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First Detection of the Plasmid-Mediated Quinolone Resistance Determinants *qnrB*, *qnr*S and *aac*(6')-Ib-cr in Extended Spectrum Beta-Lactamases-producing *Klebsiella pneumonia* in Bouaké, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CAK and SPAN designed the study. Authors MNT, AEA, ZOW, BC, PM, NFW, EMS and SLB performed the experiments, analyzed the data and drafted the manuscript. Authors MNT, AEA and CAK were involved in critically reviewing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aims of the present study were to investigate the presence of Plasmid-Mediated Quinolone Resistance (PMQR) determinants and the association of these determinants with Extended Spectrum Beta-Lactamases (ESBLs) genes in ESBL-producing *Klebsiella pneumoniae* isolates from Teaching Hospital of Bouaké, Côte d'Ivoire.

Study Design: It is a retrospective study.

Place of Study: Bacteriology-Virology Laboratory of Teaching Hospital, Bouaké, Côte d'Ivoire.

Methodology: From January 2015 to December 2016, 96 ESBL-producing *Klebsiella pneumoniae* isolates were collected from several specimens. Antimicrobial susceptibility of isolates was tested using the standard disk-diffusion method on Mueller-Hinton and interpretation according to recommendations of the 2017 EUCAST. These isolates analyzed for the detection of ESBL (bla_{CTX-M}, bla_{TEM} and bla_{SHV}) and PMQR genes (aac(6')-Ib-cr, *qnr*B and *qnr*S) using simplex PCR.

Results: Of the 96 ESBL-producing strains, 85 (88.55%) harbored at least one of the ESBL genes tested. Out of the 85 strains encoding ESBL genes, 96.47% carried blaCTX-M and 92.94% blaSHV and blaTEM genes. Eighty nine (89.6%) of the 96 ESBL producing-isolates were resistant to ciprofloxacin and 84.4% to norfloxacin. Among the 96 strains, 80 (83.33%) were found harboring at least one PMQR gene consisting of 78 (81.3%) aac(6')-Ib-cr, 61 (63.5%) *qnr*B and 15 (15.6%) *qnr*S. Among the PMQR-positive strains, 68.4% coharbored *qnr*B+acc(6')-Ib-cr genes, 10.5% *qnr*B+*qnr*S+acc(6')-Ib-cr and 6.6% *qnr*S+acc(6')-Ib-cr. The *qnr*B gene was always linked to aac(6')-Ib-cr gene. Aac(6')-Ib-cr gene showed the highest association with three ESBL genes (87.6%), followed by *qnr*B gene (70.6%), then *qnr*S (17.7%).

Conclusion: The PMQR genes were highly prevalent in ESBL-producing *Klebsiella pneumoniae*, primarily the aac(6')-Ib-cr gene. The high associated was observed between ESBL and PMQR genes, notably with the aac(6')-Ib-cr gene.

Keywords: Klebsiella pneumoniae; resistance; genes; plasmid-mediated quinolone resistance; beta-lactamases.

ABBREVIATIONS

DNA	*: Deoxyribo Nucleic Acid*
PMQR	*: Plasmid-Mediated Quinolone Resistance*
Qnr	*: Quinolone resistant*
ESBL	*: Extended Spectrum Beta-Lactamases*
ATCC	*: American Type Culture Collection*
MALDI-TOF	*: Matrix-Assisted Laser Desorption/Ionization Time of Flight*
USA	*: United States American*
UV	*: Ultra Violet*
SPSS	*: Statistical Package for the Social Sciences*

1. INTRODUCTION

After several decades of overuse of antibiotics, human pathogenic bacteria have reached alarming levels of resistance to many antibiotics. Fluoroquinolones, antibiotics that should have been used as a last resort in human medicine, have not been spared by this phenomenon. Two chromosomal mechanisms are responsible for clinical resistance to fluoroquinolones: the accumulation of mutations in the genes that code for DNA gyrase and IV topoisomerase, and the decrease in their intracellular concentration through increased efflux pump activity and/or decreased membrane permeability [1].

The discovery in 1998 of subclinical plasmid resistance to fluoroquinolones in a multi-resistant beta-lactamase-producing strain of *Klebsiella pneumoniae* lifted the veil on this phenomenon [2]. This resistance plasmid was then shown to host the qnrA gene coding for a 218-amino acid protein, *qnr*A, belonging to the family of proteins with repeated pentapeptide motifs that protects the DNA-gyrase complex from inhibition by quinolones [3]. Four other plasmid determinants also involved in quinolone resistance have been reported (*qnr*B, *qnr*S, *qnr*C and *qnr*D). Another mechanism of plasmid resistance has been related in strains isolated in China, namely inactivation of Fluoroquinolones by acetylation.

The determinant of this resistance is a variant of an aminoside N-acetyltransferase [4]. Another novel mechanism of plasmid resistance has been discovered, by active excretion of fluoroquinolones via the *qep*A efflux pump [5,6].

These PMQR mechanisms, in addition to conferring sub-clinical levels of resistance, also increase the likelihood of emergence of clinically resistant strains [7]. The co-expression of other multi-resistance genes by PMQR carrier strains and their dissemination in hospitals and in the community is a risk factor for therapeutic deadlock. Several studies, based on molecular approach, have reported a strong association between positive PMQR and ESBL positive Enterobacteriacae isolates [8-11]. ESBLs are enzymes that generate resistance to the majority of beta-lactam antibiotics, the most widely used antibiotics in human medicine. These enzymes are commonly produced by *Klebsiella pneumoniae,* an enterobacterium responsible for high morbidity and mortality worldwide. It can cause nosocomial pneumonia, sepsis, urinary tract infections, wound infections, bacteremia and liver infections [12].

Klebsiella pneumoniae is one of the multi-resistant bacteria identified as an urgent threat to human health by the World Health Organization. The purpose of this study were to investigate the presence of PMQR determinants (aac(6')-Ib-cr, *qnr*B and *qnr*S) and to examine the association of these determinants with ESBLs genes in ESBL-producing *Klebsiella pneumoniae* isolated from Teaching Hospital of Bouaké.

