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MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE AMONG CLINICAL MULTIDRUG RESISTANT *ESCHERICHIA COLI* IN TWO HOSPITALS OF NIAMEY, NIGER

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ABSTRACT

Objective: The aim of this study was to identify the multiple ESBL genes in Multidrug-resistant (MDR) *Escherichia coli* isolated in various biological samples in two hospitals of Niamey.

Methodology: A total of 195 multidrug-resistant *Escherichia coli* were included in the study. These isolates were tested using polymerase chain reaction (PCR) for detection of the presence of *bla* $_{CTX-M}$, *bla* $_{TEM}$, *bla* $_{SHV}$ and *bla* $_{OXA-1}$ beta-lactamase genes.

Results: A total of 27.7% of *Escherichia coli* isolates were ESBL producing strains. Globaly, the *bla* $_{TEM}$ gene was the most prevalent (70.3%) followed by *bla* $_{CTX-M}$ (43.1%), *bla* $_{OXA-1}$ (31.8%) and *bla* $_{SHV}$ (4.1%) genes. The four genes type of ESBL were founded simultaneously only in stool samples. Furthermore, none *bla* $_{SHV}$ gene was found in other samples type.

Conclusion: This study showed the presence of various ESBL genes among clinical MDR *Escherichia coli*. That is why a rational use of antibiotic and appropriate methods of screening ESBL genes in routine laboratories in Niger is needed to control the ESBL genes dissemination.

Keywords: MDR *Escherichia coli*, ESBL, *bla* genes, PCR, Niamey, Niger.

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CARACTERISATION MOLECULAIRE DES BETALACTAMASES A SPECTRE ETENDU CHEZ LES SOUCHES DE *ESCHERICHIA COLI* MULTI RESISTANTES DANS DEUX HOPITAUX DE NIAMEY, AU NIGER

Fody[1,2*], A. M. , Bagré[1,3], T. S. , Traoré[1], A. K., Yacouba [4], A., Dembelé[1], R., Boubou[2], L. , Inoussa[2], A., Sidikou,[5] R., Traoré,[1] A. S. , Gassama-Sow,[3] A. and Barro,[1] N.

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RESUME

Objectifs: Le but de cette étude était d'identifier les multiples gènes de BLSE chez les souches de *Escherichia coli* multi résistantes isolées de différents types d'échantillons biologiques dans deux hôpitaux de Niamey.

Méthodologie : Un total de 195 *Escherichia coli* multi résistants a été inclus dans l'étude. Ces isolats ont été testés par la réaction de polymérase en chaîne (PCR) pour détecter la présence des gènes *bla* $_{CTX-M}$, *bla* $_{TEM}$, *bla* $_{SHV}$ et *bla* $_{OXA-1}$.

Résultats : Au total, 27,7% des isolats de *Escherichia coli* multi-résistants étaient des souches productrices de BLSE. Globalement le gène *bla* $_{TEM}$ (70,3%) était le plus détecté suivi des autres gènes *bla* $_{CTX-M}$ (43,1%), *bla* $_{OXA-1}$ (31,8%) et *bla* $_{SHV}$ (4,1%). Notons que seul dans les échantillons de selles quatre types de gènes de BLSE ont été trouvés simultanément. Par ailleurs notons qu'aucun gène de type *bla* $_{SHV}$ n'a été trouvé dans les autres types d'échantillons.

Conclusion : Cette étude avait montré la présence de divers gènes de BLSE chez les souches cliniques de *Escherichia coli*. C'est pourquoi une utilisation rationnelle des antibiotiques et des méthodes appropriées de dépistage des gènes de BLSE dans les laboratoires sont nécessaires afin de contrôler la diffusion des gènes de BLSE.

