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Prevalence and Characterization of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae in Major Hospitals in Gabon

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In Gabon, few data exist on extended-spectrum beta-lactamases-producing Enterobacteriaceae (ESBL-PE). This study investigated ESBL-PE prevalence and the associated resistance genes in clinical samples ($n = 5{,}956$) and anal swabs ($n = 78$) analyzed in eight hospitals and a medical analysis laboratory in Gabon from January 2016 to March 2018. Matrix-Assisted Laser Desorption Ionization–Time Of Flight (MALDI-TOF) mass spectrometry analysis identified 790 Enterobacteriaceae isolates ($n = 712$ clinical samples and $n = 78$ fecal samples). ESBL-PE prevalence (Müller-Hinton agar disk diffusion method and double-disk synergy test) was 11.8% (84/712) in clinical samples (15.5% from inpatients and 7.1% from outpatients; $p < 0.05$) and 16.7% (13/78) in carriage isolates. Most ESBL-PE were isolated from urine samples (46/84). In clinical and carriage ESBL-PE isolates, *Escherichia coli* was predominant (42.8% and 61.5%; phylogroups A, B1, B2, and D), followed by *Klebsiella pneumoniae* (41.7% and 23.1%). Multiplex PCR and bi-directional sequencing showed that CTX-M group 1 (blaCTX-M-15) was predominant in clinical and carriage ESBL-PE (94% and 92.3%) among which 85.7% and 92.3% also harbored one to three β-lactamase-encoding genes (blaTEM-1, blaOXA-1, or blaSHV-1). Resistance genes were detected in all hospitals in Gabon. ESBL-PE prevalence in Gabon has not reached alarming levels yet, but corrective and monitoring measures are needed to curb their emergence.

Keywords: Enterobacteriaceae, ESBL-PE, CTX-M-15, phylogroups, Gabon

Introduction

Beta-lactams have a broad spectrum of activity and low toxicity, and therefore are widely used for the treatment of bacterial infections, particularly those caused by Enterobacteriaceae. This has led to the emergence of resistance mediated by enzymes, particularly extended-spectrum beta-lactamases (ESBL) that inhibit the action of broad-spectrum beta-lactam antibiotics such as third-generation cephalosporins.[1]. ESBL confer resistance to all β-lactam antibiotics, except cephamycins and carbapenems. They can be inhibited by clavulanic acid, tazobactam, and sulbactam.[2,3] There are several ESBL types, and the most common are TEM (isolated from an *Escherichia coli* strain of a patient named Temoneira), SHV (sulfhydryl variable), and CTX-M (cefotaxime-Munich). ESBL-producing isolates are often resistant also to other antibiotic families.[3–5] Indeed, ESBL-encoding genes are often carried by plasmids that also harbor other resistance genes, such as those conferring resistance to aminoglycosides and fluoroquinolones.[6] The existence of isolates that produce both ESBL and carbapenemases often leads to a therapeutic impasse, because carbapenems are the last-line treatment for severe infections caused by ESBL-producing bacteria.[7]

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ESBL-producing Enterobacteriaceae (ESBL-PE) are a real public health problem worldwide, and a major challenge for medical microbiologists, clinicians, and health care personnel who have to cope with multi-resistant bacteria and nosocomial infections.[8] ESBL-PE prevalence is increasing, and in many parts of the world 10% to 40% of *E. coli* and *Klebsiella pneumoniae* produce ESBL.[9]

In Africa, there are few data on ESBL-PE prevalence, and for some countries no information is available. The systematic review of 26 studies by Tansarli *et al.* suggests that ESBL-PE prevalence is not high in Africa (irrespective of the disparities among countries), but certainly not negligible (<15%).[10] However, these results do not allow estimating the situation in the entire African continent, because most of the included studies were performed in North and South African countries.[11] Some studies reported hospital-based ESBL-PE prevalence rates between 38.3% in Rwanda[12] and 68.8% in Cameroon.[13]

Despite some previous studies,[14–17] data on ESBL-PE prevalence in Gabon at the national level are lacking. Therefore, the aims of this study were as follows: (1) to establish ESBL-PE prevalence in clinical and carriage isolates obtained from hospitals and laboratories in seven of Gabon main cities; (2) to identify and map the geographical distribution of genes encoding ESBL, carbapenemases, 16S RNA methylases, plasmid-mediated quinolone resistance (PMQR); and (3) to determine the phylogroups of the ESBL-producing *E. coli* isolates.