2. METHODOLOGY

2.1 Strains Collection

From January 2015 to December 2016, 96 ESBL-producing *Klebsiella pneumoniae* were collected at the Bacteriology-Virology Laboratory of Teaching Hospital of Bouaké, Côte d'Ivoire. These strains were isolated from various biological products including urine, blood, pus, pleural fluid, bronchial specimen, urinary catheter, joint fluid and cephalo-rachidian fluid for diagnostic Purpose. Their identification was first carried out by the Minor's mini at Teaching Hospital of Bouaké and then confirmed at the Institute of Medical Microbiology, University Hospital Münster (Germany) by using Matrix-Assisted Laser Desorption/Ionization Time Of Flight mass spectrometry (MALDI-TOF) (Microflex, Bruker Daltonics, Bremen, Germany) [13].

2.2 Antimicrobial Susceptibility Testing

Antibiotic susceptibility of *Klebsiella pneumoniae* was tested using the standard disk-diffusion method on Mueller-Hinton agar and interpreted according to the 2017 recommendations of European Committee on Antimicrobial susceptibility Testing [14]. To do this, eighteen antibiotics were used: ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), piperacillin/tazobactam (PTZ, 30/6 µg), cefalexin (CFX, 30 µg), cefuroxim (CXM, 30 µg), cefoxitin (FOX, 30 µg), cefotaxim (CTX, 5 µg), ceftazidim (CAZ, 10 µg), cefixim (CFM, 5 µg) cefepim (FEP, 30 µg), meropenem (MEM, 10 µg), imipenem (IPM, 10 µg) aztreonam (ATM, 30 µg), gentamicin (GM, 10 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), chloramphenicol (C, 30 µg). Antibiotic disk were purchased from Oxoid Ltd (Basingstoke, Hampshire, UK). *Escherichia coli* ATCC 25922 (American Type Culture Collection) strain was used as a control for antibiotic susceptibility testing.

2.3 Extraction of Bacterial DNA

Klebsiella pneumoniae isolate DNA was extracted by the simple boiling method. Briefly, *Klebsiella pneumoniae* isolates were cultured on Mueller Hinton agar (Merck, Germany) and incubated at 37°C. After 24 hours, one to five colonies were suspended in distilled water, and suspension was boiled for 30 min at 95°C in water bath [15]. The suspension was centrifuged at 14,000 rpm for five min and the supernatant transferred to filter columns. After a final centrifugation at 1200 rpm for five min, the supernatant was transferred to a new microtube and stored at -20°C.

2.4 Molecular Detection of ESBLs and PMQR Genes

Presence of bla_{TEM}, bla_{SHV}, bla_{CTX}, *qnr*B, *qnr*S and aac (6')-Ib-cr genes were detected by simplex PCR using primers specific for each gene (Table 1) [16-19]. PCR amplification was performed in a reaction mixture of 25 µL, which contained 5 µL template DNA, 12.5 µL of 2x GoTaq® G2 Hot Start Colorless Master Mix

Table 1. Primers used for the detection of ESBL and PMQR genes

Genes	Primers	Sequences (5'→ 3')	Size (pb)	References
bla$_{TEM}$	TEM-F	ATGAGTATTCAACATTTCCGTG	840	[16]
	TEM-R	TTACCAATGCTTAATCAGTGAG		
bla$_{SHV}$	SHV-F	TTTATGGCGTTACCTTTGACC	1051	[17]
	SHV-R	ATTTGTCGCTTCTTTACTCGC		
bla$_{CTX-M}$	CTX-F	TTTGCGATGTGCAGTACCAGTAA	544	[18]
	CTX-R	CGATATCGTTGGTGGTGCCATA		
*qnr*B	*qnr*B-F	TGGCGAAAAAATTGAACAGAA	594	[19]
	*qnr*B-R	GAGCAACGATCGCCTGGTAG		
*qnr*S	*qnr*S-F	GACGTGCTAACTTGCGTGAT	388	[19]
	*qnr*S-R	AACACCTCGACTTAAGTCTGA		
aac(6')-Ib-cr	*aac(6')-Ib-cr-F*	TTGCGATGCTCTATGAGTGGCTA	482	[20]
	aac(6')-Ib-cr-R	CTCGAATGCCTGGCGTGTTT		

(Promega, Madison, USA), 2.5 µL of each primer (10 µM) and 2.5 µL nuclease free water. The amplification of all these resistance genes was performed in a thermal cycler (Applied Biosystems, Inc., CA).

For *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX}$ genes, thermal cycling conditions of consisted of an initial denaturation cycle of amplification at 94°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, extension at 72°C for 90 s, and a final cycle of amplification at 72°C for 7 min. PCR conditions for qnr genes (*qnr*B and *qnr*S) were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles consisting of 1 min at 94°C for denaturation, 1 min at 57°C for annealing and 1 min at 72°C for extension, then a final extension step at 72°C for 10 min. Primary denaturation at 95°C for 5 min; 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min; and, a final extension at 72°C for 10 min were used as thermal-cycling conditions for aac(6')-Ib-cr genes.

PCR products were analyzed on 1,5% agarose gel at 115 V for 80 min in TBE 1X containing GelRed® Nucleic Acid Stain 10,000X using 100-bp DNA ladder (Promega, USA) as a size marker. The visualization of the bands was done under UV (ultra violet) in a Gel Documentation System.

2.5 Statistical Analysis

The data were analyzed using the statistical package for windows v.19.0 (SPSS). The relationships between ESBL production and PMQR determinants were evaluated by the Pearson chi 2 test. p value <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Antibiotic susceptibility profile

All strains were resistant to cefalexin, cefuroxim, ceftazidim, cefepim and aztreonam. Resistance was 99% for cefotaxim and cefixim, 25% for cefoxitin, 4.2% for imipenem and meropenem, 91.7% for amoxicillin + clavulanic acid and 69.8% for piperacillin-tazobactam. The resistance rates to fluoroquinolones were 89.6% and 84.4% for ciprofloxacin and norfloxacin respectively. Regarding aminoglycosides, 84.3% of the strains were resistant gentamicin. Resistance to chloramphenicol and trimethoprim/sulfamethoxazole was 25% and 97.9% respectively (Fig. 1).

