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Characterization of Quinolone Resistance and ESBLs Genes Produced by *Escherichia coli* Isolated at the UHC-the National Reference in N'Djamena-Chad

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Infections caused by ESBLs-producing *E. coli* continue to represent a challenge for the implementation of appropriate treatment protocols worldwide. The objective of the present study was to assess the current profile of antimicrobial and ESBLs susceptibility, to screen the *bla-CTX*-M, *bla-TEM*, *bla-SHV* and qnrs genotypes of ESBLs-producing or non-isolated ESBLs-producing Multi-resistant *E. coli* (MDR) strains of various clinical samples in Chad. This is a prospective study conducted from September 2018 to February 2019 at the N'Djamena University Hospital in Chad. *E. coli* MDR has been isolated from urine, pus and stool cultures. Antibiotic identification and susceptibility testing was performed using an automated system, the Vitek-2. Confirmation for ESBLs production was performed by the double disc synergy assay. The *E.coli* MDR and ESBLs strains, positive or not, were screened for the genes encoding the ESBLs and qnrs by PCR. Microsoft Excel 2016 and SPSS ™ statistical software version 20.0 were used for the analysis of the results. Of the 68 MDR *E. coli* isolated from various clinical samples, 40 (58.8%) were positive for extended spectrum beta-lactamase production. 47.1% were resistant to the twelve (12) antibiotics tested. All isolates with an ESBLs phenotype were resistant to at least one antibiotic tested. The *CTX-M* gene was in the majority (72.1%), followed by *TEM* (54.4%) and *SHV* (16.2%). The qnrs genes were also detected in 52.9% (*qnrA1 to A6*), 52.9% (*qnrB1 to B6*) and 2.9% (*qnrS1 to S6*). All strains showing an ESBLs phenotype (40) were also positive in the molecular screening of ESBLs gene families. A double and triple carriage of the ESBLs and qnrs genes was observed. These results show the need for an aggressive infection control program, systematic detection of ESBLs isolates in clinical samples and antimicrobial management in hospitals in Chad.

Introduction

Enterobacteriaceae, in particular Escherichia coli, are the most frequently encountered pathogens in diseases such as abscesses, pneumonia, meningitis, septicemia, and especially enteritis and urinary tract infections (Ambler *et al.,* 1991). The ability of *Enterobacteriaceae* to become resistant to antibiotics, in particular to β-lactam antibiotics (cephalosporins and carbapenems) which are the treatments of choice for enterobacterial infections, poses a serious problem for healthcare providers. *Escherichia coli* (*E.coli*) is a commensal enterobacteria of the digestive tract of humans and animals (Schito *et al.,* 2009). However, the abusive and uncontrolled use of antibiotics will have allowed the emergence of new variants of *multiresistant E. coli* (MDR *E. coli*) strains, particularly those resistant to beta-lactams (FAO, 2017; Lucet *et al.,* 1999; Ambler *et al.,* 1991).The multidrug resistance of strains of E. coli producers of ESBL is a scourge due to the high probability of therapeutic failure and the dissemination of these bacterial strains (Bradford, 2001; Knothe *et al.,* 1983).The direct consequence of this antibiotic resistance is the increase in morbidity and mortality due to pathologies such as abscesses, pneumonia, meningitis, sepsis, enteritis and urinary tract infections (OMS, 2019; Aworh *et al.,* 2019; Hailaji *et al.,* 2016; Veenemans *et al.,* 2014).

Thus, studies conducted around the world have shown that E. coli expresses a high level of acquired resistance to most antibiotics belonging to the beta-lactam family through the expression of inactivating extended-spectrum beta-lactamases (ESBLs) last-line drugs such as third-generation cephalosporins and quinolones (OMS, 2018; El bouamri *et al.,* 2015; Rawat and Nair, 2010).

According to the World Health Organization, antimicrobial resistance is a more serious scourge than HIV/AIDS (OMS, 2018). In the UK, studies in 2016 showed that antimicrobial resistance was responsible for around 700,000 deaths a year (Humphreys and Fleck, 2016). The number of deaths attributable to antimicrobial resistance will increase to 10 million per year by 2050, with 40% of these deaths occurring in Africa (OMS, 2018).

Studies have shown that the *bla-TEM*, *bla-SHV* and *bla-CTX-M* genes coding for ESBLs are located on bacterial DNA, plasmids or transposons and are often associated with resistance to quinolones (*qnrA, qnrB* and *qnrS*), aminoglycosides, to cotrimoxazole and tetracyclines (Mariani-Kurkdjian *et al.,* 2012; Cattoir *et al.,* 2007b).

These genes can be easily transferred between two or more bacteria of the same or different species (Tansawai *et al.,* 2019; Moghaddam *et al.,* 2014; Mugnaioli *et al.,* 2006); hence the origin of the multi-resistance of *E. coli* producing extended-spectrum beta-lactamases (Rawat and Nair, 2010; Humphreys and Fleck, 2016; Mariani-Kurkdjian *et al.,* 2012; Cattoir *et al.,* 2007b; Tansawai *et al.,* 2019; Moghaddam *et al.,* 2014; Mugnaioli *et al.,* 2006; Spanu *et al.,* 2002). Understanding the effect of drug resistance is capital because of its impact on the treatment of infection. In Chad, little information on multidrug resistance in *E. coli* is known. Previous studies have reported the prevalence of high ESBLs (48%) containing the enzyme *bla-CTX-M*-15 (97%) and *bla-TEM* (14.6%) in clinical *E. coli* isolates from three major Chadian hospitals (Ouchar *et al.,* 2019; Nadlou *et al.,* 2015). The aim of this study was to characterize clinical isolates of *E. coli* from the National Reference Hospital of N'Djamena in order to (i) determine the current profiles of antibiotic susceptibility (ii) assess the current prevalence of ESBLs and (iii) identify the genes involved in resistance.

Materials and Methods

Study site and period

This prospective cross-sectional study was carried out for six months (from September 2018 to February 2019) at the Bacteriology Unit of the University Hospital Center (UHC) the National Reference of N'Djamena.

Study population

The study population was composed of all patients in the UHC departments. These are general medicine (GM), infectious diseases (ID), surgery (visceral and general), gastroenterology, pneumology, urology, otolaryngology (ORL), cardiology and diabetology. The study also looked at patients visiting the emergency department (ED).

Sample collection or sampling

A total of 808 samples were taken from patients in the various departments of the UHC. The samples taken included 257 urine samples, 210 pus samples and 341 stool samples.