Materials and Methods

Study setting

This study was performed in eight main hospitals and one medical analysis laboratory of Gabon (population of 1,811,079 inhabitants of whom more than 93% reside in urban areas) from January 2016 to March 2018. These centers are in seven of the nine provinces of the country (Fig. 1): Omar Bongo Ondimba Armed Forces Training Hospital (HIAOBO), Akanda Armed Forces Training Hospital (HIAA), and El Rapha Polyclinic (PER) in Libreville (the capital city that concentrates 49.5% of the population)[18]; Georges Rawiri Regional Hospital Center (CHRGR) in Lambaréné; Mouila Regional Hospital Center (CHREM)

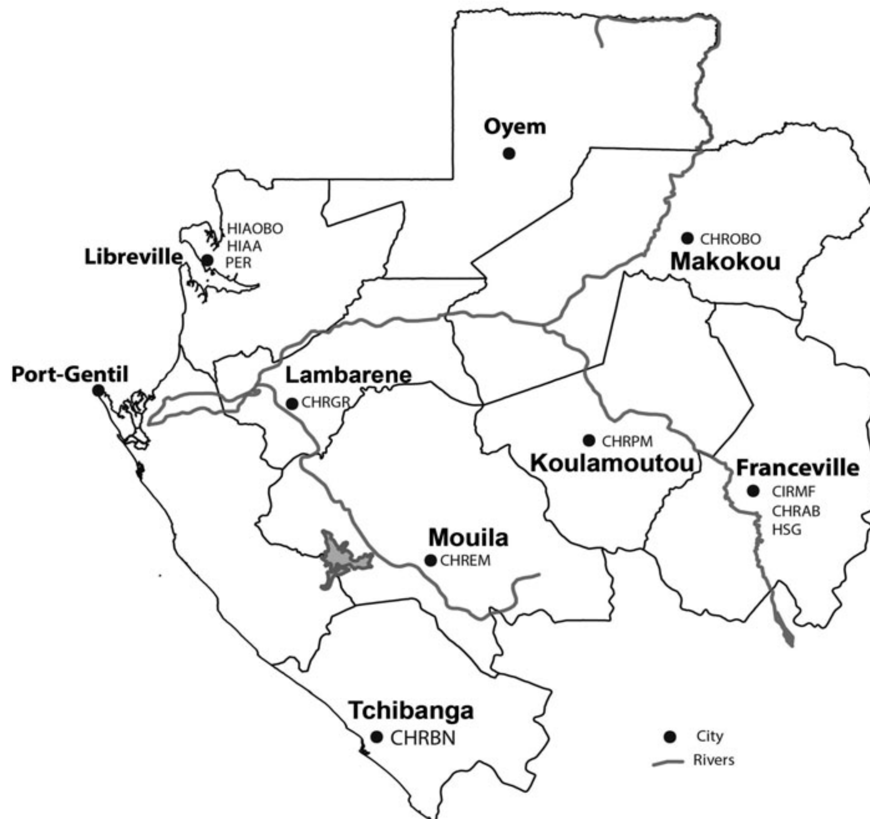

FIG. 1. Geographical distribution of the sampling sites in Gabon. HIAOBO: Hôpital d'Instruction des Armées (Omar Bongo Ondimba Armed Forces Training Hospital). HIAA: Hôpital d'Instruction des Armées d'Akanda (Akanda Armed Forces Training Hospital). PER: Polyclinique El Rapha (El Rapha Polyclinic), CHRGR: Centre Hospitalier Régional Georges Rawiri (Georges Rawiri Regional Hospital Center), CHROBO: Centre Hospitalier Régional Omar Bongo Ondimba (Omar Bongo Ondimba Regional Hospital Center), CHRPM: Centre Hospitalier Régional Paul Moukambi (Paul Moukambi Regional Hospital Center), CHREM: Centre Hospitalier Régional de Mouila (Mouila Regional Hospital Center), CHRBN: Centre Hospitalier Régional Benjamin Ngoubou (Benjamin Ngoubou Regional Hospital Center), CHRAB: Centre Hospitalier Régional Amissa Bongo (Amissa Bongo Regional Hospital Center), HSG: Hôpital Sino-Gabonais (Sino-Gabonese Cooperation Hospital Center), CIRMF: Centre Interdisciplinaire de Recherches Médicales de Franceville (Medical Analysis Laboratory of the Interdisciplinary Medical Research Center of Franceville).

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in Mouila; Benjamin Ngoubou Regional Hospital Center (CHRBN) in Tchibanga; Paul Moukambi Regional Hospital Center (CHRPM) in Koulamoutou; Omar Bongo Ondimba Regional Hospital Center (CHROBO) in Makokou; and Medical Analysis Laboratory of the Interdisciplinary Medical Research Center of Franceville (CIRMF) in Franceville. The CIRMF laboratory analyzes samples from two hospitals in Franceville: Amissa Bongo Regional Hospital Center (CHRAB) and Sino-Gabonese Cooperation Hospital (HSG). In total, these 9 hospitals have 930 beds.