3.1.2 Prevalence of ESBL and PMQR genes

Of the 96 ESBL-producing strains, 85 (88.55%) harbored at least one of the ESBL genes tested in the present study. Out of the 85 strains encoding ESBL genes, 96.47% carried *bla*$_{CTX-M}$ and 92.94% *bla*$_{SHV}$ and *bla*$_{TEM}$ genes (Fig. 2). Moreover, the 3 genes were present simultaneously in 85.06% of the strains encoding ESBL genes.

Regarding the presence of PMQR genes, electrophoresis analysis revealed 83.33% (80) of 96 ESBL-producing strains harbored at least one PMQR gene which consisted of 63.5% for *qnrB*, 15.6% for *qnrS* and 81.3% for aac(6')-Ib-cr genes. However, any PMQR genes were not detected in 16 strains (Fig. 3). There were statistically significant differences regarding the association between ESBL production and the presence of PMQR genes (p=0.001). The concomitant presence of 3 PMQR genes was detecte*d in 8.33% of ESBL-producing strains.*

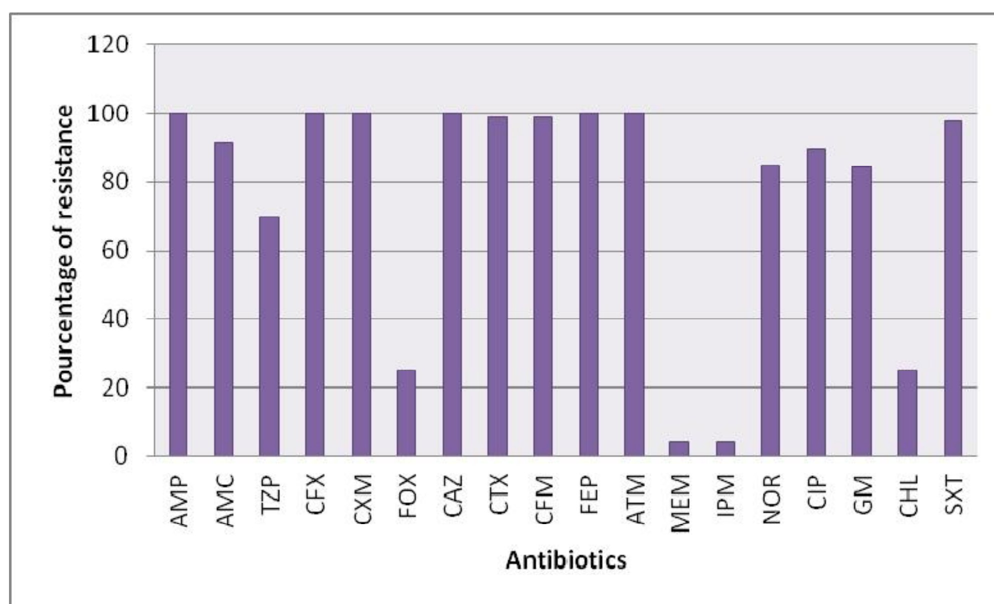

Fig. 1. Antimicrobial resistance of ESBL-producing *Klebsiella pneumoniae* isolates
AMC amoxicillin+clavulanic acid, TZP piperacillin-tazobactam, CFX cefalexin, CXM cefuroxim, FOX cefoxitin, CAZ ceftazidim, CFM cefixim, CTX cefotaxim, FEP cefepim, ATM aztreonam, IPM imipenem, MEM meropenem, CIP ciprofloxacin, NOR norfloxacin, GM gentamicin, CHL chloramphenicol, SXT trimethoprim/sulfamethoxazol

Among the PMQR-positive strains, 68.4% co-harbored *qnr*B+acc(6')-Ib-cr genes, 10.5% *qnr*B+*qnr*S+acc(6')-Ib-cr and 6.6% *qnr*S+ acc(6')-Ib-cr. The *qnrS* and aac(6')-Ib-cr genes existed alone contrary to *qnr*B gene which was always linked to aac(6')-Ib-cr.

3.1.3 Distribution of PMQR determinants among encoding ESBL genes strains

Among strains encoding ESBL genes, 89.41% carried PMQR determinants. About the distribution of the different 3 PMQR genes among strains encoding ESBL genes, aac(6')-Ib-cr gene showed the highest association with three ESBL genes (87.6%), followed by *qnr*B gene (70.6%), then *qnr*S (17.7%).

3.2 Discussion

Bêta-lactams and fluoroquinolones have been considerably used since their acceptance in therapy. Inappropriate use is responsible for the rapid emergence of resistance to these two classes of antibiotics [21,22]. This study was trying to describe the co-existence of fluoroquinolone (PMQR) and bêta-lactam (ESBL) resistance genes in clinical strains of *Klebsiella pneumoniae* isolated from patients at Teaching Hospital Bouaké. To do this, we again performed an antibiotic susceptibility test for all strains, following by the detection of resistance genes by PCR.

From this investigation, it appeared that $bla_{\text{CTX-M}}$ was the most prevalent ESBL gene followed by bla_{TEM} and bla_{SHV} with the same prevalence. Our results are in accordance with studies by Shahid et al. [23], Ahmed et al. [24], Goudarzi et al. [25] Abdallah et al. [26], Ouedraogo et al. [27], Kpoda et al. [28] and Amer et al. [29] but disagreed with those by Pishtiwan and Khadija [30], Bajpai et al. [31], Ghorbani-Dalini et al. [32], Yazdi et al. [33] and Eftekhar et al. [34] where bla_{TEM} and bla_{SHV} were most prevalent. This discrepancy could be explained by regional variation. The co-production of ESBLs genes were observed in most of the isolates meanwhile others isolates contained not none gene. The coexistence of ESBL genes were mentioned in many studies as those of Salah et al. [35], Amer et al. [36] and Amer et al. [29]. Absence of ESBL genes in some isolates would explain the presence another type of ESBL gene such as the VEB, OXA, TLA types.

