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First report of the types TEM, CTX-M, SHV and OXA-48 of beta-lactamases in *Escherichia coli*, from Brazzaville, Congo

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The objective of this study was to evaluate the emergence of beta-lactam resistance related to extended spectrum beta-lactamase (ESBL) encoding genes (TEM, SHV, CTX-M-1 and OXA-48) in *Escherichia coli* from Brazzaville. In the period between January 2016 and May 2017, 89 strains in *E. coli* were isolated from hospitalized patients, outpatients and domestic sewage. The *E. coli* strains were identified by the API 20E system. An antibiogram was performed on isolated trains by the disk diffusion method. The ESBL phenotype was detected using the synergistic technique according to CA-SFM (ESBL). Genes were detected using PCR and characterized by sequencing. An overall prevalence of 48.31 (43/89) and rates of 74.42, 23.26, 9.30, and 6.97% for blaTEM genes blaCTX-M-1, blaSHV and blaOXA-48 were detected. 25.58% were community strains and 74.42% hospital. The majority were isolated urine (81.40%) and the urology department was more represented. Except for imipenem, colistin strains of ESBL showed high resistance to other antibiotics than non-yielding ones (p <0.05). This high prevalence of detected ESBL, the high level of resistance to antibiotics raises fears of a high risk of multidrug-resistant bacteria and call on the authorities for a policy of monitoring resistance.

Key words: *Escherichia coli*, extended spectrum beta-lactamase (ESBL), carbapenemases, Brazzaville.

INTRODUCTION

Infectious diseases remain a major public health problem, especially in Africa (Ouedraogo et al., 2017). Bacterial infections are the most dominant and are responsible for most of the nosocomial and community infections. Based on their wide spectrum, mechanism of action, low toxicity and low cost, antibiotics including the beta-lactam family such as penicillins, cephalosporins and carbapenems are the most commonly used for treating infection diseases

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(Livermore, 1995). Over the past year, a significant increase in antibiotic resistance to Enterobacteriaceae has been documented as well. According to the WHO (2014) report on global surveillance of antimicrobial resistance, antibiotic resistance is a serious public health problem. In competitive environments, microorganisms including *Streptomyces*, *Nocardia*, *Actinomadura*, and *Penicillium* species, can produce natural molecules allowing the inhibition of other competitors (Baquero and Coque, 2013). To avoid self-toxicity, these microorganisms have intrinsically developed antibiotic resistance. Enterobacteriaceae are classified in different groups according to antibiotic resistance (Philippon et al., 1989). Indeed, the abuse of antimicrobials led to the selection of multi-resistant strains, particularly those resistant to beta-lactams isolated from urinary infections, pulmonary infections, and septicemia, with an increasing frequency in hospitals (Lucet et al., 1996). In Enterobacteriaceae, different enzymes are produced depending on their membership in the different groups. The most commonly cited are chromosomal penicillinase, chromosomal cephalosporinase, chromosomal cefuroximase and chromosomal extended spectrum beta-lactamase (ESBL). *Escherichia coli* is a dominant Enterobacteriacea found in the human commensal flora, especially in the digestive tract (Ahoyo et al., 2007).

ESBLs are serine-type inactivation enzymes. With the exception of OXA SSBLs (class D), ESBLs are class A beta-lactamases according to the Ambler classification. More than 300 ESBLs have been described to date (Elhani, 2012). They are characterized by a great diversity. The majority of ESBLs are derived from TEM and VHS enzymes but new ESBLs have been described such as CTX-M (for cefotaximase), OXA (for oxacillinase), PER (for *Pseudomonas aeruginosa*), VEB (for Vietnam ESBL), GES (for Guyana extended-spectrum beta-lactamase), TLA (for TEM Like activity), BES (for Brazilian extended spectrum beta-lactamases), SFO (for *Serratia fonticola*) and FEC (fecal *E. coli*) (Elhani, 2012).