Sample collection, identification, and antimicrobial susceptibility testing

During the study period, 5,956 clinical samples were sent to the microbiology laboratories for bacteriologic investigations [HIAOBO: $n=5,249$ (88.1%); HIAA: $n=7$ (0.1%); PER: $n=160$ (2.7%); CIRMF: $n=309$ (5.2%); CHRGR: $n=55$ (1%); CHREM: $n=8$ (0.2%); CHRPM: $n=50$ (0.8%); CHROBO: $n=68$ (1.1%); and CHRBN: $n=50$ (0.8%)]. A total of 974 non-duplicate and clinically significant bacterial isolates were identified, including 712 Enterobacteriaceae [bronchial aspiration: $n=4$ (0.6%); feces: $n=9$ (1.3%); urine: $n=368$ (51.7%); bedsore: $n=68$ (9.6%); blood: $n=42$ (5.9%); venous catheter: $n=11$ (1.5%); distal protected aspirate: $n=39$ (5.5%); pus: $n=36$ (5.0%); intubation catheter: $n=12$ (1.7%); urinary catheter: $n=105$ (14.7%); wound: $n=18$ (2.5%)]. The other 262 isolates included Gram-positive cocci (*Staphylococcus spp*, *Streptococcus spp*, and *Enterococcus spp*) and non-Enterobacteriaceae Gram-negative bacilli (*e.g.*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*). In the same period, fresh stool samples were collected from 78 patients hospitalized for more than 48 hr without digestive pathology at HIAOBO and CHREM to explore ESBL-PE fecal carriage [HIAOBO: $n=77$ (98.7%); CHREM: $n=1$ (1.3%)]. Briefly, 0.5 g of each fresh stool sample was suspended in 5 mL of sterile saline solution (0.9%) and 100 mL aliquots were plated on ESBL agar plates (bioMérieux, Marcy-l'Etoile, France). Plates were examined after 24 and 48 hr of incubation at 37°C.

Bacterial isolates were identified by Matrix-Assisted Laser Desorption Ionization–Time Of Flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility was tested with the disk diffusion method on Müller-Hinton agar. The following antibiotics were tested: amoxicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, cefpirome, cefpodoxime, cefoxitin, ceftazidime, cephalothin, moxalactam, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanic acid, imipenem, nalidixic acid, ciprofloxacin, levofloxacin, ofloxacin, amikacin, gentamicin, netilmicin, tobramycin, fosfomycin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and clinical breakpoints (www.eucast.org/clinical_breakpoints/). ESBL production was detected with the combined double-disk synergy method.[19] In case of high-level cephalosporinase production, the combined double-disk synergy test was performed using cloxacillin-supplemented medium. Ertapenem minimal inhibitory concentrations were determined for all multidrug-resistant enterobacteria.

Characterization of resistance genes

DNA extraction: Two to three colonies of a pure strain were placed in a tube containing 1 mL of sterile distilled water, and vortexed. After incubation at 100°C for 10 min and centrifugation at 10,000 rpm for 10 min, the supernatant containing bacterial DNA was removed.

Detection of bla_{CTX-M}, bla_{TEM}, bla_{SHV}, and $bla_{OXA-1-like}$: The multiplex PCR protocol described by Dallenne *et al.*[20] was used with the following amplification conditions: initial denaturation at 94°C for 10 min, and then 30 cycles of 40 sec at 94°C, 40 sec at 60°C, and 1 min at 72°C, with a final elongation at 72°C for 7 min. The primers used were previously described.[20]

Detection of associated resistance genes: Carbapenemase-encoding genes (bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, bla_{NDM}), PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-ib-cr*, *oqxAB*, *qepA*), and 16S RNA methylase-encoding genes (*armA*, *rmtB*, *rmtC*) were detected using different PCR protocols.[21–23]

All PCR products were visualized after migration by electrophoresis on 2% agarose gels containing ethidium bromide at 100 V for 90 min, and using a 100 base pair size marker. PCR products were bidirectionally sequenced on a 3100 ABI Prism Genetic Analyzer (Applied Biosystems), and the sequencing products were analyzed online using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Typing of *E. coli* phylogenetic groups (A, B1, B2, C, D, F) was carried out using the multiplex PCR method developed by Clermont *et al.*[24]

Statistics

Statistical analyses were performed with the R software. Different parameters were compared with the chi-square (χ^2) test. Differences were considered statistically significant at the 0.05 confidence level.