Mots clés : *Escherichia coli* multi résistantes, BLSE, gènes *bla*, PCR, Niamey, Niger

INTRODUCTION

Resistance to beta-lactam antibiotics is an increasing problem and beta-lactamase production in Gram-negative bacteria, is one of the most common mechanisms of drug resistance. Beta-Lactamases, very diversified due to their continuous mutation, belong to the Ambler classes A (1) and are usually plasmid-encoded that also harbor resistant genes to other antimicrobial classes with resulting multidrug-resistant isolates (2, 3). These multidrug resistant (MDR) Gram negative bacilli belonging to the *Enterobacteriaceae* family are increasingly responsible for hospital infections (bacteremia urinary tract and intra-abdominal infection) in many countries (4, 5). The MDR extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* have become a concern in medical bacteriology regarding antimicrobial treatment and infection control in hospitals (6). Indeed, these enzymes (ESBL) have the capacity to hydrolyze extended-spectrum cephalosporins and monobactam antibiotics, but are inhibited by clavulanic acid (7, 8). The emergence of ESBL-producing isolates has important clinical and therapeutic implications. High prevalence of ESBL-producing *Enterobacteriaceae* has been reported in the literature for clinical samples from a variety of infection sites (9). In the past decade, there has been a significant increase in the prevalence of resistance to extended spectrum cephalosporin in *Escherichia coli* (10). From the clinical and epidemiological side, the ESBL-producing *Escherichia coli* represent a significant therapeutic challenge as they are resistant to all beta-lactam antibiotics currently available and other antibiotic families alternative (fluoroquinolones, cotrimoxazole, aminosides or tetracyclines), to except cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem and ertapenem) (11, 12). Different types of ESBLs have been found in different countries. There are currently three main ESBL (TEM, SHV and CTX-M) types, which are the most widespread and clinically relevant (13). The TEM and SHV types were first reported from *Klebsiella pneumoniae* in Western Europe. In the late 1990s, the prevalence of TEM and SHV had decreased, while that of CTX-M, especially associated to species *Escherichia coli* increased (10). The CTX-M type beta-lactamases represent a rapidly emerging group worldwide, which have been found in *Enterobacteriaceae*, particularly in *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis* and *Salmonella typhimurium* (14). Genotypic tests have the potential to accurately identify different genes encoding the ESBLs (15). Several reports have described the prevalence of ESBLs genes in the Middle East North Africa region and most of the Gulf Cooperation Countries (16). So the detection of specific genes by PCR and sequencing are commonly used for final confirmation of ESBL producers. The association of ESBLs and the presence of TEM, SHV, OXA and CTX-M-type enzymes have been investigated in many studies (17). However, there are insufficient scientific data on the ESBLs gene characterization available from Niger.

To our knowledge, no data are so far available on ESBL-producing *Escherichia coli* clinical isolates in Niger. The aim of this study was to screen the presence of *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{OXA-1}$ and *bla* $_{CTX-M}$ genes among clinical MDR *Escherichia coli* isolated from various biological samples in Niamey.

MATERIALS AND METHODS

Samples collection

The present prospective study was conducted from March 2014 to June 2016 in the medical biology laboratory of "hôpital national de Niamey" and "hôpital national Lamordé" in Niamey, Niger. The isolates were obtained from various samples. Multidrug resistant *Escherichia coli* isolates, were isolated from stool (n=49), urinary tract infection

(n=134), pus (n=7), blood (n=4), and vaginal swabs (n=1) samples.

Bacterial identification and antibiotic susceptibility testing

Isolation, identification, antimicrobial susceptibility testing of isolates and phenotypic characterization of ESBL were described in our previous study (18). All isolates included in this study were multidrug resistant.

Preparation of DNA

Total DNA was extracted as previously described by Steward (19) using boiling process. Samples were cultured onto Bromo-Cresol Pourpre (BCP) agar and incubated at 37°C for 24 hours. For DNA extraction, two loopful of each strain were homogenized into 250 µl of sterile water. The mixture was boiled for 10 min and centrifuged for 10 min. After 5 min at room temperature, the supernatant was collected and used for the PCR reactions.

Detection of *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{OXA-1}$ and *bla* CTX-M genes.

The beta-lactamase genes *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{OXA-1}$, and *bla* $_{CTX-M}$ were detected by PCR using specific primers (Table 1). PCR mixtures were prepared by using 2.5 µl of template DNA, 4.0 µl of Master Mix PCR (Solis BioDyne, Estonia), 0.5 µl of Forward primer, 0.5 µl of Reverse primer in a final volume of 20 µl. Amplification conditions were 94°C for 5 min following 35 cycles of 94°C for 30 Sec, 55°C for 60 Sec, and 72°C for 60 Sec with a final extension at 72°C for 10 min. PCR amplified fragments were separated by agarose gel (1% w/v) electrophoresis in 1 $_X$ TAE buffer and visualized under UV light.