ESBLs constitute a large family of bacterial enzymes belonging to classes A, C and D based on the Ambler classification (Hall and Barlow, 2018), and capable of hydrolyzing penicillins, cephalosporins and aztreonam. They do not hydrolyze carbapenems or cephamides. This capacity to hydrolyse antibiotics has also been demonstrated in *E. coli* and *Klebsiella* species strains. Since the discovery of ESBL-producing bacteria in 1990, most of those detected are the conventional TEM and SHV types, which spread predominantly in hospital settings, including *Klebsiella pneumoniae* and *Enterobacter* species; they are associated with nosocomial outbreaks in intensive care units (Hall and Barlow, 2018). Recently, a new blaCTX-M gene encodes CTX-M enzyme has been detected. Community strains of *E. coli*, mainly responsible for urinary tract infections, could express blaCTX-M genes encoding CTX-M enzyme (Elhani, 2012)

The *blaTEM*, *blaSHV*, *blaCTX-M* and *blaOXA* genes have been described in several epidemiological studies in Europe, Asia, the USA and South America (Bush and Jacoby, 2010; Winokur et al., 2001; Villegas et al., 2008).

Organisms expressing these genes are widespread throughout the world but some geographical regions have a significantly higher prevalence rate such as South America, Asia and Europe. These prevalence rates have been evaluated by large microbial resistance surveillance programs such as SENTRY and MYSTIC and there is a steady increase in there prevalence (Goossens, 2005; Yano et al., 2013).

In Africa, prevalence has recently been estimated at less than 15% (Tansarli et al., 2014). Several previous studies reported the presence of ESBL at rates of 1.3% in Morocco (Bourjilat et al., 2011); 3.8% in Senegal (Sire et al., 2007); 4% in Central African Republic (Lovollay et al., 2006), 12 and 16% in two studies in Cameroon (Gangoue et al., 2005; Lonchel et al., 2012); 22% in Benin (Ahoyo et al., 2007).

Classes A and D enzymes are commonly found in Africa with a predominance of *bla*CTX-M-15 (Storberg, 2014). In Congo-Brazzaville, a prevalence of 73.8% of *E. coli* strains harboring beta-lactamase phenotype was reported (Moyen et al., 2014). To our knowledge, no study has been performed on the characterization of Enterobacteriaceae resistance genes.

Based on the aforementioned, this study was carried out to evaluate the emergence of beta-lactam resistance related to genes encoding ESBL (TEM, SHV, CTX-M-1 and *OXA-48*) in *E. coli* from Brazzaville.

MATERIALS AND METHODS

Bacterial strains

From January 2016 to May 2017, *E. coli* isolates were isolated from various pathological samples (urine, vaginal swabs excretions, sperm, blood and pus), environmental samples (domestic sewage).

Collected samples were inoculated on Mac Conkey agar or methylene blue eosin (EMB) agar and incubated for 24 h at 37°C in the Bacteriology-Virology Laboratory of the CHU in Brazzaville and the Exau-Kenn Laboratory.

The suspected *E. coli* isolates were purified and biochemically identified using API 20E® galleries (bioMerieux, France) and the strains were stored in Luria-Bertani (LB) liquid medium (Sigma-Aldrich, France) supplemented with 10% glycerol at -80°C for the duration of the study.

Study of the sensitivity

The antibiograms were made by diffusion in agar medium on Mueller-Hinton medium (Becton Dickinson, Le Pont de Claix, France) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) (Bonnet et al., 2010). The agar plates were inoculated with a swab from a culture inoculum calibrated at 0.5 McFarland before the deposit of the disks impregnated with antibiotic (Bio-Rad, Marnes-

Table 1. Primers used for conventional PCR and sequencing.

Gene	Primer	Sequence (5' → 3')	Accession number	Product size (bp)
*bla*TEM	F R	ATGAGTATTCAACATTTCCGTG TTACCAATGCTTAATCAGTGAG	KJ939560.1	861
*bla*SHV	F R	TTTATGGCGTTACCTTTGACC TCCCATGATGAGCACCTTTAAA	AF124984.1	1051
*bla*CTX-M1	F R	CAGCGCTTTTGCCGTCTAAG TTTGCGATGTGCAGTACCAGTAA	JQ397665.1	944
bla OXA-48	F R	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	AY236073	744

bp: Base pair; F: forward ; R: reverse.

La-Coquette, France). After incubation of agar plates at 37°C for 18 to 24 h inhibition diameters, due to antibiotic concentration gradients established from the disc, they were compared to the critical values given by CA-SFM, thus allowing definition of the susceptibility or resistance of a strain to an antibiotic.