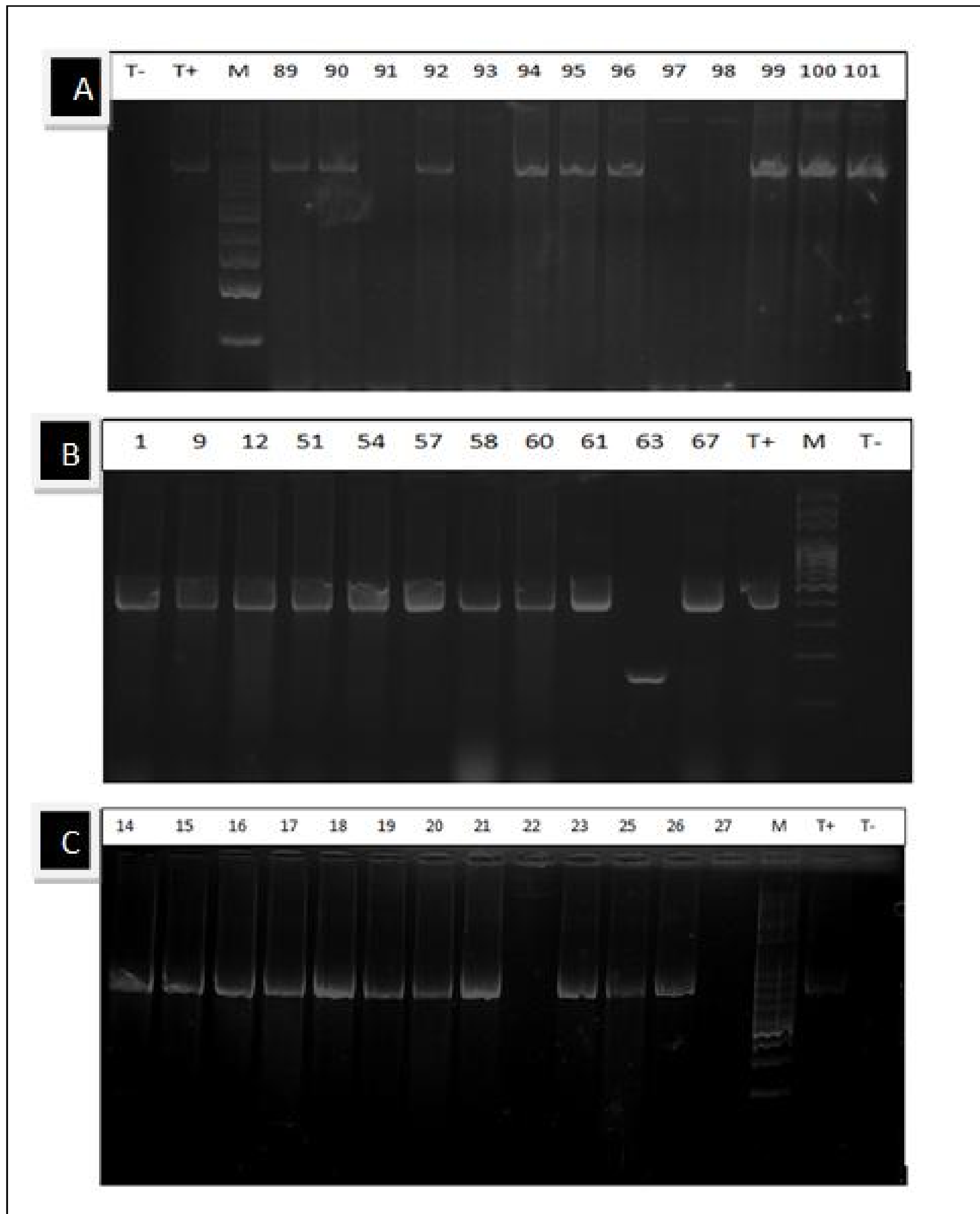

Fig. 2. Agarose gel electrophoresis of PCR ESBLs genes of clinical isolates of *Klebsiella pneumoniae*

*[Lane M: DNA Marker (100-1500 bp), T+: positive control, T-: negative control, **A**) PCR amplification of bla_{SHV} gene showing single band at 1051 bp (lanes No. 89, 90, 92, 94, 95, 96 and 99 positives isolates for bla_{SHV} isolates; lanes No. 91,93,97and 98 negatives isolates for bla_{SHV}), **B**) PCR amplification of bla_{CTX-M} gene, showing single band at 544 bp (lanes No. 1, 9, 12, 51, 54, 57, 58, 60, 61, 67: positives isolates for bla_{CTX-M} gene; lane 63: negatives isolates for bla_{CTX-M} gene), **C**) PCR amplification of bla_{TEM} gene, showing single band at 840 bp (lanes No. 14, 15, 16, 17, 18, 19, 20, 21, 23, 25, 26: positives isolates for bla_{TEM} gene; lanes 22 and 27: negatives isolates for bla_{TEM} gene)]*