TABLE 1: BETA-LACTAMASE GENES PRIMERS

Genes	Primers	Sequences (5′-3′)	Amplicon size (bp)
bla $_{TEM}$	TEM-F	ATAAAATTCTTGAAGACGAAA	1080
	TEM-R	GACAGTTACCAATGCTTAATCA	
bla $_{SHV}$	SHV-F	TTATCTCCCTGTTAGCCACC	650
	SHV-R	GATTTGCTGATTTCGCTCGG	
bla $_{OXA-1}$	OXA-1-F	ATGAAAAACACAATACATATC	890
	OXA-1-R	AATTTAGTGTGTTTAGAATGG	
bla $_{CTX-M}$	CTX-M-F	GTTACAATGTGTGAGAAGCAG	593
	CTX-M-R	CCGTTTCCGCTATTACAAAC	

Ethical considerations

All biological specimens were collected as part of the routine clinical management of patients. The study was approved by the medical establishment committee of hôpital national de Niamey" and "hôpital national Lamordé" in Niamey and permission to conduct the study was obtained from the hospital authorities of Niger.

Statistical analysis

Data analysis was carried out using Microsoft Excel 2013 and Med Cal version 11.0.1.0. $P < 0.05$ was considered to be statistically significant.

RESULTS

A total of 195 MDR *Escherichia coli* were included in this study. Among these isolates, 27.7% (54/195) were positive for the phenotypic character of extended spectrum beta-lactamases. Molecular analysis had shown the presence of various beta-lactamase genes. The genes identified by PCR method using specific primers i.e TEM, SHV CTX-M and OXA-1 on agarose gel were shown in Figures 1 and 2.

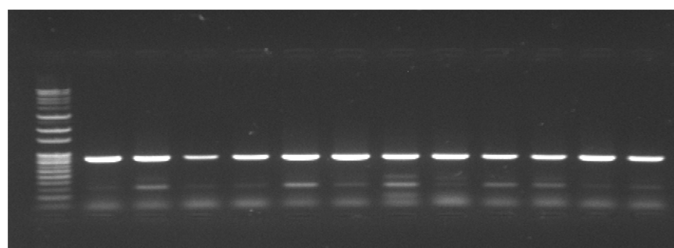

FIGURE 1: PCR AMPLIFICATION OF *E. COLI bla* $_{TEM}$ GENES

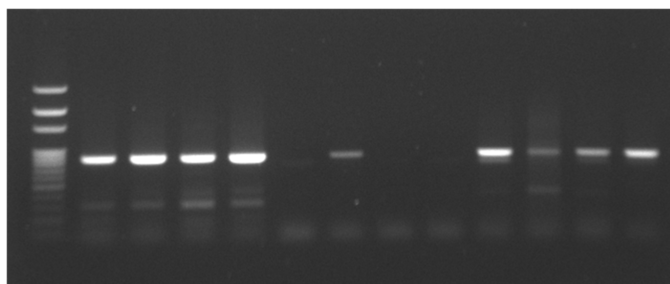

FIGURE 2: PCR AMPLIFICATION OF *E. COLI bla* $_{CTX-M}$ GENES

Forty nine (49) *MDR Escherichia coli* isolates from stool samples were tested for ESBL resistant genes. Overall, *bla* $_{TEM}$ gene was the most prevalent (93.9%) followed by *bla* $_{OXA-1}$ (71.4%), *bla* $_{CTX-M}$ (65.3%) and *bla* $_{SHV}$ (16.3%) ($P < 0.0001$) (Table 2). All the fourteen (14) ESBL isolates positive by phenotypic method, were positive for the *bla* $_{TEM}$ gene (100%), 85.7% for the *bla* $_{CTX-M}$ gene, 85.7% for the *bla* $_{OXA-1}$ gene and 21.4% for *bla* $_{SHV}$ gene ($P < 0.\ 0001$) as indicated in Table 3. Globally, *bla* $_{SHV}$ gene was only detected in stool samples.