Results

ESBL-PE prevalence

The antimicrobial susceptibility testing results and the molecular analysis of the Enterobacteriaceae isolates indicated that 11.8% (84/712) of clinical isolates and 16.7% (13/78) of carriage isolates were ESBL-PE. The percentage of ESBL-PE clinical isolates was higher among inpatients than outpatients (respectively 15.5% and 7.1% $p<0.05$) (Table 1).

More than half of clinical ESBL-PE isolates were from urine samples (46/84), and *E. coli* was the most represented species (36/84; 42.8%), followed *by K. pneumoniae* (35/84; 41.7%), *Enterobacter cloacae* (9/84; 10.7%), *Proteus mirabilis* (2/84; 2.4%), *Citrobacter sedlakii*, and *Morganella morganii* (1/84; 1.2%). Similarly, in carriage isolates, *E. coli* was detected in 61.5% of ESBL-PE isolates (8/13), followed by *K. pneumoniae* (23.1%; 3/13) and *E. cloacae* (15.4%; 2/13) (Table 2).

ESBL-PE isolates are also resistant to carbapenems and non-β-lactams

The percentages of ESBL-PE isolates resistant also to carbapenem and non-β-lactam antibiotics are shown in Fig. 2.

Table 1. Percentage of Extended-Spectrum Beta-Lactamases-Producing Enterobacteriaceae Isolates in Clinical Samples According to the Patients' Characteristics

Characteristics	*ESBL-PE isolates* n/N	%	*Statistical test*
Sex			
Male	43/350	12.29	$X^2 = 0.056$;
Female	41/362	11.32	df = 1 $p = 0.0813$
Age group			
<60 year-old	76/673	11.29	$X^2 = 1.55$;
≥60 year-old	8/39	20.51	df = 1 $p = 0.214$
Patient type			
Inpatients	62/401	15.46	$X^2 = 8.74$;
Outpatients	22/311	7.07	df = 1 **$p = 0.0031$**

n = number of ESBL-PE isolates; N = total number of isolates.
Bold value indicates statistically significant difference.
ESBL-PE, extended-spectrum beta-lactamases-producing Enterobacteriaceae.

Specifically, 1.2% and 3.6% of ESBL-PE clinical isolates from outpatients and inpatients were resistant to imipenem, 14.3% and 63.1% to gentamicin, 20.2% and 65.5% to ciprofloxacin, 22.6% and 70.2% to cotrimoxazole, and 1.2% and 17.9% to fosfomycin, respectively.

Moreover, among ESBL-PE carriage isolates, 7.7% were resistant to imipenem, 53.8% to gentamicin, 92.3% to ciprofloxacin, 84.6% to cotrimoxazole, but none to fosfomycin.

Characterization of resistance genes

PCR and sequencing analysis of the 84 clinical ESBL-PE isolates showed that 94% (79/84) harbored exclusively the $bla_{CTX\text{-}M\text{-}15}$ gene for CTX-M group 1 (Table 3). Only one isolate (1.2%) carried $bla_{CTX\text{-}M\text{-}9}$ (CTX-M group 9), and three isolates (3.6%) harbored the $bla_{TEM\text{-}1}$ gene. Genes from groups CTX-M-2, 8 and 25 were not detected in any ESBL-PE clinical isolate.

The $bla_{CTX\text{-}M\text{-}15}$ gene was found alone in 7 isolates (8.3%), or associated with other β-lactamase-encoding genes: $bla_{TEM\text{-}1}$ in 25 isolates (29.8%); $bla_{OXA\text{-}1}$ in 13 isolates (15.4%); $bla_{TEM\text{-}1}$ and $bla_{OXA\text{-}1}$ in 31 isolates (36.9%); and $bla_{TEM\text{-}1}$, $bla_{OXA\text{-}1}$, and $bla_{SHV\text{-}1}$ in 3 isolates (3.6%). The $bla_{TEM\text{-}1}$ and $bla_{OXA\text{-}1}$ association was detected in one *E. coli* isolate (1.2%).

Like in clinical isolates, CTX-M group 1 was predominant in the 13 ESBL-PE carriage isolates (12/13; 92.3%), and was exclusively represented by the $bla_{CTX\text{-}M\text{-}15}$ gene that was associated with $bla_{TEM\text{-}1}$ in 5 isolates (38.5%), with $bla_{OXA\text{-}1}$ in 4 isolates (30.8%), and with $bla_{TEM\text{-}1}$ and $bla_{OXA\text{-}1}$ in 3 isolates (23%). Only one *K. pneumoniae* isolate carried $bla_{TEM\text{-}1}$ alone (7.7%).

Overall (clinical and carriage ESBL-PE isolates), $bla_{TEM\text{-}1}$ was detected in all species, and $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}1}$ were found in all species but for *P. mirabilis*. $bla_{CTX\text{-}M\text{-}9}$ was found only in one *E. coli* isolate, and $bla_{SHV\text{-}1}$ only in one *K. pneumoniae* isolate (Table 3).

