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Original Research Paper

Bacteriology

MOLECULAR ASPECT OF MULTI-DRUG RESISTANT EXTENDED SPECTRUM BETA-LACTAMASES (ESBL) SECRETING ENTEROBACTERIACEAE STRAINS ISOLATED AT BOTH BACTERIOLOGY LABORATORY OF THE TEACHING HOSPITAL ARISTIDE LE DANTEC AND AT THE PRIVATE MEDICAL BIOLOGY LABORATORY BIO-24 OF DAKAR IN SENEGAL

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ABSTRACT The objective of this work was to study at a molecular level the resistance genes of the ESBL-producing enterobacterial strains isolated at the Laboratory of Bacteriology and Virology of the CHNU Aristide Le Dantec and at the private laboratory Bio-24.The CTXM genes of group 1 (CTXM1) and the CTXM of group 9 (CTXM9) encoding for ESBL, OXA1 and CMY1 encoding for penicillinase and cephamycinase respectively were searched by PCR followed by nucleotide sequencing. The resulting sequences were submitted to the NCBI-Blast site for gene typing.The CTXM genes in Group1, CTXM in Group 9, OXA1 and CMY1 were found in 96%, 18%, 85% and 56% of the strains, respectively. Nucleotide sequencing of 15 CTXM1 allowed the CTXM15 to be recovered.These resistance genes can be associated with those of quinolones and/or carbapenems and thus lead to a therapeutic impasse, hence the need to strengthen the fight against antibiotic resistance.

KEYWORDS : *Enterobacteria*, ESBL, CTXM1, CTXM9

INTRODUCTION

Urinary tract infections (UTIs) are the most important cause of bacterial infections today, with a global annual incidence of around 250 million cases. Approximately 50% of women will have at least one episode of UTI in their lifetime.UTIs caused by Gram-negative, antibiotic-resistant bacteria are a growing concern due to limited treatment options and only a few bacterial species are considered to be true uropathogens (*Escherichia coli, Staphyllococcusspp, Proteus mirabilis, Klebsiella pneumoniae*) (**12,2**). Excessive and/or inappropriate use of antibiotics in the treatment of urinary tract infections leads to the emergence and spread of multidrug-resistant uropathogenic bacteria, primarily through the production of ESBLs(**10**).Research has shown that 44.1% of urinary tract infections (UTIS) caused by E. coli are attributable to extended spectrum beta-lactamase (ESBL) producers(9).ESBL provide enterobacteria with resistance to all beta-lactam antibiotics with the exception of cephamycins and carbapenems (**4**). Transmission, mainly plasmid, of genes coding for ESBL is responsible for the release and rapid increase of ESBL-producing enterobacteria worldwide **(1)**. This resistance to beta-lactam strains may be associated in some cases with resistance to quinolones, which are typically used for the treatment of severe urinary tract infections. This can lead to a therapeutic impasse, resulting in increased morbidity and mortality.

In Senegal the resistance genotype of ESBL-producing enterobacteria is not well known. It is in this context that we propose to characterize at the molecular level, resistance genes of strains of enterobacteriauropathogenic ESBL secretions.

METHODOLOGY

Sample collection

This prospective study was carried out at both bacteriology laboratory of the Aristide Le Dantec teaching hospital and at the medical laboratory Bio-24 from 2016 to 2018.

Enterobacteria strains suspected to be ESBL, isolated during this period from urine were transplantedin 15% glycerol supplemented brain broth and stored at -20° pending handling.

Confirmation of identity of strains and ESBLphenotype

The strains were first re-isolated to verify the purity of the collections and their identity was determined using Api 20E. The production of ESBL was confirmed by the agar diffusion method while respecting the recommendations of the French microbiology society's (CA-SFM) 2018 Antibiogram Committee. The detection of the secretion of these ESBL was to demonstrate a synergy between clavulanic acid inhibitor and two third-generation cephalosporins (Cefotaxime and ceftazidim) (Figure 1).

Gene detection (Group 1 CTXM, Group 9 CTXM, OXA1 and CMY1)

Bacterial DNA was extracted using the Qiagen kit (Qiagen, Germantown, USA)according tomanufacturer's instructions. Polymerase Chain Reaction (PCR) were used to amplify *bla*OXA1, *bla*CMY1, *bla*CTXMencoding for penicillinase,cephamycinase, andESBLrespectively.The amplification program was one cycle of 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 57°C for 1 minute and 72°C for 1 minute and one cycle of 72°C for 7 minutes. PCR products were revealed on gel stained by ethidium bromide. The characteristics of the primers used, and the reaction mixture are described in the tables below (Table 1, 2,).

Nucleotide sequencing

Nucleotide sequencing was also performed using the ABI 3130 XL sequencer. The resulting sequences were edited and corrected with the DNA STAR software. These sequences were then typed at the NCBI database.

RESULTS

The study included 83 strains of uropathogenicen terob acteriawithextended spectrum beta-lactamases (ESBL) secretions. Of these 83 strains, 43 were isolated at Bio-24 and 40 at the CHNU Le Dantec bacteriology laboratory. Isolated strains were: *Klebsiellapneumoniae* (n=13, 15.66%), *Escherichia coli* (n=68, 81.9%), *Citrbacterkoseri* (n=1, 1.4%), and*Enterobacter cloacae* (n=1, 1.4%). Strains were isolated in outpatient (78.3%) and hospitalized patients (21.7%). The confirmations of the identity and phenotype ESBL of the strains were all conclusive.

Following the PCR reactions, CTXM1, CTXM9, OXA1 and CMY1 were found in 96.4% (80/83), 18.07% (15/83), 85.5 (71/83) and 56.6% (47/83) of the strains, respectively (Figure 2).