TABLE 2: DISTRIBUTION OF BETA-LACTAMASE GENES FROM MDR *E. COLI*

	Clinical source				
ESBL genes	**Stools N=49**	**Urine N=134**	**Pus N=7**	**Blood N=4**	**Vaginal swabs N=1**
bla $_{TEM}$ **n (%)**	46 (93.9)	82 (61.2)	4 (57.1)	4 (100)	1 (100)
bla $_{SHV}$ **n (%)**	8 (16.3)	0 (0)	0 (0)	0 (0)	0 (0)
bla $_{OXA-1}$ **n (%)**	35 (71.4)	20 (14.9)	5 (71.4)	2 (50)	0 (0)
bla $_{CTX-M}$ **n (%)**	32 (65.3)	45 (33.6)	5 (71.4)	2 (50)	0 (0)

About the 134 MDR *Escherichia coli* isolated from urine samples, *bla* $_{TEM}$ gene was the most prevalent (61.2%), followed by *bla* $_{CTX-M}$ (33.6%) and *bla* $_{OXA-1}$ (14.9%) respectively (Table 2). Out of the thirty-seven (37) ESBL isolates positive by phenotypic method, 27 (73.0%) were positive for *bla* $_{TEM}$ gene, 13 (35.1%) for *bla* $_{CTX-M}$ gene, and 3 (8.1%) for *bla* $_{OXA-1}$ gene ($P < 0.0001$) (Table 3). None of the isolates was positive for *bla* $_{SHV}$ genes.

The study of seven (7) MDR *Escherichia coli* isolated from pus samples, showed the presence of *bla* $_{CTX-M}$ gene, *bla* $_{OXA-1}$ gene and *bla* $_{TEM}$ gene with a prevalence of 71.4%, 71.4% and 57.1% respectively. The *bla* $_{SHV}$ gene was not found in any of the isolates (Table 2). There was no significant difference between the expressing of these genes in pus samples ($P = 0.3735$). Only the *bla* $_{CTX-M}$ gene (50%) and *bla* $_{OXA-1}$ gene (50%) were found in *Escherichia coli* isolates positive for ESBL by phenotypic method (Table 3).

In the four (4) MDR *Escherichia coli* isolated from blood culture samples, *bla* $_{TEM}$, *bla* $_{OXA-1}$ and *bla* $_{CTX-M}$ genes were found in 100%, 50%, and 50% respectively (Table 2). However, *bla* $_{TEM}$ (100%), *bla* $_{OXA-1}$ (100%) and *bla* $_{CTX-M}$ (100%) were found in *Escherichia coli* isolates producing ESBL by phenotypic method (Table 3).

Concerning vaginal swabs, only *bla* $_{TEM}$ gene was found in *Escherichia coli* isolates as indicated in Table 2 and Table 3.

TABLE 3: DISTRIBUTION OF ESBL GENES FROM MDR *E COLI* POSITIVE TO ESBL

ESBL genes	Stool ESBL + N=14	Stool ESBL – N=35	Urine ESBL + N=37	Urine ESBL- N=97	Pus ESBL+ N=2	Pus ESBL - N=5	Blood ESBL+ N=1	Blood ESBL - N=3	Vaginal swabs ESBL+ N=0	Vaginal swabs ESBL - N=1
bla $_{\mathbf{TEM}}$ **n (%)**	14 (100)	32 (91.4)	27 (73.0)	55 (56.7)	0 (0)	4 (80)	1 (100)	3 (100)	0 (0)	1 (100)
bla $_{\mathbf{SHV}}$ **n (%)**	3 (21.4)	5 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
bla $_{\mathbf{OXA\text{-}1}}$ **n (%)**	12 (85.7)	23 (65.7)	3 (8.1)	17 (17.5)	1 (50)	4 (80)	1 (100)	1 (33.3)	0 (0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **n (%)**	12 (85.7)	20 (57.1)	13 (35.1)	32 (33.0)	1 (50)	4 (80)	1 (100)	1 (33.3)	0 (0)	0 (0)