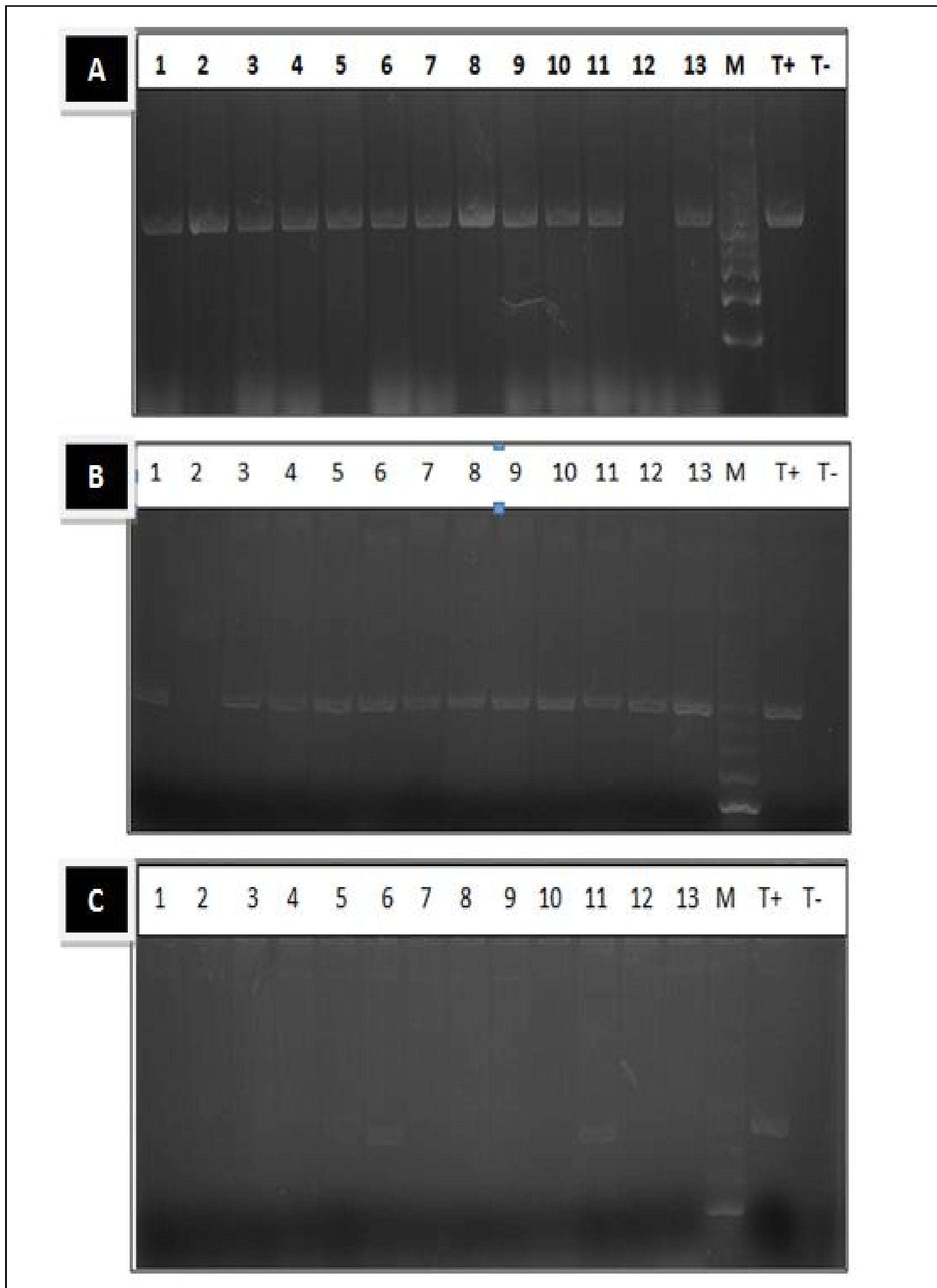

Fig. 3. Agarose gel electrophoresis of PCR PMQR genes of isolates of *Klebsiella pneumoniae*

*[Lane M: DNA Marker (100-1500bp), T+: positive control, T-: negative control, **A**) PCR amplification of qnrB gene showing single band at 594 bp (lanes No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 13 positives isolates for qnrB; lanes No. 13 negatives isolates for qnrB), **B**) PCR amplification of aac(6')-Ib-cr gene, showing single band at 594 bp (lanes No. 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 positives isolates for aac(6')-Ib-cr gene; lane 2: negative isolate for aac(6')-Ib-cr gene), **C**) PCR amplification of qnrS gene, showing single band at 388 bp (lanes No. 6 and 11: positives isolates for qnrS gene; lanes 1, 2, 3, 4, 5, 7, 8, 9, 10, 12 and 13 negatives isolates for qnrS gene)]*

The presence of resistance to fluoroquinolones in ESBL-producing isolates has been previously related [37]. In this study, high level of resistance to fluoroquinolones was observed. This prevalence was higher than those in Abidjan, Côte d'Ivoire (73.3%), Iran (68%) and Spain (19.4%) [38-40].

This high level of resistance to fluoroquinolones thus confirms high prevalence of PMQR determinants obtained in this study. These results were comparable with those noted in Tunisia (80%) [41] but higher than those reported in 2017 by Tahou et al. in Abidjan (Côte d'Ivoire), in 2016 by Domokos et al. in Hungary, in 2015 by Eftekhar and Seyedpour inTehran (Iran), in 2014 by Guillard et al. in eastern France and in 2004 by Wang et al. which were 50.54%, 75%, 59.5%, 27.3% and 11% respectively [42-45]. A lower prevalence of PMQR determinants was also noted in Argentina (66.6%), Mexico (61.1%) and China (13.5%) [46-48]. However, a high prevalence (88.5%) that ours was observed in a study conducted in Iran [49].

In our investigation, the most prevalent gene among all PMQR genes was aac(6')-Ib-cr, followed by *qnr*B and *qnr*S. Several studies have reported high levels of aac(6')-Ib-cr genes compared to *qnr* genes [42-44,49-52]. Yet, the results of studies conducted in India, which indicated that qnrB was the most widespread PMQR [53]. This difference could be due to the variation in the type of samples processed. The high prevalence of aac(6')-Ib-cr among *Klebsiella pneumoniae* isolates in our study may be related to clonal spread of a single clone.

Frequencies of *qnr* (B, S) genes found in this study are lower than those reported by other in Côte d'Ivoire in 2017 which were 71.73% for *qnr*B and 26.08% for *qnr*S [38] but higher than those obtained in Morocco where *qnr*B was found at 23% and *qnr*S at 3% [19]. Different prevalence of *qnr* genes were reported (64.3% for *qnr*S and 26.2% for *qnr*B) in Niger as well as in Togo (47.74% for *qnr*B and *qnr*S 47.10% [54,55]. The low prevalence of *qnr*S was observed in our series was similar in a study conducted in Hungary [42]. We observed in this study that *qnr*S and aac(6')-Ib-cr genes existed alone contrary to *qnr*B gene which was always linked to aac(6')-Ib-cr reason for the high prevalence of the association *qnr*B+aac(6')-Ib-cr. These two genes would exist on the same plasmid.