ESBL production was confirmed phenotypically according to CA-SFM recommendations, including the MH agar synergy test between clavulanic acid disc and cephalosporin discs of the 3rd (ceftriaxone: CRO, cefipime: FEP). The carbapenemase OXA-48 phenotype was determined when there was a decrease in inhibition diameter around the Ertapenem disk on MH agar in an imipenem-susceptible strain (Yagoubat et al., 2017). Sixteen (16) antibiotics were tested: amoxicillin AMX (25 µg), amoxicillin/clavulanic acid AMC (20/10 µg), cefipime FEP (30 µg), Piperacillin-Tozobactam TZP (75/10 µg), cefalotin CF (30 µg), ceftriaxone CRO (30 µg) ERT ertapenem (10 µg), imipenem IMP (10 µg), fosfomycin FF (50 µg), nitrofuran FNitro (300 µg), trimithoprim + sulfaphametxazole SXT (1.25/23.75 µg), amykacin (30 µg), ciprofloxacin CIP (5 µg), doxicycline DO (30UI), CT colistin (50 µg) and gentamycin GEN (15 µg). The different strains tested were classified into Sensitive (S), Intermediate (I) and Resistant (R) categories. The strain of *E. coli* ATCC 25922 was used as a sensitive reference for susceptibility testing.

DNA isolation

Bacteria isolates were separately grown overnight on a MacConkey agar plates at 37°C. Subsequently, colonies were picked up using a sterile 10 µL plastic loop and transferred into 200 µL sterile water (Nouria et al., 2016). Total DNA was extracted with the NucliSENS easyMag automat (Biomérieux, France) according to the manufacturer's protocol. DNA was eluted in a final volume of 200 µL. The extracted DNA was stored at -70°C for further analysis.

PCR Detection of ESBL and carbapenemase genes

Molecular detection of genes was performed on all the strains by the conventional PCR technique using a T100 thermocycler (Bio-Rad, France) using the specific primer pairs for the *bla*TEM genes (Kruger et al., 2004), blaCTX-M1 (Roschanski et al., 2014), *bla*SHV (Yagi et al., 2000) and *bla*OXA-48 (Poirel et al., 2011) shown in Table 1.

The PCR was carried out in a final volume of 50 µL containing: 1 µL of each primer at 20 µM, 5 µL of 10X PCR reaction buffer, 1 µL of the dNTPs, 0.25 µL of Taq DNA polymerase (Eurogentec, Belgium), 36.75 µL of distilled water sterile, and 5 µL of crude DNA extract. Standard PCR conditions at 95°C for 15 min; followed by 35 cycles of amplifications each comprising a hybridization step at 55°C for 50 s, an elongation step at 72°C for 1 min, and a final extension step at 72°C for 7 min.

The amplification products were detected by electrophoresis using 1.5% agarose gels containing BET ethidium bromide diluted to 125 µL per 50 ml agarose gel heated in an electrophoresis vat containing buffer TBE (TRIS, Borate, EDTA) at 0.5%. The migration is done for 25 min at a voltage of 135V (Invitrogen, Leek, The Netherlands), as well as a DNA molecular weight marker (BenchTop pGEM®DNAMarker, Promega, Madison, Wisconsin, USA). Visualization of the gels was performed using the leaning ADNPPEM marker (Promega, Madison, Wisconsin, USA) under ultraviolet illumination.

DNA sequencing

Classical PCR products positive for ESBL genes were purified using the NucleoFast 96 PCR plate (Machery-Nagel EURL, France) and sequenced using BigDye terminator chemistry on a automatic sequencer ABI3730 (Applied Biosystems, Foster City, Californie, États-Unis).

The sequences obtained were assembled and corrected with Codon Code Aligner software. Sequence alignment and analyses were performed using the basic local alignement search tool (BLAST) software available on the website of the National Information Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 (Kumar et al., 2017).

Statistical analysis

For the data analysis, intermediate category strains were counted among the resistant (I + R). The data were analyzed using GraphPad Prism 7 software. The Chi-square test (χ^2) was used to compare the resistance frequencies between the different parameters studied. The difference between frequencies was considered significant when the *p*-value was less than 0.05.

Ethical clearance

The study was approved by the ethics committee of Marien

Table 2. Antibiotic susceptibility of ESBL-producing isolates (n=43) and no ESBL-producing isolates (n=46).