Once collected, the samples were immediately transported to the laboratory for processing.

Biochemical isolation identification of *E. coli*

The E. coli strains were isolated in the laboratory of the National Reference Hospital of N'Djamena, following the methods in force in this center. The urine, stool and pus samples were respectively inoculated onto agar media: CLED, Hektoen and Mac Conkey from BioMérieux.

After Gram staining of suspicious *E. coli* colonies, subculture on EMB was performed and strain identification is carried out by the VITEK® 2TM 15 compact automaton.

Antibiotic sensitivity tests

Antimicrobial susceptibility of E. coli isolates was performed using the VITEK® 2TM 15 compact automaton. Antibiotic susceptibility disks (AST N 233) of Gram-negative bacteria were used.

Categorization criteria were defined as susceptible (S), intermediate (I) and resistant (R) for each antibiotic used. Twelve antibiotics (Amoxycillin + clavulanic acid (AUG, 30 µg), Imipenem (IPM, 10 µg), Ceftriaxone (CRO, 30 µg), Cefepime (FEP, 30 µg), Azteronam (ATM, 30 µg), Gentamicin (CN, 10 µg), Nalixidic acid (NA, 30 µg), Norfloxacin (NOR, 10 µg), Ciprofloxacin (CIP, 5 µg), Trimetroprim-sulfonamide (SXT, 25 µg), Tetracycline (TET, 30 µg) and azithromycin) have been tested. Isolates resistant to at least two unrelated classes of antibiotics were considered multidrug-resistant (Kiratisin *et al.,* 2008).

Detection of extended-spectrum beta-lactamase production

The production of extended-spectrum beta-lactamases (ESBLs) has been demonstrated by the double-disc synergy technique. This test is based on the detection of a synergy between Ceftriaxone, Aztreonam and Cefepime disks arranged around a disk of amoxicillin + clavulanic acid separated by 20 to 30 mm on a plate of Müller-Hinton agar.

After an incubation of 18 to 24 hours, a dome-shaped zone without culture appears between the discs of the AMC and those of the C3Gs, known as a “champagne cork”.

Isolates presenting the ESBL phenotype were collected for the detection of ESBLs genes (*TEM, SHV*and *CTX-M*) and quinolone resistance genes (*qnrA1* to *A6*, *qnrB1* to *B6* and *qnrS1* to *S6*).

Detection of β-lactam and quinolone resistance genes by classical PCR

DNA extraction

DNA extraction was performed by the heat shock method. Using a sterile loop, colonies of each bacterial strain on Müller-Hinton agar were scraped and suspended in 150 µl of PCR water (Solis BioDyne) in Eppendorf tubes. Then they were homogenized.

The mixtures were incubated at -20°C for 10 minutes, 100°C for 10 minutes, and -20°C for 10 minutes to create bacterial cell lysis. The suspensions obtained were centrifuged for 10

minutes at 13,000 g. DNA supernatants were collected in new clean and sterile Eppendorf tubes for PCR.

Characterization of β-lactamase genes

The bla-TEM, *bla-SHV* and *bla-CTX-M* genes were detected by conventional PCR using the specific primer pairs *bla-TEM-F* and *bla-TEM-R*, *bla-SHV-F* and *bla-SHV-R* and *bla-CTX-MF* and *bla-CTX-MR* (Table 1). The 20 µl reaction mix consisted of 5 µl DNA, 4 µl Master mix (5x Fired Pol, Solis BioDyne with 12.5 mM), 2 µl each primer, GENECUST SAS (1 µl forward primer, 1 µl of reverse primer) and 9 µl of PCR water (Solis BioDyne).

The conditions under which the *bla-TEM*, *bla-SHV* and *bla-CTX-M* genes were amplified are shown in Table 2.

Characterization of quinolone resistance genes

Conventional PCR was used to detect the genes qnrA1 to A6, qnrB1 to B6 and qnrS1 to S6 with the specific primer pairs *Qnr AF* and *qnrQnrA-R*, *QnrBm-F* and *QnrBm-R* and *QnrS-F* and *qnrQnrS-R* (Table 1). The 20 µl reaction mix consisted of 5 µl DNA, 4 µl Master mix (5x Fired Pol, Solis BioDyne with 12.5 mM), 2 µl each primer, GENECUST SAS (1 µl forward primer, 1 µl of reverse primer) and 9 µl of ultra-pure water. The conditions used for the amplification of qnrs genes are shown in Table 2.

Ethical considerations

The study was approved by the research committee of the UHC laboratory of N'Djamena. The UHC authorities authorized the authors to conduct the study. All biological samples were collected as part of the routine clinical management of patients.

Statistical analysis

Data analysis was performed using Microsoft Excel 2016 and Statistical Software for the Social Sciences (SPSS™) version 20.0 (IBM, Armonk, NY, USA) and presented as percentage of baseline distribution. Data with a p-value less than 0.05 (95% CI) were considered significant.

Results and Discussion

During the six-month study period, 808 samples were taken and cultured in the laboratory bacteriology unit of the UHC. These are 210 (26%) pus samples, 341 (42%) stool samples and 257 (32%) urine samples. A total of 194 strains of E. coli were isolated from 194 positive cultures, 68 of which were multiresistant either a prevalence of *E. coli* MDR of 35% (68/194).

Prevalence of *E. coli* strains MDR depending on the origin, type of samples and production or not of ESBLs

Table 3 shows that among the 68 *E. coli* MDR isolates, the highest prevalence was observed in urine specimens (61.8%), followed by pus specimens (23.5%) and stool specimens (14.7%).In relation to the various services, the distribution of isolates of *E. coli* multidrug-resistant, the highest were observed in the urology departments (n=18), followed by the infectious diseases departments (n=13), emergency room (n=9) and gastroenterology (n=8).

The ESBLs phenotype observed in the samples analyzed, a prevalence of MDR *E. coli* of 58.8% (40/68) was observed. The majority of ESBLs-producing multidrug-resistant *E. coli* were isolated respectively from urine (n=24), pus (n=10) and stool (n=6) samples.

There was no significant difference between the ESBLs production of the different *E.coli* isolated in the biological fluids ($p > 0.05$). In addition, in the various departments of the UHC, the highest distribution of ESBL phenotypes were observed respectively in the urology department (14), infectious diseases (6), emergency departments (5) and the gastroenterology department (4). No significant difference was observed between the

phenotypic distribution of ESBLs in the departments of the University Hospital at the threshold of P>0.05 (Table 3).