High frequency of PMQR determinants among our isolates carrying ESBL genes supports suggestions in which PMQR genes are co-associated with genes encoding ESBLs. Previous reports showed that PMQR genes were associated with diverse ESBL types. Korean researchers noted that SHV-12 and DHA-1 were co-produced with qnrA1 and qnrB4 [56]. In a Taiwanese Hospital, all *qnr*-positive ESBL-producing *Enterobacter cloacae* isolates possessed the bla_{SHV-12} and *qnr*B2-like genes [57]. In the current study, we noted a strong association between three ESBLS genes and aac(6')-Ib-cr gene. Our findings corroborate those of Guillard et al. [44]. The presence of three ESBL genes and aac(6')-Ib-cr genes in the same mobile genetic elements could explain the co-resistance of these genes. It could be the same strain of bacteria circulating. Molecular typing could confirm that, hence the limitation of our study.

Other potential limitations of this study were the absence of data on minimal inhibiting concentration (MIC) for norfloxacin and ciprofloxacin to determine the level of bacterial resistance to these antibiotics, exclusion of non ESBL-producing strains and sequence analysis of the different genes.

4. CONCLUSION

ESBLs have been extensively reported for the past years in both community and nosocomial settings and a strong linkage between their production and fluoroquinolones resistance has been described in Enterobacteriaceae. Our findings indicated high prevalence of PMQR determinants in ESBL-producing *Klebsiella pneumoniae* from Bouaké, Côte d'Ivoire. The high associated was observed between ESBL and PMQR genes, notably with the aac(6')-Ib-cr gene.

ETHICAL APPROVAL

The study was carried out with the approval of the Medical and Scientific Department of Bouaké Teaching Hospital, who acts as the institutional ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Drlica K, Hooper DC. Mechanisms of quinolone action. In Hooper DC, Rubinstein E, (Ed), Quinolone Antimicrobial Agents, 3rd Edition. ASM Press, Washington, DC. 2003;19-40.
DOI: 10.1128/9781555817817.ch2.
2. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet. 1998; 351(9105):797-799
DOI: 10.1016/S0140-6736(97)07322-4
PMID: 9519952
3. Hooper DC. Mechanisms of fluoroquinolone. Resistance Drug Resist Updat. 1999;2(1):38-55.
DOI: 10.1054/drup.1998.0068
PMID: 11504468
4. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother. 2006;50: 3953-3955.
DOI: 10.1128/AAC.00915-06
PMID: 16954321
PMCID: PMC1635235
5. Périchon B, Courvalin P, Galimand M. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to Hydrophilic Fluoroquinolones by QepA-Mediated Efflux in *Escherichia coli.* Antimicrobial Agents and Chemotherapy. 2007;51(7):2464-9.
DOI: 10.1128/AAC.00143-07
PMID: 17470656
PMCID: PMC1913276
6. Yamane K, Wachino J-I, Suzuki S, Kimura K, Shibata N, Kato H, et al. New plasmid-mediated fluoroquinolone efflux pump, QepA, Found in an *Escherichia Coli* clinical isolate. Antimicrob Agents Chemother. 2007;51(9):3354-60.
DOI: 10.1128/AAC.00339-07
PMID: 17548499
PMCID: PMC2043241
7. Muylaert A, Mainil JG. Résistances aux fluoroquinolones: la situation actuelle. Ann. Méd. Vét. 2013;157:15-26.
8. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J Antimicrob Chemother. 2005;56(3):463-9.
DOI: 10.1093/jac/dki245
9. Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, et al. *qnr*B, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother. 2006;50(4):1178-82.
DOI: 10.1128/AAC.50.4.1178-1182.2006
PMID: 16569827
PMCID: PMC1426915
10. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 2006;6(10):629-40.
DOI: 10.1016/S1473-3099(06)70599-0
PMID: 17008172
11. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. Microbiol Spectr. 2014;2(5):1-42.
DOI:10.1128/microbiolspec.PLAS-0006-2013
PMID: 25584197
PMCID: PMC4288778
12. Janda JM, Abbott SL. The genera *Klebsiella* and *Raoultella.* The Enterobacteria, 2nd Ed. Washington, USA: ASM Press. 2006;115-129.
13. Seng P, Rolain JM, Fournier PE, La SB, Drancourt M, Raoult D. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol. 2010;5(11): 1733-1754.
DOI: 10.2217/fmb.10.127
PMID: 21133692
14. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1; 2017.
15. Zaniani FR, Meshkat Z, Naderi NM, Khaje-Karamadini M, Ghazvini K, Rezaee A, et al. The prevalence of TEM and SHV genes among extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae.* Iran J Basic Med Sci. 2012;15(1):654–60.
PMCID: PMC3586863
PMID: 23493850
16. Essack SY, Hall LM, Pillay DG, McFadyen ML, Livermore DM. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum beta-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. Antimicrob Agents Chemother. 2001;45(1): 88-95.
DOI: 10.1128/AAC.45.1.88-95.2001
PMID: 11120950
17. Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of

Klebsiella pneumoniae and *Escherichia coli* in Japan. FEMS Microbiol Lett. 2000;184(1):53-6.
DOI: 10.1111/j.1574-6968.2000.tb08989.x
PMID: 10689165