In addition, 9.3% (9/97) of ESBL-PE isolates co-expressed the $bla_{NDM\text{-}5}$ or $bla_{OXA\text{-}48}$ carbapenemase-encoding gene, and 78.3% (76/97) co-expressed one to three

Table 2. Extended-Spectrum Beta-Lactamases-Producing Enterobacteriaceae Distribution According to Bacterial Species and Sample Type

	Clinical samples												
	Bronchial aspirate	*Stool*	*Urine*	*Bedsore*	*Blood*	*Venous catheter*	*Distal protected aspirate*	*Pus*	*Intubation catheter*	*Urinary catheter*	*Wound*	Total clinical isolates	Anal swabs
Species	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*	n *(%)*
Citrobacter sedlakii	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)
Enterobacter cloacae	0 (0)	0 (0)	5 (5.9)	1 (1.2)	1(1.2)	0 (0)	2 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	9 (10.7)	2 (15.4)
Escherichia coli	0 (0)	1 (1.2)	26 (30.9)	5 (5.9)	0 (0)	1 (1.2)	1 (1.2)	0 (0)	0 (0)	1 (1.2)	1 (1.2)	36 (42.8)	8 (61.5)
Klebsiella pneumoniae	1 (1.2)	0 (0)	15 (17.9)	1 (1.2)	7 (8.3)	1 (1.2)	0 (0)	4 (4.8)	1 (1.2)	4 (4.8)	1 (1.2)	35 (41.7)	3 (23.1)
Morganella morganii	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)
Proteus mirabilis	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	1 (1.2)	0 (0)	0 (0)	1 (1.2)	0 (0)	2 (2.4)	0 (0)
Total	1 (1.2)	1 (1.2)	46 (54.7)	9 (10.7)	8 (9.5)	2 (2.4)	4 (4.8)	4 (4.8)	1 (1.2)	6 (7.2)	2 (2.4)	84 (100)	13 (100)

n, number.

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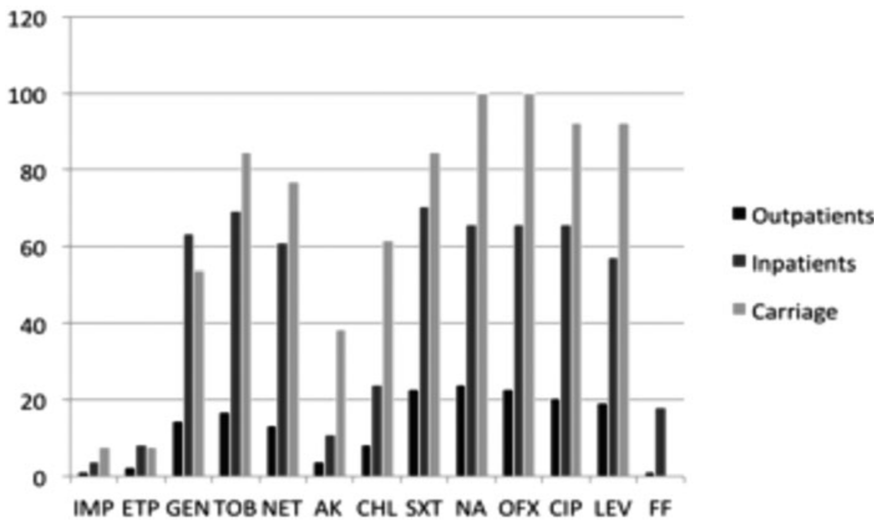

FIG. 2. Percentage of antibiotic resistances in ESBL-PE isolates from inpatients and outpatients (clinical isolates) and from anal swabs. IMP: imipenem; ETP: ertapenem; GEN: gentamicin TOB: tobramycin; NET: netilmicin; AK: amikacin; CHL: chloramphenicol; SXT: trimethoprim+ sulfamethoxazole; NA: nalidixic acid; OFX: ofloxacin; CIP: ciprofloxacin; LEV: levofloxacin; FF: fosfomycin; ESBL-PE, extended-spectrum beta-lactamases-producing Enterobacteriaceae.