Antibiotic	*E. coli* ESBL+ *(N=43)*	*E. coli* ESBL- *(N=46)*	*p-v*alue
	% (n)		
	R	R	
AMX	100 (43)	100 (46)	-
AMC	100 (43)	93.48 (43)	0.330
TZP	46.51 (20)	0	0.0001**
CF	95.6 (22)	67.39 (31)	0.119
CRO	79.07 (34)	17.39 (8)	0.0001**
FEP	37.20 (16)	0	0.00001**
ERT	9.30 (3)	0	0.0684
IMP	0	0	-
CS	0	0	-
AK	9.30 (4)	0	0.0344**
GEN	34.88 (15)	0	0.00001**
CIP	13.95 (6)	0	0.0095**
FF	46.51 (20)	0	0.00001**
F	69.77 (30)	0	0.00001**
DO	53.49 (23)	97.82 (45)	0.00001**
SXT	93.02 (40)	95.65 (44)	0.590

AMX: Amoxicillin; AMC: amoxicillin/acid clavulanic; TZP: piperacillin-tozobactam; CF: cefalotin; CRO: ceftriaxon; FEP: cefipim; ERT: ertapenem; IMP: imipenem; CS: colistin; AK: amykacin; GEN: gentamycin; CIP: ciprofloxacin; FF: fosfomycin; F: nitrofurantoin; DO: doxycillin; SXT: trimethoprim+sulfaphametaxazol. N: strains number. R: resistance. (**): $\alpha < 0.05$ (exact Fisher test, 2-tailed) indicates statistically significant differences in resistance rates between ESBL *E. coli* and no-ESBL *E. coli.*

Ngouabi University. The protocol was reviewed and accepted by the authorities of Brazzaville hospitals (this allowed us to conduct our study). During the study period, we ensured the confidentiality of the results and the anonimate of the patients.

RESULTS

Bacterial strains

Eighty-nine (89) isolates of *E. coli* non-redundant were collected from two types of samples: an environmental sample consisting of household wastewater collected in a few neighborhoods of Brazzaville and a clinical sample taken from samples for the diagnostic purpose of hospitalized patients and those seen externally at the Makelekele base hospital and at the University Hospital Center of Brazzaville.

These samples consisted of five (5) blood cultures, sixty four (64) urine, one (1) vaginal sample, one (1) spermoculture and four (4) pyocultures, distributed as follows (Table 4): 14 *E. coli* originated from household sewage and 75 from hospital, twenty 20 strains were from outpatients and fifty five (55) straines from hospital services including 3 surgical, 9 urology care, 5 pediatric care (infant and grandchild), 4 infectious deseases, 10 cardiology care, 9 metabolic deseases, 9 general medicine, 3 neonatal and 3 intensive care unit (Table 3).

The resistance profiles of 89 isolates of *E. coli* studied on the 16 antibiotics used show a 100% sensitivity for imipenem and colistin, in *E. coli* ESBL+ and *E. coli* ESBL- isolates.

Low resistance was observed for Ertapenem 9.30% (3/43). The frequencies of resistance to amoxicillin, amoxicillin/clavulanic acid and cephalothin, which are antibiotics used in the first-line treatment of urinary tract infections, are relatively high in the two groups, but moreso for the strains of ESBL+ than ESBL- (Table 2). This difference is not statistically significant ($p > 0.05$). Aside from amoxicillin, amoxicillin/clavulanic acid, cephalothin, ertapenem, imipenem, colistin, and trimethoprim + sulfaphametxazole, there is a statistically significant difference between *E. coli* ESBL+ and *E. coli* ESBL- (Table 2).

Regarding to aminoglycosides, there was fairly marked resistance for gentamicin in ESBL *E. coli* isolates only at 34.88%, but it was only 9.30% for amykacin. In addition, with respect to fluoroquinolones, a resistance level of 13.95% was detected for ciprofloxacin; Furans remain resistant to 69.77%; as for the sulfamides and tetracyclines, the latter remain resistant in the two groups of strains studied (Table 2).

Table 3. Prevalence of *E. coli* isolates ESBL-producing based on their origin (N=89).

Origin	*E. coli* ESBL+ % (n)	*E. coli* ESBL- % (n)	Total
surgery	100 (3)	0	3
Infectious diseases	75 (3)	25 (1)	4
Pediatrics	100 (5)	0	5
Urology	77.78 (7)	22.22 (2)	9
Cardiology	40 (4)	60 (6)	10
Metabolic diseases	55.56 (5)	44.44 (4)	9
Intensive care	33.33 (1)	66.67 (2)	3
General medicine	33.33 (3)	66.67 (6)	9
neonatology	33.33 (1)	66.67 (2)	3
communal	32.35 (11)	67.65 (23)	34
Total	48.31 (43)	51.69 (46)	89

χ^2=18.11; ddl=9; α =0.0339.