Antibiotic sensitivity test

During the study period, sixty-eight (68) *E. coli* MDR were isolated in the N'Djamena University Hospital. The antimicrobial susceptibility test results of these 68 multidrug-resistant *E. coli* including 40 ESBL-producing isolates have been reported in figure 2.

The antibiotic resistance profile of these isolates revealed that 47.1% (32/68) were resistant to the twelve (12) antibiotics tested. All of these isolates exhibited the extended-spectrum beta-lactamase producing phenotype. The 8 ESBL positive strains also showed resistance to 5 antibiotics. Figure 2 presents the results of antibiotic susceptibility of the 68 MDR *E. coli* tested.

The results show that 47.1% (32/68) of the isolates were resistant to the twelve (12) antibiotics tested, 51.5% (35/68) were resistant to at least three (3) antibiotics and 1.5% (1/68) was resistant to two (2) antibiotics.

High resistance to beta-lactams (amoxicillin + clavulanic acid (92.6%), ceftriaxone (91.1%), cefepime (83.9%), aztreonam (88.7%) and imipenem (77.8 %)), macrolides (azithromycin (91.2%)), cyclines (tetracycline (88.3%)), quinolones (nalidixic acid (88.2%), norfloxacin (83.8%) and ciprofloxacin (79.4%)), aminoglycosides (gentamicin (79.4%)) and sulfonamides (trimethoprim-sulfonamide (83.8%)) were observed.

Global characterization of extended-spectrum β-lactamase genes

A total of 68 MDR E coli were the subject of molecular characterization in this study. All strains with an ESBLs phenotype (40) were also positive in the molecular screen for common ESBLs gene families. Molecular analysis of the isolates showed the presence of various beta-lactamase genes (Table 4).

Table 4 shows that the majority of isolates of *E. coli* multidrug-resistant carried the bla-CTX-M gene (72.1%), while 54.4% and 16.2% carried the bla-TEM and bla-SHV genes respectively. No significant difference between the carriage of these genes was observed (P>0.05).

Forty-two (42) *E coli* MDR isolates from urine samples were tested for ESBLs resistance genes. Among these isolates, the bla-CTX-M gene was the most prevalent (71.4%), followed by the bla-TEM gene (54.8%), and bla-SHV (14.3%).

The study of sixteen (16) *E. coli* MDR isolated from pus samples, showed the presence of bla-CTX-M gene, bla-TEM gene and bla-SHV gene with a prevalence of 75%, 50% respectively and 12.5%.

Of the ten (10) *E. coli* MDR isolated from stool samples, the bla-CTX-M gene was the most prevalent (70%), followed by the bla-TEM gene (60%) and the bla-SHV gene (30%) respectively). There is no significant difference between the expression of these genes in the different samples studied (P >0.05).

Distribution of genes according to ESBLs phenotypes of strains of E. coli MDR

The study also revealed the existence of different ESBLs genes in the same strain. In fact, a double carriage was noted in 20.5% of the strains which simultaneously carried the TEM and CTX-M-13 genes, 6% which carried the TEM and SHV genes. 17.6% of the strains carried 3 genes at the same time (TEM, SHV and CTX-M). Table 5 shows that all the twenty-four (24) urinary isolates presenting an ESBL phenotype were positive for the bla-CTX-M gene (100%), 63% for the bla-TEM gene and 21% for the bla-SHV gene. For the sixteen E coli MDR isolates positive for ESBLs production, a prevalence of 90% for the bla-CTX-M gene, 60% for the bla-

TEM gene and 20% for the SHV gene was observed. Of the six (6) ESBL isolates positive by phenotypic method, 66.7% were positive for the bla-CTX-M gene and 66.7% for the bla-TEM gene. None of the isolates were positive for bla-SHV genes.

Furthermore, the coexistence of bla-TEM and bla-CTX-M genes was observed in stool, pus, and urine samples with a prevalence of 40% (4/10), 25% (4/16), and 14.3% (6/42) respectively. While bla-TEM + bla-SHV genes were detected in 20%, 6.25%, and 2.4% in stool, pus, and urine samples, respectively. The coexistence of three ESBLs genes belonging to the bla-TEM + bla-SHV gene cluster was observed in 30%, 50%, 18.8% and 14.3%.

Distribution of ESBLs genes according to clinical services

The distribution of ESBLs genes observed in the different departments of the UHC is presented in Table 6.The latter shows that, in this study, the highest overall ESBLs gene carriage was observed in the urology department 26.5% (n=18) followed by the infectious diseases department 22.1% (n=15), the emergency room 13.2%(n=9), gastroenterology and emergencies 11.8% (n=8), otolaryngology service 7.4% (n=5), surgery service 5.9% (n=4), Hematology 4.4% (n=3), cardiology 2.2% (n=2), diabetology 2.2% (n=2) and internal medicine 2.2% (n=2) services (Table 5). No significant difference was observed between the presence of these genes in the departments of the UHC ($P > 0.05$).

Profile of ESBLs genes amplified by PCR

The genes identified by the PCR method using specific primers i.e. *TEM, SHV and CTX-M* on agarose gel are shown in Figures A, B and C.

Prevalence of quinolone genes

Concerning the search for the three quinolone resistance genes of the qnrA, qnrB and qnrS type of this study, their carriage by the isolates of *E. coli* MDR is represented in table 6.

Table 6 shows that the *qnrs* genes were present in 60% (41/68) of the strains studied. The results showed that 36 isolates (52.9%) of E. coli MDR carry the qnrA1-like gene to A6, 36 isolates (52.9%) carry the *qnrB1*-like gene to *B6*, and 2 isolates (2.9%) carry the *qnrS1*-like gene for *S6*.

Indeed, 24 strains out of the 41 strains carrying qnr genes, i.e. 58.5% (24/41), were positive for ESBLs phenotypes. Moreover, among all the strains carrying the *qnrs* genes, 87.8% (36/41) were resistant to at least one antibiotic belonging to the quinolone family such as nalidixic acid (NA), ciprofloxacin (CIP) and norfloxacin (NOR). These strains also co-expressed the qnr and ESBLs genes; of which 6 isolates expressed the *CTX-M* gene, 5 isolates expressed the *TEM* gene and 1 isolate expressed the *SHV* gene.