The nucleotide sequencing of 15 CTXM1s yielded only CTXM15s

Group 1 CTXM, OXA1 and CMY1 were widely distributed in all species. CTX-M9 was found in only 22% of *E.coli* strains (Table 3).

The distribution of CTXM1 and Group 9 was almost uniform in both inpatients and outpatients (Table 4).

The CTXM1 gene was mainly found associated with OXA1 (82%) and CMY1 (47%) (table5).

DISCUSSION

The objective of the study was to describe the aspect of beta-lactam resistance of uropathogenic*enterobacteria* isolated in a private laboratory and an University teaching hospital in Dakar.A total of 4 genes (CTXM of group 1, CTXM of group 9, OXA 1 and CMY 1) of beta-lactam resistance were detected in ESBLstrains. For the genes that encode for ESBL, CTX-M from Group 1 was predominant in the study especially CTX-M-15. CTXMs are non-TEM non-SHV ESBL. They were first described in Germany in strains of *E. coli*.These enzymes are characterized by their preferential activity for cefotaxime rather than ceftazidim **(15)**. Shortly after being described, CTXM spread worldwide until it became the predominant ESBL in *enterobacteria***(6).** Some authors even report that these CTXM genes are endemic in most European, Asian and South American countries, with high prevalence rates ranging from 30 to 90% for *E.coli* **(13)**. Among the CTXM, the CTXM15 belonging to group 1 and the CTXM14 are the most found in humans, animals and the environment, worldwide **(5, 11)**. This CTXM15 was described in Senegal in the study of Diaand al in 2016 with a prevalence of 90.63% **(7)**. It has also been found in other studies in Senegal such as that of Moquet and al in 2011 **(14)**, Diene and al in 2014 at a strain of *Morganellamorganii* at the main hospital in Dakar **(8)**.

In our study, CTXM1s were often found in association with OXA1 (82%). Barguagui and al found CTX-M-15 associated with OXA 1 in 10 strains (100%) of *E. coli***(3)**. The same association was also described in Senegal with a prevalence of 63.33% by Dia and al **(7)**.

CONCLUSION

These results show the multigenic aspect of beta-lactam resistance. These resistances associated with those of quinolones can lead to a therapeutic deadlock, hence the need to find ways of combating them. The good use of antibiotics is one of the best ways of responding.

In perspective a larger study should be done for the typing of all resistance genes (CTXMs).

Table 1: Characteristics of primers used.

Genes	sequence of primers	size	Annealing
blaOXA1	F ATGAAAAACACAATACATATC	890	55
	R AATTTAGTGTGTTTAGAATGG		
blaCTXM1	F GGTTAAAAAATCACTGCGTC	800	57
	R TTGGTGACGATTTTAGCCGC		
blaCTXM9	F ATGGTGACAAAGAGAGTGCA	1000	57
	R CCCTTCGGCGATGATTCTC		
blaCMY1	F GACAGCCTCTTTCTCCACA	1000	57
	R TGGAACGAAGGCTACgTA		

Table 2: Mixing for PCR.

Reagents	Volume for one reaction
H_2O RNaseDNase free qsp 48 µl	29,85 µl
Taq Buffer 5 X Promega	10µl
$MgCl_2$ 25 mM	5µl
Forward primer 20 µM	0,5µl
Reverse primer 20 µM	0,5µl
dNTPs (5 mMchacun)	2µl
GoTaq Flexi Pol (5 U/µl) Promega	0,15µl
DNA extract	2 µl

Table 3: Distribution of strains by bacterial species.

	E. coli	*K. pneumoniae*	*C. koseri*	*E. cloacae*
CTX-M1	97% (66/68)	92% (12/13)	100% (1/1)	100% (1/1)
CTX-M9	22%	0	0	0
OXA1	88% (60/68)	69% (9/13)	100%	100%
CMY1	58% (40/68)	54% (7/13)	0	0

Table 4: Distribution of CTX-M according to hospitalized and external characteristics.

	CTX-M1	CTX-M9
Isolated strains in hospitalized patients	94,44% (17/18)	17% (11/65)

Table 5: Association of CTX-M-1 and other genes.

Prevalence	Genes
15% (13/83)	CTX-M-1, CTX-M-9, OXA1, CMY1
47% (39/83)	CTX-M-1, CMY1
82% (68/83)	CTX-M-1, OXA1

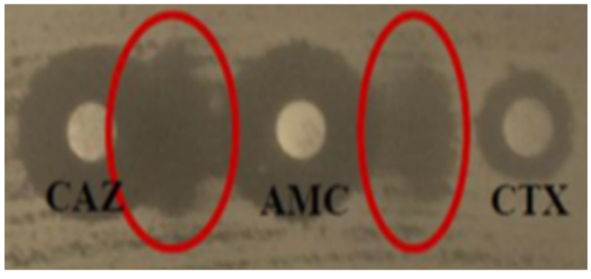

Figure 1: image of synergy between beta-lactamase inhibitor and third generation cephalosporins

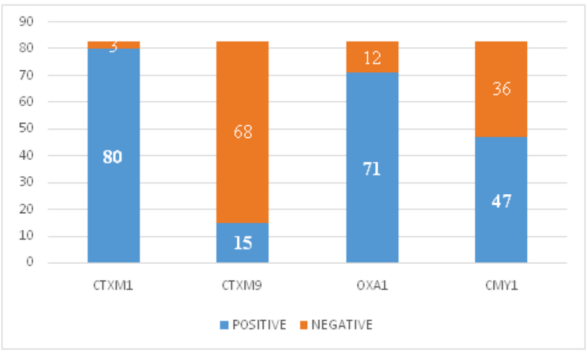

Figure 2: overall results of PCR resistance gene research

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