Table 4. Frequency of *E. coli* (N=89) and nature of samples.

Nature of samples	*E. coli* ESBL+	*E. coli* ESBL-	*p*-value
	% (n)	% (n)	
Urine	81.40 (35)	63.04 (29)	
Sewage	2.32 (1)	30.23 (13)	
Blood	6.97 (3)	4.35 (2)	
Pus	6.97 (3)	2.17 (1)	0.00001
PV	0	2.17 (1)	
Sperms	2.32 (1)	0	
Total	48.31 (43/89)	51.69 (46/89)	

χ^2=26.19; ddl=5; α=0.00001. Pv: Vaginal collection ; ESBL: extended spectrum beta-lactamase.

Characterization of ESBL genes

Figure 1 shows the *bla*TEM, *bla*SHV, *bla*CTX-M-1 and *bla*OXA-48 genes with bands of respective sizes of 861, 1051, 944 and 744 base pairs.

Figure 2 shows the evolutionary history of ESBL genes (*bla*TEM, *bla*SHV, *bla*CTX-M-1). The optimal tree with the sum of branch length = 3.01339548 is shown. Evolutionary distances were calculated using the composite maximum likelihood method and are expressed in units of the number of base substitutions per site. All positions with gaps and missing data have been eliminated. There was a total of 479 positions in the final dataset.

The prevalence of ESBL genes on 89 isolates of *E. coli* was 48.31% (43/89). 25.58% (11/43) were community strains and 74.42% (32/43) were hospital strains. With a statistically significant difference in the production of genes that code for ESBLs between community strains and hospital strains, *p*-value < 0.05 (*p* = 0.0178).

The *bla*TEM gene was the most common with 74.42% (32/43), followed by *bla*CTX-M1 23.26% (12/43) and *bla*SHV 9.30% (4/43) and then *bla*OXA-48 6.97% (3/43) (Table 5). The following set: a triple expression *bla*TEM-SHV-OXA-48 was identified in a strain (2.33%) isolated from urine in a patient hospitalized in general practice, followed by a double expression *bla*TEM-CTX-M1 respectively in six isolates (13.95%). The *bla*TEM gene alone was present in twenty five (25) strains (58.14%), blaCTX-M1 in six (6) isolates (13.95%) and *bla*SHV in thee (3) isolates (6.97%) and then *bla*OXA-48 in two isolates (4, 65%) (Table 5).

The distribution of strains according to the hospital service shows a predominance of ESBL producing strains for the surgical and pediatric departments at 100%. The urology department comes in second place with 77.78%; followed by infectious diseases 75%, metabolic diseases (55.56%) and cardiology (40%). General medicine, pediatric intensive care and neonatology departements come last (33.33%) (Table 3).

Sequencing of PCR amplification products and after comparison based on NCBI data revealed that: all 32 *bla*TEM-positive strains were all TEM-1 AAR25033.1 (Table 5); the 12 CTX-M strains of group 1 were all CTX-