Double carriage of ESBLs genes was observed in 18 isolates that expressed *TEM* + *CTX-M* genes and 2 isolates expressed *SHV* + *CTX-M* genes. 9 isolates simultaneously expressed three ESBL genes (*TEM* + *SHV* + *CTX-M*). A correlation was observed between *qnrS* gene carriage and quinolone resistance ($P < 0.05$).

Distribution of *qnrs* genes according to susceptibility to ciprofloxacin

The distribution of *qnrs* genes according to the susceptibility of isolates to ciprofloxacin is presented in Table 7. Table 7 shows the distribution of *qnrS* genes according to ciprofloxacin susceptibility. Indeed, 75.6% (31/41) of ciprofloxacin-resistant strains carried at least one *qnr* gene. Whereas, 24% (10/41) of the ciprofloxacin sensitive strains had a *qnr* gene. There is a significant difference between the resistance of the isolates to ciprofloxacin and the carriage of the *qnrs* genes ($p<0.05$).

Profile of qnrs genes amplified by PCR

The qnrs genes identified by the PCR method using specific primers i.e. *qnrB1 to B6, qnrS1 to S6 and*

qnrA1 to A6 on agarose gel are shown in Figures A, B and C.

The main objective of our study was to assess the prevalence of isolates of multiresistant ESBL-producing *E. coli* and determine the carriage of ESBLs and quinolone resistance genes circulating at the CHU-RN in Chad.

The isolates of *E. coli* in this study were distinguished by a high carriage of resistance genes to major antibiotics such as beta-lactams, quinolones, macrolides and aminoglycosides commonly used to treat enterobacterial infections.

Thus, the study showed that of the 68 *E.coli* MDR isolated, 58.8% (40/68) were ESBLs producers. These results show a high prevalence of ESBLs; this explains the high rate of resistance to antibiotics ordinarily used to treat enterobacteria infections in the CHU-RN of N'Djamena.

In Chad, Ouchar *et al.,* (2019), reported somewhat similar results to our study. Indeed, their study focused on three hospitals in the city of N'Djamena where 94 strains of ESBL-producing *Enterobacteriaceae* were isolated and of which E. coli was the predominant species with a frequency of 63.83% (n = 60).A similar prevalence was also found in some African countries such as Iran (58%) (Pourakbari *et al.,* 2019), Burkina Faso (62.6%) (Kpoda *et al.,* 2017) among children admitted to the Saint Camille Hospital and in France (76%) (Holstein *et al.,* 2010) at Bretonneau Hospital.

However, other authors have revealed high proportions of ESBL-producing *E. coli.* Dembele *et al.,* (2020) and Diagne *et al.,* (2018) reported a proportion of 100% carriage of ESBL-producing *E.coli* respectively in rural areas of Burkina Faso and patients seen at Fann Hospital in Senegal.

This large variation in the carriage of ESBLs observed could testify to an increase in the frequency of ESBLs within our university hospital center, or difference in the type is the number of samples collected, reflecting the average level of hygiene or even a greater sensitivity to our method of analysis. These results should draw the attention of the nursing staff to the observation of hygiene measures in the hospital environment.

Lucet *et al.,* (1999) showed that it is possible to effectively control situations in which ESBLs are epidemic or hyper-endemic by the correct identification of pathogens, hand hygiene, isolation of patients, wearing of gloves and blouses.

This study showed that ESBL-producing *E. coli* were isolated from different types of clinical samples. Thus, it was found that urine samples had the highest ESBLs carriage (62%), followed by pus (56%) and stool (50%) samples. Similar results were obtained in Ouagadougou in Burkina Faso (62.4%) (Kpoda *et al.,* 2017) in urine samples.

Moreover, this carriage remains weak compared to the results obtained by Fody *et al.,* (2017) in Niger; 26.7%, 26.3% and 25% respectively in urine, pus and stool. This difference in carriage of ESBL-producing *E. coli* in clinical samples could be explained by the more variable number of samples.

However, high levels of isolate ESBL-producing *E. coli* in the urology department (35%) and in the urine samples (62%) observed in this study could be elucidated by the nature of the samples from the patients studied, the length of stay in the hospital, the invasive procedures and devices (catheters, probes, intubation, etc.) and long-term exposure of patients to antibiotics as reported by Rodríguez-Baño *et al.,* (2010) on the one hand and the combined therapy of aminoglycosides and cephalosporins in the treatment of urinary tract infections at the CHU-RN (Cattoir, 2007b) on the other hand.

It should be noted that if the current practices of lack of systematic monitoring of ESBLs and the absence of an ESBLs control program in the CHU-RN continue, a rapid increase in the prevalence of ESBLs can be observed in the years to come.

Table.1 Primers of beta lactamases and quinolones genes

Target genes	Primers	Sequences of primers sens,antisens (5' → 3')	Size (pb)	References
***bla*$_{TEM}$**	TEM-F	ATAAAATTCTTGAAGACGAAA	1080	Mabilat *et al.*, (1990) ; [56]
	TEM-R	GACAGTTACCAATGCTTAATC		
***bla*$_{SHV}$**	SHV-F	TTATCTCCCTGTTAGCCACC	795	[56]
	SHV-R	GATTTGCTGATTTCGCTCGG		
***bla*$_{CTX-M}$**	CTX-M-F	GTTACAATGTGTGAGAAGCAG	1041	Jouini.(2007)
	CTX-M-R	CCGTTTCCGCTATTACAAAC		
qnrB1 to qnrB6	QnrB-F	GGMATHGAAATTCGCCACTG[a]	264	Cattoir *et al.*, (2007a)
	QnrB-R	TTTGCYGYYCGCCAGTCGAA[b]		
qnrS1 to qnrS6	QnrS-F	GCAAGTTCATTGAACAGGGT	428	Cattoir *et al.*, (2007b)
	QnrS-R	TCTAAACCGTCGAGTTCGGCG		
qnrA1 to qnrA6	QnrA-F	AGAGGATTTCTCACGCCAGG	580	Cattoir *et al.*, (2007b)
	QnrA-R	TGCCAGGCACAGATCTTGAC		

NB:aM = A ou C;aH = A ou C ou T; bY =C ou T.

Table.2 Conditions for amplification of beta lactamases and quinolones resistance genes

Conditions for amplification of beta lactamases resistance genes			
Cycle	Number of cycles	Temperature	Time
Initial denaturation		94°C	5mn
Amplification	35 cycles	94°C	30 sec
		55°C	60 sec
		72°C	90 sec
Final elongation		72°C	10 min
Conditions for amplification of quinolones resistance genes			
Initial denaturation		95°C	10mn
Amplification	35 cycles	95°C	60 sec
		54°C	60 sec
		72°C	60 sec
Final elongation		72°C	10 min

Fig.1 Positive double-disc synergy test performed on a 90 mm Mueller-Hinton agar plate.