18. Birkett CI, Ludlam HA, Woodford N, Brown DFJ, Brown NM, Roberts MTN, et al. Real-time TaqMan PCR for rapid detection and typing of genes encoding CTX-M extended-spectrum beta-lactamases. Med Microbiol. 2007;56(1):52-55.
DOI: 10.1099/jmm.0.46909-0
PMID: 17172517
19. Bouchakour M, Zerouali K, Claude JDG, Amarouch H, El Mdaghri N, Courvalin P, et al. Plasmid-mediated quinolone resistance in expanded spectrum beta lactamase producing Enterobacteriaceae in Morocco. J Infect Dev Ctries. 2010;4(12):779-803.
DOI: 10.3855/jidc.796
PMID: 21252459
20. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, et al. Fluoroquinolone-modifying enzyme: A new adaptation of a common aminoglycoside acetyltransferase. Nat Med. 2006;12(1):83-8.
DOI: 10.1038/nm1347
PMID: 16369542
21. Rodriguez-Martinez JM, Poirel L, Pascual A, Nordmann P. Plasmid-mediated quinolone resistance in Australia. Microb Drug Resist. 2006;12(2):99-102.
DOI: 10.1089/mdr.2006.12.99
PMID: 16922624
22. Pitout JDD, Wei Y, Church DL, Gregson DB. Surveillance for plasmid mediated quinolone resistance determinants in Enterobacteriaceae within the Calgary Health Region Canada: The emergence of aac(6')-Ib-cr. J Antimicrob Chemother. 2008;61(5):999-1002.
DOI:10.1093/jac/dkn068
PMID: 18296438
23. Shahid M, Singh A, Sobia F, Rashid M, Malik A, Shukla I, et al. Bla(CTX-M), bla(TEM), and bla(SHV) in enterobacteriaceae from North-Indian tertiary hospital: High occurrence of combination genes. Asian Pac J Trop Med. 2011;4(2):101-5.
DOI: 10.1016/S1995-7645(11)60046-1
PMID: 21771430
24. Ahmed AB, Omar AO, Asghar AH, Elhassan MM. Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella spp.* urinary isolates from Sudan with confirmed ESBL phenotype. Life Sci J. 2013;10(2):191-5.
25. Goudarzi M, Azad M, Seyedjavadi SS. Prevalence of plasmid-mediated quinolone resistance determinants and oqxab efflux pumps among extended-spectrum-lactamase producing *Klebsiella pneumoniae* isolated from patients with nosocomial urinary tract infection in Tehran, Iran. Scientifica (Cairo). 2015;518167.
DOI: 10.1155/2015/518167
PMID: 26301114
PMCID: PMC4537771
26. Abdallah HM, Wintermans BB, Reuland EA, Koek A, Al Naiemi N, Ammar AM. Extended spectrum β-actamase and carbapenemase producing enterobacteriaceae isolated from Egyptian patients with suspected blood stream infection. PLoS ONE. 2015;10(5): e0128120.
DOI: 10.1371/journal.pone.0128120
PMCID: PMC4441433
PMID: 26001049
27. Ouedraogo A-S, Sanou M, Kissou A, Sanou S, Solaré H, Kaboré F, et al. High prevalence of extended spectrum ß-lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. BMC Infect Dis. 2016;16:326.
DOI: 10.1186/s12879-016-1655-3
PMCID: PMC4939587
PMID: 27400864
28. Kpoda DS, Ajayi A, Somda M, Traore O, Guessennd N, Ouattara AS, et al. Distribution of resistance genes encoding ESBLs in enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. BMC Res Notes. 2018;11:471.
DOI: 10.1186/s13104-018-3581-5
PMCID: PMC6045822
PMID: 30005695
29. Amer RMMA, El-Baghdady KZ, Kamel IKF, El-Shishtawy HM. Prevalence of extended spectrum beta-lactamase genes among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates. Egypt J Microbiol. 2019;54(1):91-101.
DOI: 10.21608/ejm.2019.16460.1113
30. Pishtiwan AH, Khadija KM. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated

from Thalassemia patients in Erbil, Iraq. Mediterr J Hematol Infect Dis. 2019;11(1):e2019041.
DOI: 10.4084/MJHID.2019.041
PMCID: PMC6613628

31. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of TEM, SHV, and CTX-M beta-lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med. 2017;7(1):12-16.
DOI: 10.4103/2231-0770.197508
PMCID: PMC5255976
32. Ghorbani-Dalinia S, Kargarb M, Doostic A, Abbasid P, Sarshare M. Molecular epidemiology of ESBLs genes and multi-drug resistance in diarrheagenic *Escherichia coli* strains isolated from adults in Iran. Iranian Journal of Pharmaceutical Research. 2015;14(4):1257-1262.
PMCID: PMC4673955
33. Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M, Sharifi SH. Genotypic versus phenotypic methods to detect extended-spectrum beta lactamases (ESBL's) in uropathogenic *Escherichia coli*. Ann Biol Res. 2012;3(5):2454-8.
34. Eftekhar F, Rastegar M, Golalipoor M, Mansoursamaei N. Detection of extended spectrum b-lactamases in urinary isolates of *Klebsiella pneumoniae* in relation to blaSHV, and blaCTX gene carriage. Iran J Public Health. 2012;41(3):127-32.
PMCID: PMC3481709
35. Salah M, Azab M, Halaby H, Hanora A. Mutations in β-lactamases detected in multidrug resistant gram negative bacteria isolated from community acquired urinary tract infections in Assiut, Egypt. Afr J Microbiol Res. 2016;10(46):1938-1943.
DOI: 10.5897/AJMR2016.8150
36. Amer SAE, El-Hefnawy AM, Abouseada NM, Elshehy ER. Detection of extended spectrum beta-lactamase producing strains among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Alexandria using Chrom-ID ESBL agar and molecular techniques. Egyptian Journal of Medical Microbiology. 2017;26(2):9-17.
37. Cremet L, Caroff N, Dauvergne S, Reynaud A, Lepelletier D, Corvec S. Prevalence of plasmid-mediated quinolone resistance determinants in ESBL Enterobacteriaceae clinical isolates over a 1-year period in a French hospital. Pathol Biol (Paris). 2011;59(3):151-6.
DOI: 10.1016/j.patbio.2009.04.003
PMID: 19481883
38. Tahou JE, Guessennd N, Sokouri PD, et al. Antimicrobial resistance of *Klebsiella pneumoniae*-ESBL producing strains isolated from clinical specimens in Abidjan (Côte d'Ivoire). Microbiol Res J Int. 2017;20(2):1-7.
DOI: 10.9734/MRJI/2017/34085
39. Goudarzi M, Fazeli M. Quinolone resistance determinants *qnr, qep*, and aac(6')-Ib-cr in extended-spectrum B-lactamase producing *Escherichia coli* isolated from urinary tract infections in Tehran, Iran. Shiraz E-Med J. 2017;18(5):e13186.
DOI: 10.5812/semj.44498
40. Briales A, Rodriguez-Martinez JM, Velasco C, de Alba PD, Rodriguez-Bano J, Martinez-Martinez L, et al. Prevalence of plasmid-mediated quinolone resistance determinants qnr and aac(6')-Ib-cr in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Spain. Int J Antimicrob Agents. 2012;39(5):431-4.
DOI: 10.1016/j.ijantimicag.2011.12.009
PMID: 22365240
41. Ferjani S, Saidani M, Amine FS, Boubaker IB-B. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in a Tunisian hospital. Microb Drug Resist. 2015;21(2):158-66.
DOI: 10.1089/mdr.2014.0053
PMID: 25247633
42. Domokos J, Kristok K, Szabo D. Plasmid-mediated quinolone resistance among extended-spectrum beta-lactamase producing *Enterobacteriaceae* from bloodstream infections. Acta Microbiol Immunol Hung. 2016;63(3):313-323.
DOI: 10.1556/030.63.2016.002
PMID: 27539331
43. Eftekhar F, Seyedpour SM. Prevalence of *qnr* and aac(6')-Ib-cr genes in clinical isolates of *Klebsiella pneumoniae* from Imam Hussein Hospital in Tehran. Iran J Med Sci. 2015;40(6):515-521.
PMCID: PMC4628142
PMID: 26538780
44. Guillard T, Bertrand X, Cholley CCP, Bajolet O, Gbaguidi-Haore H. aac(6')-Ib-cr is the major plasmid-mediated quinolone resistance determinant in extended-spectrum β-lactamase-producing