Table 3. Resistance Genes in Extended-Spectrum Beta-Lactamases-Producing Enterobacteriaceae Isolates

		Associated resistance genes		
Species (n)	*β-lactamase-encoding genes* (n)	*Carbapenemase-encoding genes* (n)	*PMQR genes* (n)	*16S RNA methylase-encoding genes*
Escherichia coli (36)	$bla_{\text{CTX-M-15}}$ (3)	$bla_{\text{NDM-5}}$ (1)	*aac(6′)-ib-cr* (16)	—
	$bla_{\text{CTX-M-9}}$ (1)		*aac(6′)-ib-cr/qnrC* (1)	
	$bla_{\text{CTX-M-15/TEM-1}}$ (8)		*aac(6′)-ib-cr/qnrD* (1)	
	$bla_{\text{CTX-M-15/OXA-1}}$ (11)		*aac(6′)-ib-cr/qnrS* (4)	
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (11)		*qnrS* (1)	
	$bla_{\text{TEM-1/OXA-1}}$ (1)			
	$bla_{\text{TEM-1}}$ (1)			
Klebsiella pneumoniae (35)	$bla_{\text{CTX-M-15}}$ (2)	$bla_{\text{NDM-5}}$ (2)	*aac(6′)-ib-cr* (1)	—
	$bla_{\text{CTX-M-15/TEM-1}}$ (17)	$bla_{\text{OXA-48}}$ (4)	*aac(6′)-ib-cr/qnrB* (23)	
	$bla_{\text{CTX-M-15/OXA-}}$1 (2)		*aac(6′)-ib-cr/qnrB/qnrD* (1)	
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (11)		*qnrB* (10)	
	$bla_{\text{CTX-M-15/TEM-1/OXA-1/SHV-1}}$ (3)			
Enterobacter cloacae (9)	$bla_{\text{CTX-M-15}}$ (2)	$bla_{\text{NDM-5}}$ (1)	*aac(6′)-ib-cr* (6)	—
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (7)		*aac(6′)-ib-cr/qnrB* (1)	
			aac(6′)-ib-cr/qnrB/oqxAB (1)	
Citrobacter sedlakii (1)	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (1)	—	—	—
Morganella morganii (1)	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (1)	—	*aac(6′)-ib-cr/qnrB/qnrA* (1)	—
Proteus mirabilis (2)	$bla_{\text{TEM-1}}$ (2)	—	*qnrD* (2)	—
Carriage				
E. coli (8)	$bla_{\text{CTX-M-15/TEM-1}}$ (3)	$bla_{\text{NDM-5}}$ (1)	*aac(6′)-ib-cr* (3)	—
	$bla_{\text{CTX-M-15/OXA-1}}$ (4)		*aac(6′)-ib-cr/qnrS* (1)	
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (1)			
K. pneumoniae (3)	$bla_{\text{CTX-M-15/TEM-1}}$ (1)	—	*aac(6′)-ib-cr/qnrB* (2)	—
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (1)		*qnrA* (1)	
	$bla_{\text{TEM-1}}$ (1)			
E. cloacae (2)	$bla_{\text{CTX-M-15/TEM-1}}$ (1)	—	*aac(6′)-ib-cr* (2)	—
	$bla_{\text{CTX-M-15/TEM-1/OXA-1}}$ (1)			

PMQR, plasmid-mediated quinolone resistance.

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PMQR genes (*aac(6′)-ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxAB*). No ESBL-PE isolate co-expressed *16S* RNA methylase-encoding genes (*armA*, *rmtB*, *rmtC*) (Table 3).

Geographical distribution of resistance genes in Gabon

At all sampling sites in Gabon, ESBL-PE isolates carried the $bla_{CTX\text{-}M\text{-}15}$ gene alone or with $bla_{TEM\text{-}1}$ and/or $bla_{OXA\text{-}1}$ and/or $bla_{SHV\text{-}1}$ (Fig. 3A). $bla_{SHV\text{-}1}$ was only detected in Libreville, while $bla_{OXA\text{-}1}$ was not found in Lambaréné, Makokou, and Koulamoutou. ESBL-PE isolates co-harbored the carbapenemase-encoding gene $bla_{OXA\text{-}48}$ in Libreville, and $bla_{NDM\text{-}5}$ in Libreville, Mouila and Tchibanga (Fig. 3B). ESBL-PE isolates co-expressed one or more PMQR genes (*aac(6′)-ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxAB*) at all studied sites (Fig. 3C).

Phylogenetic grouping

Among the 36 ESBL-producing *E. coli* clinical isolates, phylogroups A and B2 were predominant (36.1%; $n=13$/each), followed by phylogroups D (11.1%; $n=4$) and B1 (2.8%; $n=1$). Moreover, two *E. coli* Clade I were identified (Table 4). Phylogroup A was predominant among inpatients (8/19), and B2 among outpatients (8/14). Among the 27 *E. coli* isolates that caused urinary tract infections, 13 (48.1%) belonged to phylogroup A, and 10 (37%) to phylogroup B2. Phylogroup B2 was predominant in ESBL-producing *E. coli* isolates from anal swabs (4/8; 50%), followed by phylogroup A (3/8; 37.5%).