Champagne cork

Table.3 Distribution of multidrug resistant *E. coli*

Origin	Effective	Services	MDR *E. coli*	*E. coli* BLSEs	*E. coli* not BLSEs
Stool	14,7 (n=10)	Gastro	11,8 (n=8)	5,9 (n=4)	5,9 (n=4)
		ID	2,9 (n=2)	2,9 (n=2)	0
Urine	61,8 (n=42)	Uro	26,5 (n=18)	20,6 (n=14)	5,9 (n=4)
		Hemato	4,4 (n=3)	2,9 (n=2)	1,5 (n=1)
		ID	14,7 (n=10)	2,9 (n=2)	11,8 (n=8)
		Cardio	2,9 (n=2)	1,5 (n=1)	1,5 (n=1)
		PU	13,2 (n=9)	7,4 (n=5)	5,9 (n=4)
Pus	23,5 (n=16)	Surgery	5,9 (n=4)	1,5 (n=1)	4,4 (n=3)
		Diabetes	2,9 (n=2)	2,9 (n=2)	0
		Int Med	2,9 (n=2)	2,9 (n=2)	0
		ID	4,4 (n=3)	2,9 (n=2)	1,5 (n=1)
		ORL	7,4 (n=5)	4,4 (n=3)	2,9 (n=2)
Total	**100 (n=68)**	**10**	**100 (n=68)**	**58,8 (n=40)**	**41,2 (n=28)**

Legend: Uro= Urology; Cardio= Cardiology; Diabetes= Diabetology; Hemato= Hematology; Med Int= Internal Medicine; ID= Infectious Diseases; ORL= Otorhinolaryngology; ER= Emergency room, Gastro=gastrology.

Table.4 Carrying of ESBLs resistance genes by multiresistant *E. coli*

ESBLs genes	Clinical origin of samples			
	Urine (N=42)	Pus (N=16)	Stools (N=10)	Total (N=68)
bla-CTX-M (%)	30 (71,4)	12 (75)	7 (70)	49 (72,1)
bla-TEM (%)	23 (54,8)	8 (50)	6 (60)	37 (54,4)
bla-SHV (%)	6 (14,3)	2 (12,5)	3 (30)	11 (16,2)

Table.5 Distribution of ESBLs genes according to the clinical origin of the samples

ESBLsgenes	Clinical origin of samples					
	Urine (N=42)		Pus (N=16)		Stools (N=10)	
	$ESBL^+$ N=24	$ESBL^-$ N=18	$ESBL^+$ N=10	$ESBL^-$ N=6	$ESBL^+$ N=6	$ESBL^-$ N=4
bla-CTX-M (%)	24 (100)	6 (33,3)	9 (90)	3 (50)	4 (n=66,7)	3 (75)
bla-TEM (%)	15 (63)	8 (44,4)	6 (60)	2 (33,3)	4 (n=66,7)	2 (50)
bla-SHV (%)	5 (21)	1(5,6)	2 (20)	0 (0)	0 (0)	3 (75)
blaTEM+blaCTX-M (%)	3 (13)	3 (11,1)	2 (20)	2 (33,3)	3 (n=33,3)	1 (25)
blaTEM+blaSHV (%)	0 (0)	1 (5,6)	0 (0)	1 (16,7)	0 (0)	2 (50)

Table.6 Carrying of quinolone resistance genes by *E. coli* MDR isolates

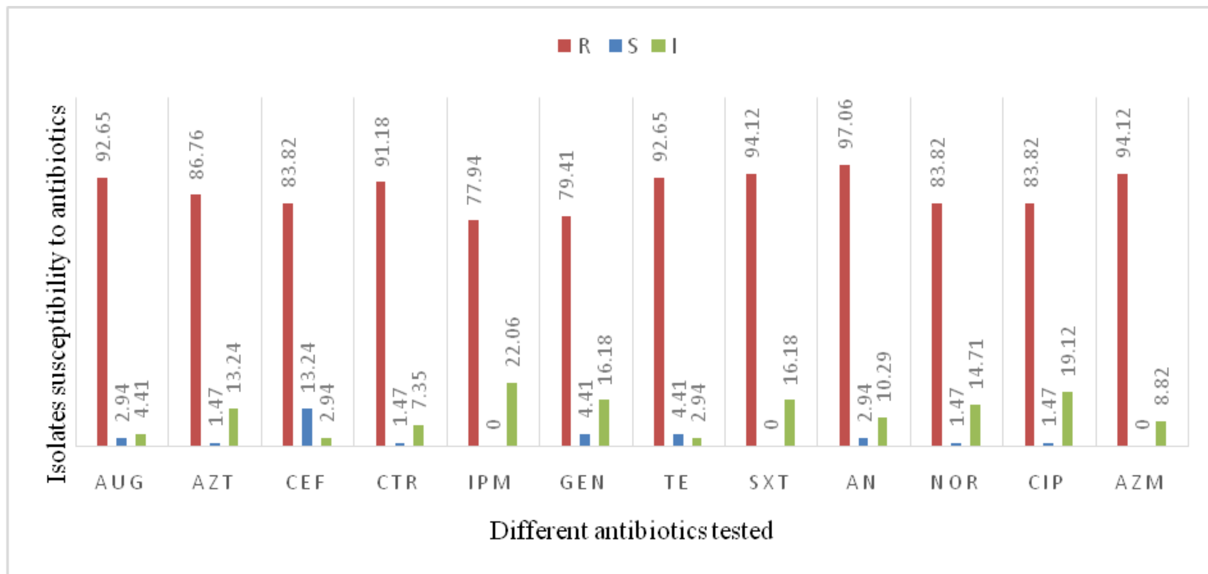
Genes	*qnr B1 to B6* (%)	*qnrS1 to S6*(%)	*qnrA1 to A6*(%)
Presence	52,9 (n=36)	52,9 (n=36)	2,9 (n=2)
Absence	47,1 (n=32)	47,1 (n=32)	97,1 (n=66)
Total	100 (n=68)	100 (n=68)	100 (n=68)

Table.7 Distribution of qnr genes according to susceptibility of isolates to ciprofloxacin