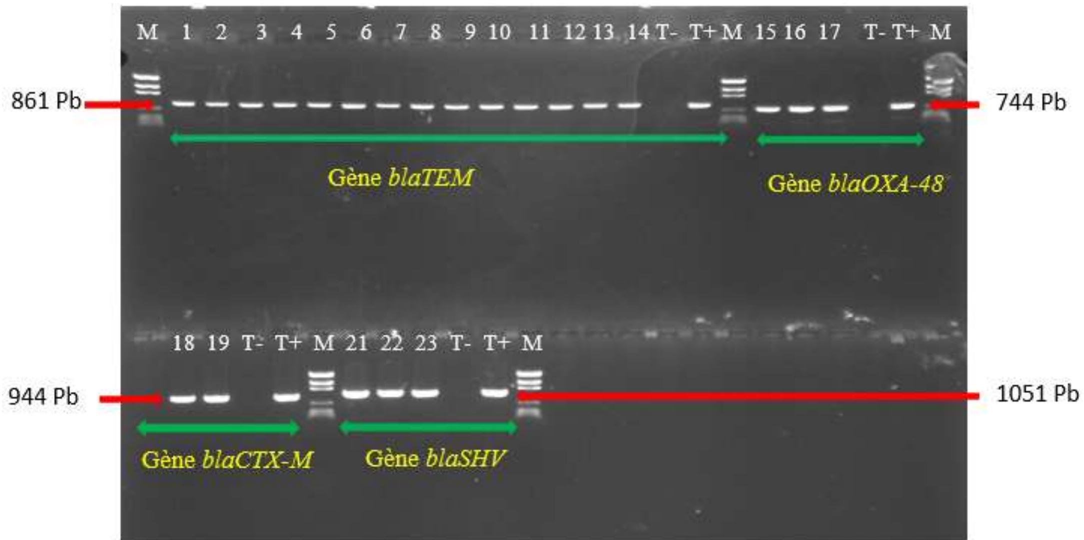

Figure 1. 1.5% agarose gel electrophoresis showing simplex PCR amplification products for detection of the genes ESBL. Lane T: Negative control; Lane M: molecular weight marker (Invitrogen, 100 bp DNA Ladder); Lane T+: Positive Control (blaTEM, blaCTX-M-1, blaSHV and blaOXA-48); Lane 1-14: positive samples for *bla*TEM (861 bp); Lane15-17: positive samples for *bla*OXA-48 (744 bp); Lane 18-19: positive samples for *bla*CTX-M-1 (944 bp); Lane 21-23: positive samples for *bla*SHV (1051 bp).

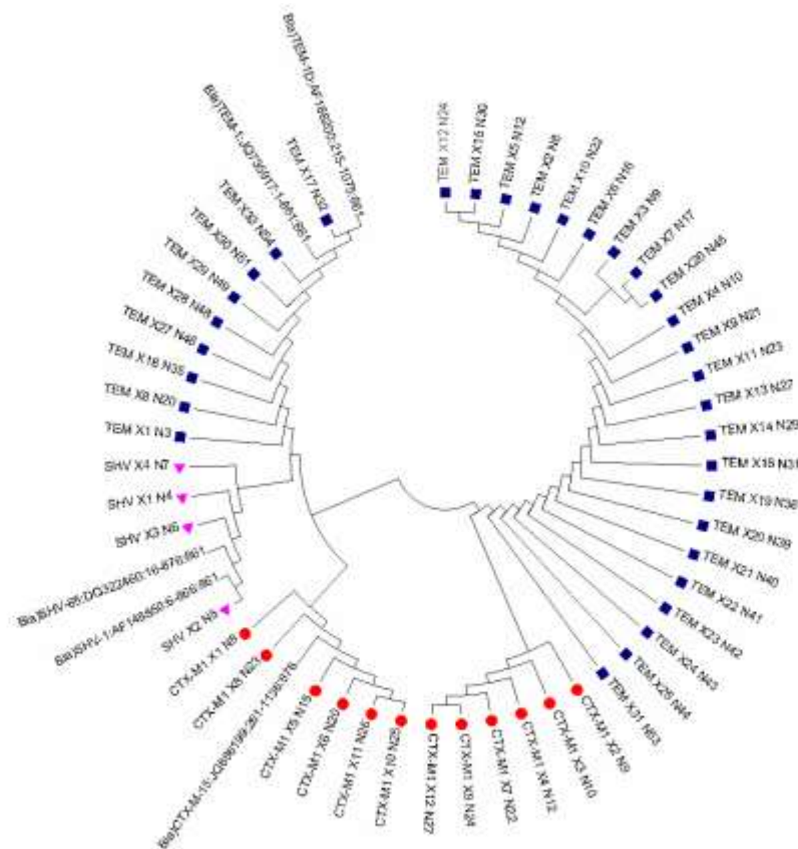

Figure 2. Evolutionary relationships of ESBL genes. Dendrogram generated using BioEdit and MEGA7.1 from the blaTEM gene set (blue square), *bla*CTX-M-1 (Red dot) and *bla*SHV (Pink Triangle) by comparing their relationship to each other and to other genes ESBL deposited at GenBank (black text). The lengths of branches are indicative of kinship.

Table 5. Characterization of the ESBL genes of the 43 *E. coli* isolates of hospital and community origin.