Escherichia coli in eastern France. J Glob Antimicrob Resist. 2014;2(2):111-113. DOI: 10.1016/j.jgar.2014.01.006 PMID: 27873588

45. Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the qnr gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob Agents Chemother. 2004;48(4):1295–1299. DOI: 10.1128/aac.48.4.1295-1299.2004 PMCID: PMC375335 PMID: 15047532.
46. Cruz GR, Radice M, Sennati S, Pallecchi L, Rossolini GM, Gutkind G, Di Conza JA. Prevalence of plasmid-mediated quinolone resistance determinants among oxyiminocephalosporin-resistant *Enterobacteriaceae* in Argentina. Mem Inst Oswaldo Cruz. 2013;108(7):924-927. DOI: 10.1590/0074-0276130084 PMCID: PMC3970647 PMID: 24037111
47. Silva-Sanchez J, Barrios H, Reyna-Flores F, Bello-Diaz M, Sanchez-Perez A, Rojas T. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum b-lactamase-producing *Enterobacteriaceae* isolates in Mexico. Microb Drug Resist. 2011;17(4): 497–505. DOI: 10.1089/mdr.2011.0086
48. Jiang Y, Zhou Z, Qian Y, Wei Z, Yu Y. Plasmid-mediated quinolone resistance determinants *qnr* and aac (6′)-Ib-cr in extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* in China. Journal of Antimicrobial Chemotherapy. 2008;61(5):1003-1006. DOI: 10.1093/jac/dkn063
49. Shams E, Farzaneh F, Moniri R, Zibaei M. Prevalence of plasmid-mediated quinolone resistance genes among extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* human isolates in Iran. J Pathog. 2015;2015:1-7. DOI: 10.1155/2015/434391 PMCID: PMC4649097 PMID: 26618005
50. Magesh H, Kamatchi C, Vaidyanathan R, Sumathi G. Identification of plasmid-mediated quinolone resistance genes *qnr*A1, *qnr*B1 and aac(6')-1b-cr in a multiple drug-resistant isolate of *Klebsiella pneumoniae* from Chennai. Indian J Med Microbiol. 2011;29(3):262-8. DOI: 10.4103/0255-0857.83910 PMID: 21860107
51. Cruz GR, Radice M, Sennati S, Pallecchi L, Rossolini GM, Gutkind G, et al. Prevalence of plasmid-mediated quinolone resistance determinants among oxyiminocephalosporin-resistant *Enterobacteriaceae* in Argentina. Mem Inst Oswaldo Cruz. 2013;108(7):924-927. DOI: 10.1590/0074-0276130084 PMCID: PMC3970647 PMID: 24037111
52. Dasgupta N, Dhar (Chanda) D, Kharkongor N, Chakravarty A, Bhattacharjee A. Molecular detection of aac(6')-Ib-cr among clinical enterobacterial isolates conferring quinolone resistance:- A study from North East India. J Microbiol Infect D. 2016;6(3):97-102. DOI: 10.5799/ahinjs.02.2016.03.0225
53. Tripathi A, Dutta SK, Majumdar M, Dhara L, Banerjee D, Roy K. High prevalence and significant association of ESBL and qnr genes in pathogenic *Klebsiella pneumoniae* isolates of patients from Kolkata, India. Indian J Microbiol. 2012;52(4):557-564. DOI: 10.1007/s12088-012-0281-z PMCID: PMC3516640 PMID: 24293710
54. Moumouni A, Diagbouga S, Nadembèga C, et al. Quinolone Resistance (*qnr*) genes in fecal carriage of extended Spectrum beta-lactamases producing Enterobacteria isolated from children in Niger. Curr Res Microbiol Biotechnol. 2017;5(1):953-957.
55. Salah FD, Soubeiga ST, Ouattara AK, Sadji AY, Metuor-Dabire A, Obiri-Yeboah D, et al. Distribution of quinolone resistance gene (*qnr*) in ESBL-producing *Escherichia coli* and *Klebsiella spp*. in Lomé, Togo. Antimicrob Resist Infect Control. 2019;8(104):1-8. DOI: 10.1186/s13756-019-0552-0 PMCID: PMC6582466 PMID: 31244995.
56. Jeong HS, Bae IK, Shin JH, Jung HJ, Kim SH, Lee JY, et al. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and ampc beta-lactamase in *Enterobacteriaceae.* Korean J Lab Med. 2011;31(4):257-264. DOI: 10.3343/kjlm.2011.31.4.257 PMCID: PMC3190004

57. Wu JJ, Ko W-C, Tsai SH, Yan JJ. Prevalence of plasmid-mediated quinolone resistance determinants QnrA, QnrB and QnrS among clinical isolates of *Enterobacter cloacae* in a Taiwanese hospital. Antimicrob Agents Chemother. 2007;51(4):1223-1227.
DOI: 10.1128/AAC.01195-06
PMCID: PMC1855486
PMID: 17242140

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