Antibiotic	Number	*qnrs genes*				
		qnrA1-qnrA6	*qnrB1- qnrB6*	*qnrS1- qnrS6*	*qnrB1-qnrB6 qnrS1-qnrS6*	*qnrA1-qnrA6 qnrB1-qnrB6*
CIP-R	31	1	3	4	23	0
CIP-S	10	0	1	0	8	1
Total	**41**	**1**	**4**	**4**	**31**	**1**

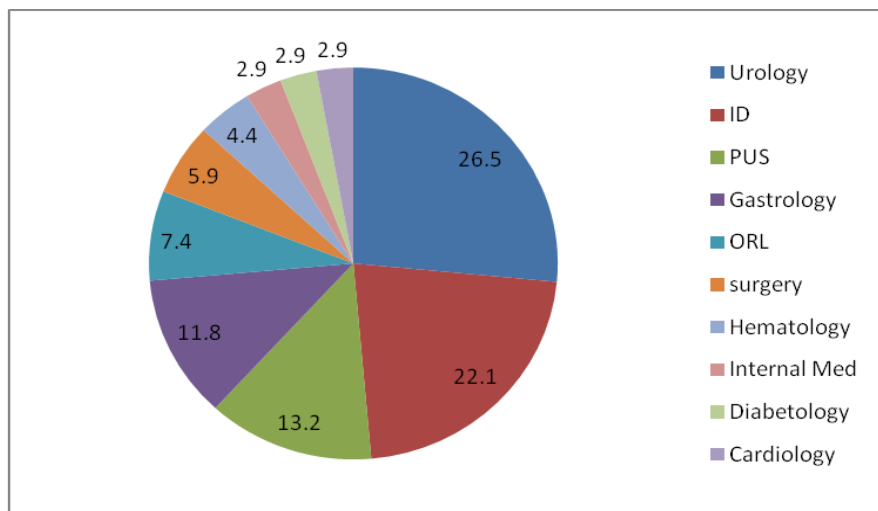
Legend: CIP-R= ciprofloxacin resistant, CIP-S= ciprofloxacin sensitive

Fig.2 Antibiotic resistance susceptibility of multidrug-resistant *E. coli* strains isolated.



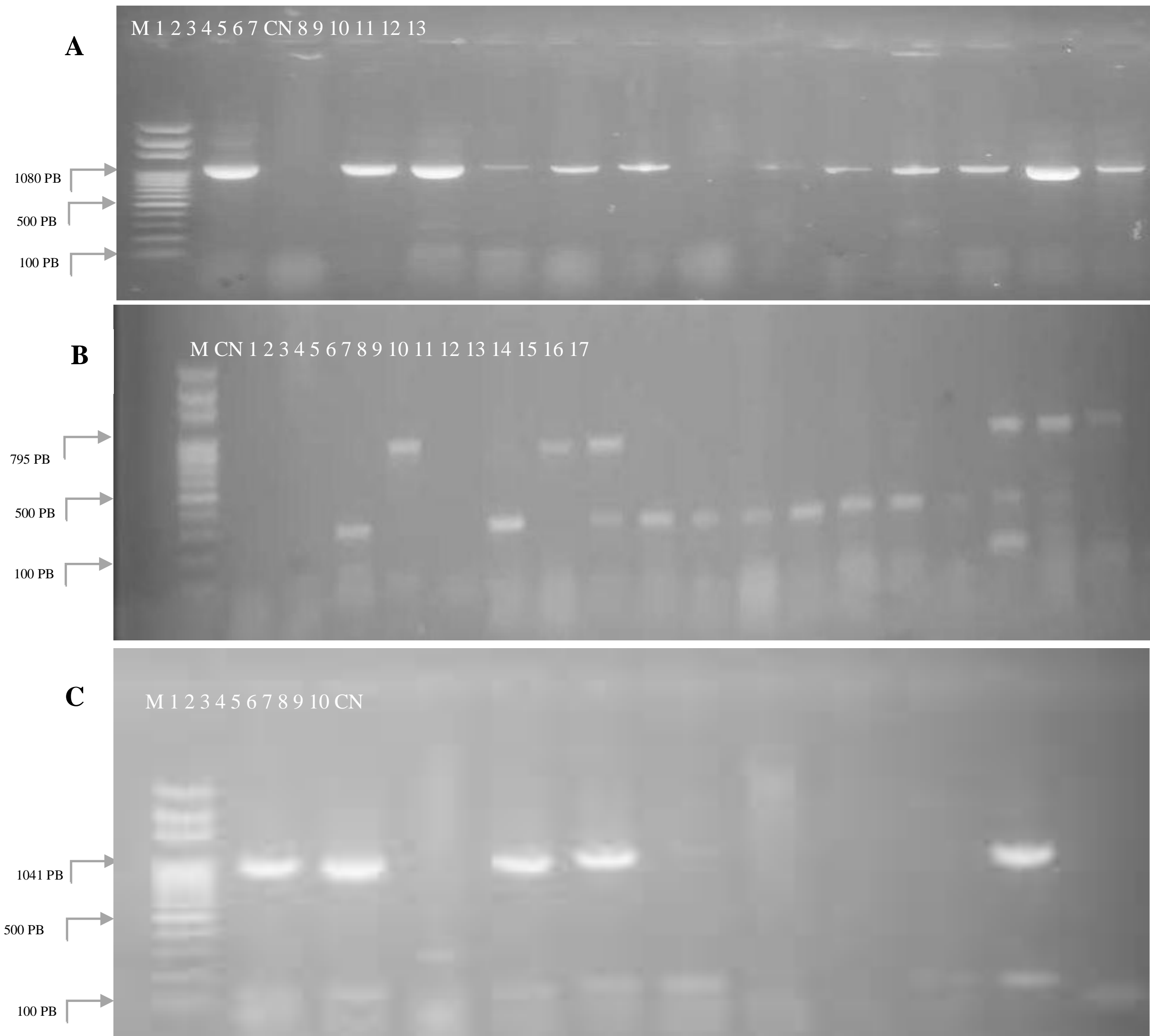
R: Resistance percentage (%), S: Sensitivity percentage (%), I: Intermediate percentage; Gentamicin: GEN, Aztreonam: AZT, Imipenem: IPM, Amoxicillin–clavulanic-acid: AMC, Sulfamethoxazole–trimethoprim: SXT, Ceftriaxon: CTR, Ciprofloxacin: CIP, Nalidixic acid: NA, Tetracycline: TE, Norfloxacin: NOR, Cefepime: CEF ,Azithromycin:AZM