Discussion

This study on ESBL-PE prevalence in the main hospitals of Gabon showed ESBL-PE rates of 11.8% and 16.7% in clinical and carriage isolates, respectively.

In 2005 Gangoué-Piéboji *et al.* found a similar ESBL-PE prevalence in clinical isolates (12%) at the central hospital of Yaoundé, Cameroon.[25] Yala *et al.* also showed an ESBL-PE prevalence of 15% to 18% (depending on the used method) in clinical isolates at HIAOBO.[15] Nevertheless, it remains lower than the rates observed in Central African Republic (19.3%), Nigeria (20.9%), and Burkina Faso (58%).[26–28] Tansarli *et al.* highlighted disparities among countries and the increase of ESBL-PE rate in Africa; however, they estimated that overall, this rate was lower than 15% for clinical samples.[8] In our study, ESBL-PE rate was higher in clinical samples from inpatients than outpatients (15.5% and 7.1% $p<0.05$), as previously reported by Ouedraogo *et al.* in Burkina Faso (70% and 45%, respectively).[28] Hospitalized patients are more likely to meet the two criteria that Sharif *et al.* in Iran[29] consider as risk factors of infection by broad-spectrum β-lactamase-producing bacteria: prolonged hospital stay (≥7 days), and consumption of antibiotics. Patients in intensive care units are particularly at risk because of the high antibiotic pressure, and generally long hospital stay.[30] Hospitalization is a risk factor because resistance genes are mostly carried by plasmids that can easily pass from one bacterium to another and infect patients in hospital.[31,32] Moreover, Filippini *et al.*[33] demonstrated the well-known link between selection pressure exerted by antibiotic use and prevalence of resistance at the population level.

As previously described by Mahamat *et al.* in Chad,[34] in our study sex was not significantly associated with ESBL-PE presence.

Overall, our study revealed that the ESBL-producing species were mainly represented by *E. coli* (42.8%), *K. pneumoniae* (41.7%), and *E. cloacae* (10.7%), as previously reported by Toudji *et al.* in Lomé (Togo) in 2017.[35] Moreover, in our study, 4.8% (46/84) of ESBL-PE were isolated from urine samples, and 56.5% and 32.6% of these urinary ESBL-PE were *E. coli* and *K. pneumoniae*, respectively. This is in agreement with the fact that urinary tract infections are the most common bacterial infection worldwide, and are mostly caused by the *E. coli* and *K. pneumoniae*.[36,37]

ESBL-PE prevalence in carriage isolates was 16.7%. This rate is higher than that described by Herindrainy *et al.* (10.1%) in Antananarivo (Madagascar) in 2011.[38] Schaumburg *et al.* in 2013 found an even higher ESBL-PE carriage rate (45%) at the Albert Schweitzer Hospital in Lambaréné (Gabon), and confirmed that length of hospital stay is one of the risk factors of ESBL-PE infection.[17]

Among the ESBL-PE detected in carriage isolates, *E. coli* (61.5%), *K. pneumoniae* (23.1%), and *E. cloacae* (15.4%) were the most common, as previously described by Mahamat *et al.* in Chad in 2019.[39]

ESBL production by bacteria is often associated with other resistance factors.[40] Our study showed that, in addition to their high resistance to β-lactam antibiotics conferred by ESBL, ESBL-PE (clinical and carriage isolates) were also resistant to several other families of antibiotics, such as aminoglycosides, quinolones, and cotrimoxazole, as previously reported by Moutachakkir *et al.* for ESBL-PE isolated in the laboratory of Marrakech University Hospital (Morocco) in 2014.[41] Some plasmids carrying ESBL-encoding genes, such as $bla_{CTX\text{-}M}$, often co-harbor other genes responsible for resistance to aminoglycosides, quinolones, and cotrimoxazole. This finding led to the concept of co-infection and co-expression.[42] Similarly, the association of *qnr* genes and *aac(6′)-ib-cr* with ESBL-encoding genes reinforces the possibility of co-selection.[43–45] All this confirms ESBL-PE multiresistant nature that strongly limits the therapeutic arsenal and promotes the massive use of carbapenems.[46] However, in our study, ESBL-PE showed imipenem resistance rates between 1.2% and 3.6% for clinical isolates and of 7.7% for carriage isolates. This highlights the emergence of resistance to carbapenems that have always been fully active against ESBL-PE. The same finding was reported by Toudji *et al.* in Lomé (Togo).[35] Although still low, resistance to carbapenems can lead to therapeutic impasse, resulting in excess mortality[47,48] because carbapenems are the treatment of choice for ESBL-PE infections.[49] Conversely, fosfomycin retained good activity against ESBL-PE detected in carriage samples (0% resistance). This is a good thing because fosfomycin may be useful in simple urinary tract infections (cystitis).[40]