Isolate	Number of ESBL producers (%)	Positive genes detected by simplex PCR assays (n)	ESBL variant detected (n)	Other β-lactamase genes detected
E. coli hospitable (n=32)	2.33 (1/43)	TEM, SHV, OXA-48	TEM-1 (1)	SHV-85; OXA-181
	9.30 (4/43)	TEM, CTX-M-1	CTX-M-15 (4)	TEM-1
	44.19 (19/43)	TEM	TEM-1 (19)	None
	13.95 (6/43)	CTX-M-1	CTX-M-15(6)	None
	4.65 (2/43)	SHV	SHV-1(2)	None
E. coli community (n=11)	4.65 (2/43)	TEM, CTX-M-1	CTX-M-15 (2)	TEM-1
	13.95 (6/43)	TEM	TEM-1(6)	none
	2.33 (1/43)	SHV	SHV-1 (1)	none
	4.65 (2/43)	OXA-48	OXA-181(2)	none

M15 JQ686199. Two types of enzyme were detected for blaSHV: SHV-85 WP_063864713 (n = 1) and SHV-1 YES11730.1 (n = 3). Strains OXA-48 were OXA-181 variant with accession number HM992946.

DISCUSSION

ESBL-producing bacteria are a major concern in community and hospital settings because of their epidemic spread and multidrug resistance. In fact, ESBLs are found in a large proportion of Gram-negative bacilli, but enterobacteria are the most incriminated organisms (Gniadkowski, 2001).

The present study, the first conducted in the Republic of Congo, detected 43 strains of *E. coli* producing ESBL on 89 strains tested, a prevalence of 48.31% between January 2016 and May 2017.

The obtained prevalence is relatively high compared to some studies, especially in Benin (22%) and Cameroon (14.3%) (Ahoyo et al., 2007; Gangoue et al., 2005). However, it is quite similar to that reported by Djukoué in Cameroon (45.3%) (Djuikoue et al., 2017).

The high prevalence of ESBL-producing *E. coli* isolates observed in the present study is probably a consequence of selection pressure due to inappropriate prescribing and misuse of broad-spectrum antibiotics in both hospital and community setting (dispensing without prescription, self-medication).

The rate of isolation of ESBL *E. coli* isolates was greater in the hospital (74.42%) than in the community (25.58%) with a statistically significant difference (*p*-value <0.05). These results corroborate with the literature on the epidemiology of ESBLs. The duration of hospitalization, the severity of the disease, the surgical intervention, the wearing of arterial or urinary catheters are the risk factors for the acquisition of ESBL in hospitalized patients (Bradford et al., 1997; Paterson, 2000).

Also, these species are distributed differently according to the services and sites of sampling. Urinary tract infection is a common pathology in daily practice. The main bacterial species involved in this infection is *E. coli* since it represents 50 to 80% of the agents involved (Matute et al., 2004). This corresponds to the results obtained in the present study with a rate of 81.40%. This is related to the physiopathology of urinary tract infection, which is usually ascending, and there is a strong colonization of the perineum by Enterobacteriacae of digestive origin, and in particular *E. coli*. In addition, there are specific factors of uropathogenicity. Thus, *Escherichia coli* has adhesins, capable of binding the bacterium to the urinary epithelium and preventing its removal (Matute et al., 2004).

In the present study, most ESBL producers were collected from patients in the surgical ward and the pediatry than other reported services. In these wards, isolates are exposed to great antibiotic pressure. Furthermore, many of these patients are particularly vulnerable to infection because they are immunocompromised or have an easy avenue of access for bacteria (Xiong et al., 2002).

In addition, the problem related to ESBL is mainly the frequent presence of co-resistances making multiresistant strains (Touati et al., 2012).

Indeed, ESBLs are usually carried by large plasmids which also carry resistance genes to non-β-lactam antibiotic classes, such as aminoglycosides, quinolones and trimethoprim/sulfamethoxazole. As well, the use of these antibiotics contributes to the selection of producing strains from ESBL (Paterson and Bonomo, 2005).

The incidence of occurrence of resistances aminoglycoside has increased in recent years and particularly in producer of ESBL (Spanu et al., 2002). The resistance levels of these strains in the present study is more important for gentamycin (34.88%). Amikacin remains the most effective molecule with 90.7% of susceptible strains as reported in several studies (Gangoue et al., 2005; Touati et al., 2012).

The fluoroquinolones show a fairly good activity, the

overall sensitivity of the strains falling considerably in the case of strains producing ESBL. For the majority of strains, acquired resistance is the consequence of a mutation, which limits its diffusion (Larabi et al., 2003).