Fig.3 ESBLs genes carriage rate in the different CHU departments



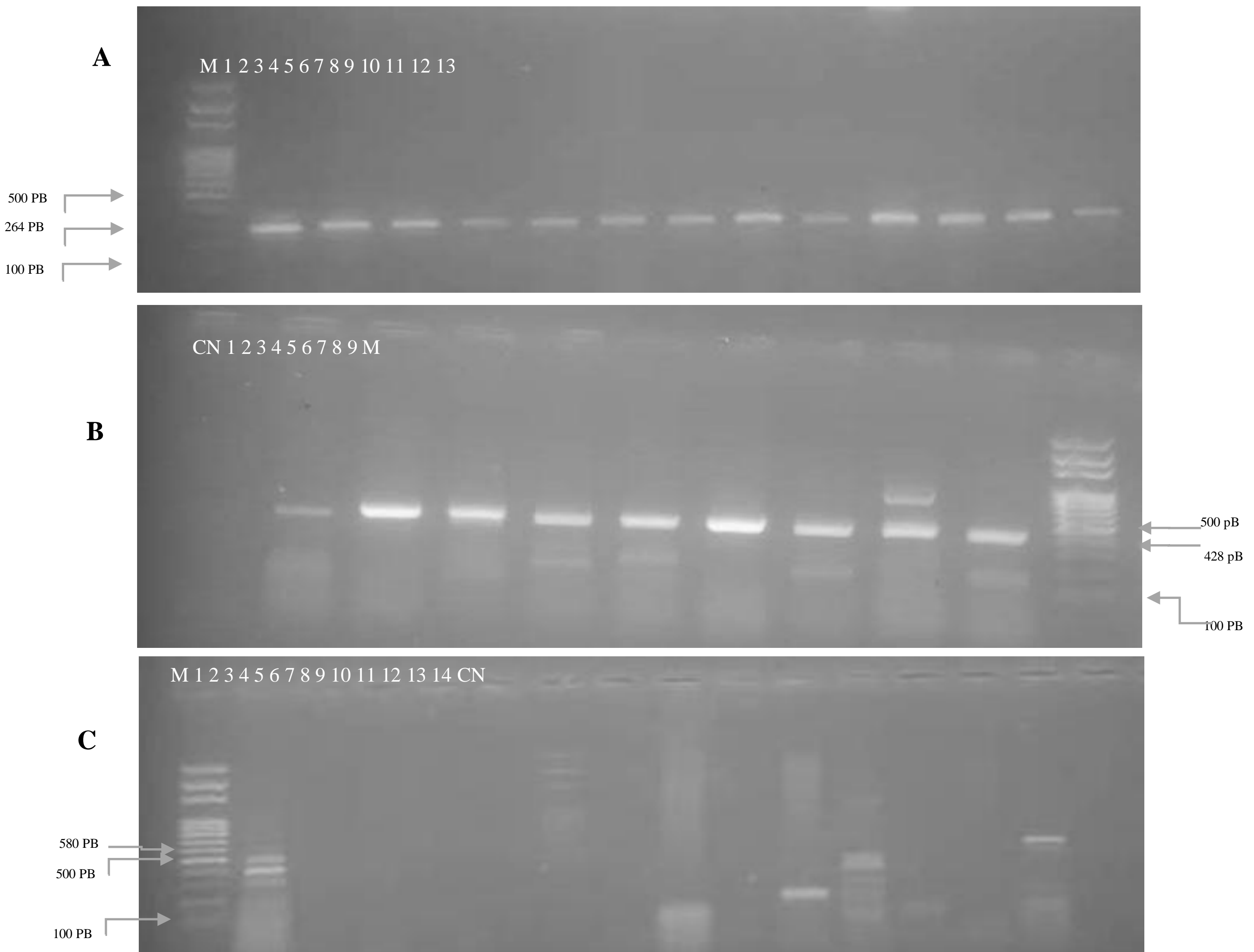
Legend :ID= Infectious Diseases; ORL= Otorhinolaryngology.

Fig.4 Profile of ESBLs genes amplified by PCR



A:PCR amplification of the *bla-TEM* gene (1080bp). Legend: M = 100 bp ladder molecular weight marker; CN = negative control, from 1 to 13 samples tested for the *TEM* gene; 1, 3 to 13 = positive samples; 2 = negative sample.
B:PCR amplification of the *bla-SHV* (795bp) gene. Legend: M = 100 bp ladder molecular weight marker; CN = negative control, from 3, 6, 7, 15, 16 and 17 samples tested positive for the *SHV*gene; 1, 2, 4, 5 and 8 to 14 = negative samples
C:PCR amplification of the *blaCTX-M* gene (1041bp). Legend: M = 100 bp ladder molecular weight marker; CN = negative control, from 1, 2, 4, 5 and 10 samples tested positive for the *CTX-M* gene; 3, 6, 8 and 9 = negative samples.

Fig.5 Profile of qnrs genes amplified by PCR



A :PCR amplification of *qnrB1 toqnrB6* genes (264 bp). Legend: M = 100 bp ladder molecular weight marker; 1 to 13 samples tested positive for *qnrB1 to qnrB6* genes.
B:PCR amplification of qnrS1 to qnrS6 genes (428bp). Legend: M = 100bp ladder molecular weight marker; CN = negative control, from 1 to 9 samples tested positive for the *qnrS1 to qnr S6* gene.
C :PCR amplification of genes from *E.coliqnrA1 to qnrA6* (580bp). Legend: M = 100bp ladder molecular weight marker; CN = negative control, from 1 and 14 samples tested positive for *qnrA1 to qnrA6* genes; 2 to 13 = negative samples.

This situation will result in increased infection treatment costs and treatment failures. The European Antimicrobial Resistance Surveillance Network reports that extended-spectrum β-lactamases (ESBLs) are of great clinical importance as they represent the main cause of multiple antibiotic resistance in *Enterobacteriaceae* (Inserm, 2020).

The resistance phenotypes of all the ESBL *E.coli* isolates studied were very diverse depending on the antibiotic family (fig2). Thus, 47.1% (32/68) of the isolates were resistant to all the antibiotics tested (El bouamri *et al.,* 2015). In addition to their strong resistance to beta-lactams, there was resistance to other families of antibiotics commonly used in

CHU-RN such as macrolides, quinolones, fluoroquinolones, cyclins and aminoglycosides. Similar observations have been made for beta-lactams, quinolones, fluoroquinolones, sulfonamides, cycles and aminoglycosides in Chad by Ouchar *et al.,* (2019) and by Linefiene *et al.,* (2017) and for beta-lactams, fluoroquinolones and sulfonamides in Benin (Anago *et al.,* 2015) in hospitals.