Overall, resistance rates to other antibiotics were higher in ESBL-PE isolates of inpatients than outpatients, and in ESBL-PE isolates from carriage samples (except for gentamicin and fosfomycin). As anal swabs were taken exclusively from inpatients, these findings confirm hospitalization as one of the risk factors for the development of resistance, due to the high selection pressure of antibiotics.[50]

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FIG. 3. Geographical distribution of resistance genes detected in ESBL-PE isolates in Gabon. **(A)** ESBL-encoding genes; **(B)** carbapenemase-encoding genes; **(C)** PMQR-encoding genes. PMQR, plasmid-mediated quinolone resistance.

Table 4. Phylogenetic Groups of ESBL-Producing *Escherichia coli* Isolates

Samples	*Phylogenetic group*	*Patient type*	*Origin*
Clinical (n=36)	*A* (n=13)	Inpatients (n=08)	Urine (n=08)
		Outpatients (n=05)	Urine (n=05)
	B1 (n=01)	Inpatients (n=0)	
		Outpatients (n=01)	Stool (n=01)
	B2 (n=13)	Inpatients (n=05)	Urine (n=02) Bedsore (n=02) Venous catheter (n=01)
		Outpatients (n=08)	Urine (n=08)
	D (n=04)	Inpatients (n=04)	Urine (n=01) Bedsore (n=02) Wound (n=01)
		Outpatients (n=0)	
	Clade I (n=02)	Inpatients (n=02)	Urine (n=01) Bedsore (n=01)
		Outpatients (n=0)	
	Undefined (n=03)		
Anal swabs (n=08)	*A* (n=03)	Inpatients (n=03)	
		Outpatients (n=0)	
	B1 (n=0)		
	B2 (n=04)	Inpatients (n=04)	
		Outpatients (n=0)	
	D (n=0)		
	Clade I (n=01)	Inpatients (n=01)	

In our study, most ESBL-PE isolates (both clinical and carriage samples) produced CTX-M group 1 (94% and 92.3%, respectively), represented exclusively by the $bla_{\text{CTX-M-15}}$ gene. In ESBL-PE isolates, $bla_{\text{CTX-M-15}}$ is the most frequently detected in Africa[51,52] and also worldwide, with the exception of Western Pacific where $bla_{\text{CTX-M-14}}$ is the predominant gene.[53,54] High $bla_{\text{CTX-M-15}}$ frequency was previously observed in Gabon (84.1%), Angola (98%), Chad (96.7%), and Algeria (89.2%).[17,34,55,56] In 85.7% and 92.3% of clinical and carriage ESBL-PE isolates, $bla_{\text{CTX-M-15}}$ was associated with $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, and/or $bla_{\text{SHV-1}}$. These combinations of β-lactamase-encoding genes reduce the therapeutic choice.[57] The present study highlights not only the countrywide dissemination of ESBL-PE, but also the co-expression of PMQR genes throughout Gabon. In some cities (Libreville, Mouila, and Tchibanga), ESBL-PE isolates carried also the $bla_{\text{OXA-48}}$ or $bla_{\text{NDM-5}}$ carbapenemase-encoding gene. A similar distribution of different resistance genes was described by Tani *et al.* in the northern regions of Algeria.[58] This means that bacterial resistance concerns all hospitals and all environments (rural or urban).

Our study revealed that ESBL-producing *E. coli* isolates belonged to four different groups (A, B1, B2, D) and that phylogroups A and B2 were the most common. Similarly, Smati *et al.* in 2013[59] showed that in France, *E. coli* belonged to these four phylogenetic groups, and that phylogroups A and B2 were proportionally more important (74% and 70%, respectively).

Conclusion

This study highlights ESBL-PE ubiquity throughout Gabon, with a particularly high prevalence in hospitalized patients, and also their multiresistant character through the co-expression of several other antibiotic resistance genes. Carriage of the same resistance genes in the digestive tract of inpatients confirmed the intestinal microbiota as a site of emergence of many antibiotic resistance factors, and of amplification and dissemination within the population. To reduce the risk of antibiotic resistance, health care centers must reinforce hospital hygiene measures, put in place a monitoring system for circulating resistance genes, and regulate the use of antibiotics by reasoned prescription.

Ethics Approval

This study was approved by the ethics board of each hospital and by the Staff of the Gabonese military health service (No. 00000228/MDN/DGSSM/DCP).

Authors' Contributions

Conceptualization: S.G., Visualization: M.B., Methodology: E.-B.N., Investigation: A.-C.D., Formal analysis: L.B., Supervision: R.O., Writing-original draft preparation: A.-C.D., Validation: S.G.

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