A very low activity was detected for trimethoprim/sulfamethoxazole (93.02%) and tetracyclines (with resistant strains). This correlates with other studies, where many ESBL producers are multi-resistant to non-β-lactam antibiotics, including fluoroquinolones and aminoglycosides (Livermore et al., 2007), Consequently, effective antibiotic therapy for treating these infections is limited to a small number of drugs, such as carbapenems and thus increasing the chance of resistance to carbapenems among the Enterobacteriaceae (Pitout, 2010).

As far as colistin is concerned, it is one of the molecules with the highest sensitivity levels on naturally occurring species sensitive (apart from *Proteus*). This is likely related to a lower use of this antibiotic in the current practice. This correlates with results of the susceptibility rate to this molecule were also reported by Nouria et al. (2016).

Carbapenems are the treatment of choice for severe infections caused by ESBL-producing enterobacteria (Nordmann et al., 2008). The resistance of enterobacteria to these molecules is still a marginal phenomenon as in the epidemiological data obtained for a large number of strains, with sensitivity percentages of 99 to 100% (Nordmann et al., 2008). In the present study, 9.3% strains had resistance to ertapenem and 100% were sensitive to imipenem. This decrease in sensitivity to ertapenem may be related to carbapenemase production since carbapenem activity may be compromised by the emergence of these enzymes (Munoz-price et al., 2013 ; Sekhri et al., 2010).

ESBLs are divided into five types: TEM, SHV, CTX-M, OXA-48, and others, based on the homogeneity of coding genes. Most ESBLs derived from plasmid-mediated penicillinases belonging to TEM or SHV famillies (Xue et al., 2012). Recently, the CTX-M group with a typical ESBL resistance phenotype but does not originate from TEM or SHV families have been described. The CTX-M group is a new family of plasmid-mediated ESBLs that preferentially hydrolyse cefotaxime (Xiong et al., 2002).

The prevalent genotypes vary in different contries such as the major genotypes TEM-10, TEM-12 and TEM-26 in U.S (Jacoby and Medeirof, 1991), TEM-10 and TEM-12 in United Kingdom. SHV-3, SHV-4 and TEM-4 in France. Previously, the most prevalent ESBLs in *E. coli* isolates from Korea as SHV-12 and CTX-M, as well as a prototype of Beta-lactams, TEM-1 (Xue et al., 2012). In Cameroon, Gangoue et al. (2005) report predominance of blaSHV-12 in Enterobacteriacea (Gangoue et al., 2005), as well as Benin, Ahoyo et al. (2007) reported a predominance of the *bla*SHV gene in *E. coli*.

TEM and SHV-type ESBLs remain more common in North American and in Africa. CTX-M type ESBLs have been mainly in South America, Eastern Europe, Japan and more recently in Spain, Kenya (Xue et al., 2012) and Algeria (Nouria et al., 2016).

In this study, it was determined that most of 32 *E. coli* isolates were prevalent TEM-type ESBLs. TEM was the main type of beta-lactamase and CTX-M was the second. SHV was detected in five isolates. In addition to OXA-48 was detected in 3 isolates. Another interesting point is that the results modify the current epidemiology of ESBL in Enterobacteriaceae, the CTX-M that represented the majority of ESBLs in any region of the world, both in hospitals and community settings, to such an extent that their spread of pandemic (Elhani, 2012).

The genetic diversity of ESBLs and carbapenemases can be attributed to genes already detected and the emergence of new clusters in our geographical area (TEM-1, SHV-1, SHV-85, CTX-M-15 and OXA-181) (Lonchel et al., 2012; Ouédraogo et al., 2016; Nouria et al., 2016).

Conclusion

This can be the first study revealed the real existence of the genes encoding ESBL in *E. coli* isolates from the community and hospital patients in Brazzaville. A high prevalence (48, 31%) of ESBL *E. coli* isolates with predominance of the *bla*TEM gene. Fighting against this phenomenon is multidisciplinary and should integrate the rationalization of district compliance with the prescription of antibiotics, with hygiene measures. Justification of antibiotherapy policy and/or a restriction of the prescription of third-generation cephalosporin and even all beta-lactams would lead to a significant decrease in the frequency of ESBL.

Monitoring of antibiotic resistance of bacterial strains should be continuous and systematic to define therapeutic strategies adapted to local epidemiology data.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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