The high and variable resistance rates of MDR *E. coli* to widely prescribed antimicrobials in Chad observed in this study could be explained by the uncontrolled and inappropriate use of antimicrobials (distribution without a prescription, self-medication, etc.), to the availability of certain antimicrobial molecules in galenic form tablets and capsules (beta-lactams) on the one hand and non-compliance with regulations, sale, use of antibiotics in livestock sectors (Ministry of Public Health, 2018; Yandai *et al.,* 2014) and prescription of antibiotics with antagonistic effect of 'somewhere else. This worrying observation corroborates the high carriage (58.8%) of ESBLs phenotype observed in this study.

In this study, the *CTX-M* gene was mainly present in *E. coli* MDR strains with a carriage rate of 72.1%. Then come in second and third position the *TEM* and *SHV* genes with 54.4% and 16.2% respectively.

Indeed, as our study shows, other work had shown that the *CTX-M* gene was the most widespread and dominant gene throughout the world. The latter had been reported in Iran (88%) (Pourakbari *et al.,* 2019); in Brazil (90.32%) (Dexheimer *et al.,* 2015) and in France (96.4%) (Holstein *et al.,* 2010). Studies carried out in Senegal by Diagne *et al.,* (2018) on 32 strains of E. coli producing ESBL had shown that 90.62% of the strains had the *CTX-M* gene, followed respectively by the *TEM* (28%) and *SHV* (3.12%) genes. Similar results were observed in India (*CTX-M*: 87.5%, *TEM*: 68.4% and *SHV*: 3.1%) (Nisha *et al.,* 2017) and Brazil (*CTX-M*: 90, 32%, *TEM*: 70.96% and *SHV*: 56.45%) (Dexheimer *et al.,* 2015). In Chad, Ouchar *et al.,* (2019) obtained 97% of the genes from *CTX-M*.

The high carriage of the *blaCTX-M* gene in this study (72.1%) confirms its increasing prevalence among clinical strains of *E. coli* MDR as reported in numerous studies. Moreover, the high level of the *blaTEM* gene (54.4%) may be due to its presence in highly mobile genetic elements that promote its horizontal spread among bacteria worldwide (Daoud *et al.,* 2015). This prevalence also explains the strong resistance of the isolates to antibiotics (Cattoir, 2007b; Canton and Coque, 2006) observed in this study.

Our study showed that 16.2% of *E. coli* MDR isolates carried the *blaSHV* gene. Results inconsistent with those reported by Fody *et al.,* (2018) in Niger, Mensah *et al.,* (2016) in Ghana and Udomsantisuk *et al.,* (2011) in Thailand, where none of the isolates of *E. coli* harboring the *blaSHV* gene was observed. This difference could be due to the types of sampling and the different geographical areas.

The study showed that of the 68 strains analyzed, 41 carried the *qnrS* genes, i.e. a prevalence of 60%, of which 36 possess the *qnrB1 to B6* gene (i.e. 52.9%), 36 other strains have the *qnrS1 to qnrS6* gene (52.9%) and 2 isolates carry the gene *qnrA1 to A6* (2.9%). A predominance of *qnrB and qnrS* type genes was observed. However, no *qnrA* gene was detected by Abbasi and Ranjbar. (2018) in Iran.

The qnrs gene carriage obtained in this study is slightly higher than that in Nigeria (11%) Onanuga *et al.,* (2019) and Niger (7%) Aïssatou *et al.,* (2017), respectively for *qnrA* and *qnrB*. On the other hand, the *qnrS* carriage rate (82%) found by Aïssatou *et al.,* (2017) is superior to ours. Furthermore, the coexistence of two *qnrs* genes was detected in this study with a prevalence of 75.6% (31/41) of *qnrB1 to B6* + *qnrS1 to S6* and 2.4% (1/41) of qnrA1 *to A6* + *qnrB1 to B6*genes. The concurrent presence of three (3) *qnrS* genes in one isolate was not detected in this study.

The present study results showed that 36 (87.8%) of the strains harboring the *qnrS* genes were resistant to

at least one antibiotic belonging to the quinolone and fluoroquinolone family such as nalidixic acid (NA), ciprofloxacin (CIP) and norfloxacin (NOR), of which 24 (58.5%) presented the ESBL phenotype. A correlation was observed between the presence of qnr genes and the resistance to quinolones of *E. coli* MDR isolated ($P < 0.05$). These results are similar to those observed by Onanuga *et al.,* (2019) and Ogbolu *et al.,* (2011) in Nigeria and Namboodiri *et al.,* (2011) in Ghana. These different results clearly explain that the accumulation of mutations within the genes coding for the DNA gyrase and topoisomerase IV enzymes (the main targets of these antibiotics) remains the main mechanism for the acquisition of resistance to fluoroquinolones in *E. coli.* (Muylaert and Mainil, 2013; Drlica and Hooper, 2003).

In fact, multiple mutations are generally necessary to determine a clinical level of resistance to quinolones because wild *E. coli* are very sensitive to these molecules (Hooper, 1999). The acquisition of *qnrs* genes by ESBL-producing strains sensitive to quinolones could lead to the selection of ciprofloxacin and strains resistant to cephalosporins and an increase in resistance to fluoroquinolones (Salah *et al.,* 2019). The presence of these genes in patients is of concern, due to the potential spread of plasmids which may compromise therapeutic options and therefore a public health concern.

The global spread of beta-lactam resistance has become a major public health problem. In Chad, resistance to beta-lactams in *Enterobacteriaceae*, in particular E coli, is constantly increasing. This study showed that there is a high incidence of ESBLs and quinolone resistance genes among clinical isolates of *E.coli* isolated in the bacteriology laboratory of the UHC of N'Djamena. Several strains containing more than one ESBLs determinant and quinolone resistance genes were detected.

These determinants are at the origin of strains resistant to most beta-lactams and quinolones and challenge conventional treatments. Given these results, regular and active monitoring of multi-resistant bacteria in bacteriology laboratories and care units and better management of antibiotic consumption are necessary to prevent, monitor, reduce and control transmission and promote success in the treatment of these bacterial infections.

Conflict of interest

We declare that there is no conflict of interest.

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