In this study, there was co-existence of ESBL genes in some *Escherichia coli* isolates. The co-existence of *bla* $_{TEM}$ and *bla* $_{CTX\text{-}M}$ genes was found in stool, blood, pus and urine samples in 63.3%, 50%, 42.9%, 22.4% (P = 0. 0002) respectively, while *bla* $_{CTX\text{-}M}$ gene and *bla* $_{OXA\text{-}1}$ gene were detected in 57.1%, 50.0%, 71.4%, and 4.5% respectively. The co-existence of three ESBL genes belonging to group *bla* $_{CTX\text{-}M}$ + *bla* $_{TEM}$ + *bla* $_{OXA\text{-}1}$ genes was observed in 57.1%, 50%, 28.6% and 3% respectively from stool, blood, pus and urine samples (P < 0. 0001). However, other multiple co-detection of ESBL genes belonging to different groups: *bla* $_{CTX\text{-}M}$ + *bla* $_{SHV}$ (14.3%), *bla* $_{CTX\text{-}M}$ + *bla* $_{TEM}$ + *bla* $_{SHV}$ (12.2%), *bla* $_{CTX\text{-}M}$ + *bla* $_{SHV}$ + *bla* $_{OXA\text{-}1}$ (14.3%) were observed in stool samples. A combination of four genes (12.2%), *bla* $_{CTX\text{-}M}$ + *bla* $_{TEM}$ + *bla* $_{SHV}$ + *bla* $_{OXA\text{-}1}$ genes was found only in *Escherichia coli* isolated from stool samples (Table 4).

TABLE 4: DISTRIBUTION OF VARIOUS ESBL GENES COMBINATIONS ACCORDING TO CLINICAL SAMPLES

ESBL genes combination	Stool N=49	Urine N=134	Pus N= 7	Blood N=4	Vaginal swabs N=1
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{TEM}}$ **n (%)**	31 (63.3)	30 (22.4)	3 (42.9)	2 (50.0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{SHV}}$ **n (%)**	7 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{OXA\text{-}1}}$ **n (%)**	28 (57.1)	6 (4.5)	5 (71.4)	2 (50.0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{TEM}}$ **+** ***bla*** $_{\mathbf{SHV}}$ **n (%)**	6 (12.2)	0 (0)	0 (0)	0 (0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{TEM}}$ **+** ***bla*** $_{\mathbf{OXA\text{-}1}}$ **n (%)**	28 (57.1)	4 (3.0)	2 (28.6)	2 (50.0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{SHV}}$ **+** ***bla*** $_{\mathbf{OXA\text{-}1}}$ **n (%)**	7 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)
bla $_{\mathbf{CTX\text{-}M}}$ **+** ***bla*** $_{\mathbf{TEM}}$ **+** ***bla*** $_{\mathbf{SHV}}$ **+** ***bla*** $_{\mathbf{OXA\text{-}1}}$ **n (%)**	6 (12.2)	0 (0)	0 (0)	0 (0)	0 (0)

DISCUSSION

Over the last decades, many studies have demonstrated the presence of ESBL resistance genes in bacteria isolated from patients during various infections (20 -22). This study reported the results of molecular characterization of ESBL resistance genes in MDR *Escherichia coli* strains isolated in two hospitals of Niamey, Niger.

The study of *Escherichia coli* producing ESBL (14 isolates) isolated from stool samples, had shown that the most frequent ESBL gene was *bla* $_{TEM}$ followed by *bla* $_{CTX\text{-}M}$, *bla* $_{OXA\text{-}1}$ and *bla* $_{SHV}$ genes. However, previous studies from Qatar reported a higher prevalence of *bla* $_{CTX\text{-}M}$ gene (66.1%), *bla* $_{SHV}$ (53.2%) and *bla* $_{TEM}$ (40.4%) (9). In another study, the predominant ESBL genes, were *bla* $_{CTX\text{-}M}$, *bla* $_{TEM}$ and *bla* $_{SHV}$ (23). Other studies in clinical isolates showed that *E. coli* harbored *bla* $_{SHV}$ gene (88.9%), *bla* $_{OXA}$ (44.4%) and *bla* $_{CTX\text{-}M}$ genes (44,4), and *bla* $_{TEM}$ gene (22.2%) (24). These results were contradictory to our results. The difference would be due to the sampling from different hospitals and from different geographic locations, whereas in our study, samples were collected only from two hospital setting.

