2%) followed by the qnr A gene (3.3%). The study showed as well the presence of the qnr B6 and qnr B7 genes for the first time in Côte d'Ivoire. Given the importance of fluoroquinolones in the treatment of many bacterial infections, the presence of resistance genes is a concern. Therefore, monitoring the prescription of antibiotic is necessary to limit the risk of spreading resistance genes.

COMPETING INTEREST

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

It is not applicable

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ABBREVIATIONS:

ESBL: Extended-Spectrum Beta-Lactamases
Qnr: Quinolone resistance