This study revealed that, out of the 37 *E coli* producing ESBL isolated from urine samples, the percentage of *bla* $_{TEM}$ was 73.0% followed by *bla* $_{CTX\text{-}M}$ (35.1%) and *bla* $_{OXA\text{-}1}$ (8.1%). Karimian et al. in Iran, reported similar data: *bla* $_{TEM}$ and *bla* $_{CTX\text{-}M}$ genes were observed in 71.2% and 37.3% of *E. coli* isolates, respectively (25). In our study, none of the isolate harbored *bla* $_{SHV}$ gene. A similar finding was reported

from Thai studies. (26). But our results contradicted those reported in some studies conducted by Egyptian and Indian authors, in which *bla* $_{SHV}$ gene was detected among urinary *E. coli* isolates (27, 28). Also, in East Africa, ESBL genes, *bla* $_{CTX-M}$, *bla* $_{TEM}$, *bla* $_{SHV}$ and *bla* $_{OXA}$ were found frequently (23). Thus, this required a better understanding of the ESBL genes in order to give useful data about their epidemiology.

In this study, only 4 MDR *Escherichia coli* isolated from blood culture were tested for ESBL genes. Thus, the co-existence of *bla* $_{TEM}$, *bla* $_{OXA-1}$ and *bla* $_{CTX-M}$ was only seen in one strain producing EBSL. Our results correlated with other results reported in some African's countries which showed a significantly lower proportion of ESBL in blood cultures than in other specimens (29). The previous finding was contradicted with the finding of a Burkina Faso study which had shown the highest proportion of ESBL genes in blood samples (30). Whereas, none of ESBL-producing isolates carried *bla* $_{SHV}$ or *bla* $_{CTX-M}$ genes as reported in Ghana (31). This discrepancy can be explained by differences in the antimicrobials use between countries.

The present study reported the multiple co-existence of ESBL genes. Thus, 63.3% of isolates from stool samples harbored two ESBL genes belonging to groups of *bla* $_{CTX-M}$ + *bla* $_{TEM}$, followed by blood samples (50%), pus samples (42.9%) and urine samples (22.4%). The greatest frequency of beta lactamase gene in *E. coli* samples was related to TEM/CTX-M in previous study as reported in Iran (32). The co-existence of *bla* $_{TEM}$ + *bla* $_{CTX-M}$ genes and *bla* $_{SHV}$ + *bla* $_{CTX-M}$ genes in two strains were reported in India (33).

This study revealed that the co-existence of three (3) ESBL genes (*bla* $_{CTX-M}$ + *bla* $_{TEM}$ + *bla* $_{OXA-1}$) was observed in 57.1%, 50%, 28.6% and 3% of the *Escherichia coli* isolates respectively in stool, blood, pus and urine samples. This result was also found in another study conducted in Bangladesh (24). These results indicated that ESBL-producing *Escherichia coli* frequently harbored more than one beta-lactamase genes.

Overall, only isolates from stool samples harbored simultaneously four (4) ESBL genes (*bla* $_{CTX-M}$ + *bla* $_{TEM}$ + *bla* $_{SHV}$ + *bla* $_{OXA-1}$). According to a previous study in Lebanon, 15.9% of *Escherichia coli* harbored the four ESBL genes (34). The genetic diversity of *bla* genes in isolates, suggested that resistance genes can easily move from one specie to another with the possibility of easy interspecies transfer.

This study was conducted in two hospitals of Niamey; it may reflect the local antibiotic resistance patterns and this may be a limitation.

CONCLUSION

This first study in Niger showed a high incidence of ESBL resistance genes among clinical *Escherichia coli* isolates from two laboratories in two hospitals (Hôpital National de Niamey and Hôpital National Lamordé) of Niamey. Many isolates harboring more than one ESBL resistance genes, were also detected. Therefore, immediate implementation and recommendations for antimicrobial management and infection control measures were necessary to prevent the spread of these genes across the whole country.

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