Annales Africaines de Médecine
Article original

Phenotypic detection of Extended-spectrum beta-lactamases and antibiotic resistance of *Enterobacteriaceae* strains isolated from hospitalized patients in the Ngaliema Clinic of Kinshasa, the Democratic Republic of Congo

Détection phénotypique des Bétalactamases à Spectre Elargi et Résistance aux antibiotiques des souches d'entérobactéries isolées des malades hospitalisés à la Clinique Ngaliema de Kinshasa, République Démocratique du Congo

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Résumé

Contexte et objectifs: La prévalence croissante des entérobactéries productrices de bêta-lactamases à spectre étendu (BLSE) devient un problème de santé publique mondial. L'objectif de la présenté étude était de détecter les BLSE et d'évaluer la résistance aux bêta-lactamines des souches d'entérobactéries isolées chez des patients hospitalisés.

Méthodes : Les entérobactéries ont été identifiées par des méthodes conventionnelles. Le profil de résistance aux antibiotiques a été déterminé à l'aide de la méthode de diffusion sur disque. La production de BLSE a été confirmée à l'aide d'une méthode de disque combiné.

Résultats : Sur un total de 230 souches isolées, les pathogènes prédominants étaient *Escherichia coli* 105/230 (45,7 %), suivi de *Klebsiella pneumoniae* 34/230 (14,8 %) et de *Citrobacter diversus* 24/230 (10,4 %). Parmi ces 230 souches, 157 (68,3 %) avaient un phénotype β-lactamase à spectre étendu. *Escherichia coli* 72 (72,3 %), *Klebsiella pneumoniae* 17 (50,0 %), *Enterobacter agglomerans* 15 (75,0 %) et *Citrobacter diversus* 18 (75,0 %) étaient les plus importants producteurs de BLSE. La majorité des souches étaient hautement résistantes aux pénicillines et aux céphalosporines.

Conclusion : Les résultats obtenus ont montré une prévalence élevée d'entérobactéries productrices de BLSE. Les carbapénèmes étaient les antibiotiques bêta-lactamines actifs contre les entérobactéries productrices de BLSE.

Mots-clés : Bétalactamases à spectre étendu, *Enterobacteriaceae*, Résistance aux antibiotiques, République Démocratique du Congo

Summary

Context and objective: The increasing prevalence of extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae* is becoming a global public health concern. This study aimed to detect ESBLs and to evaluate the resistance to beta-lactams of the strains of *Enterobacteriaceae* isolated from hospitalized patients.

Methods: Clinical samples were collected from hospitalized patients at Clinique Ngaliema, Kinshasa. *Enterobacteriaceae* were identified by microbiological conventional methods. Antibiotic resistance pattern was determined using the disk diffusion method. ESBLs production was confirmed using a combination disk method.

Results: In a total of 230 enterobacterial strains isolated, the predominant pathogens were *Escherichia coli* 105/230 (45.7 %), followed by *Klebsiella pneumoniae* 34/230 (14.8 %), and *Citrobacter diversus* 24/230 (10.4 %). Among these 230 strains, 157 (68.3 %) had an ESBL phenotype. *Escherichia coli* 72 (72.3 %), *Klebsiella pneumoniae* 17 (50.0 %), *Enterobacter agglomerans* 15 (75.0%), and *Citrobacter diversus* 18 (75.0 %) were the most important ESBL producers. All strains were highly resistant to penicillins (88.3 % - 99.6 %, with the exception for piperacillin+ tazobactam (39.1 %), and cephalosporins (Resistance rate 83.5 % - 96.5 %; exception for cefoxitin 62.2 %, and cefepime 37.8 %).

*Conclusion***:** The results of the present study showed a high prevalence of ESBL producing-*Enterobacteriaceae*. Carbapenems were the active beta-lactam antibiotics against ESBL-producing *Enterobacteriaceae*.

Keywords: Extended-Spectrum β-lactamase, β-lactams, *Enterobacteriaceae*, antibiotic resistance, Democratic Republic of the Congo

https://dx.doi.org/10.4314/aamed.v17i2.2

Received: July 28th, 2023
Accepted: January 14th, 2024

Annales Africaines de Médecine
Article original

https://dx.doi.org/10.4314/aamed.v17i2.2
Reçu le 28 juillet 2023
Accepté le 14 janvier 2024

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Introduction

The Family of *Enterobacteriaceae* encompasses many Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella* spp and *Enterobacter* spp, which are involved in community-associated as well as healthcare-associated infections displaying high level of antimicrobial resistance (1). *Enterobacteriaceae* had become resistant to β-lactam antibiotics due to the production of extended-spectrum beta-lactamases (ESBLs). ESBLs are a group of enzymes that can hydrolyze a variety of β-lactams including fourth generation cephalosporins and compromise the efficacy of all β-lactams, except cephamycins and carbapenems (2). ESBLs were found first in *Klebsiella pneumonia* and then predominantly in *E. coli*. The most frequently encountered ESBLs belong to the CTX-M, SHV, and TEM families (3). ESBL-producing Gram-negative pathogens are a major cause of resistance to expanded-spectrum β-lactam antibiotics. Since their discovery in the early 1980s, they have spread worldwide and are now endemic in *Enterobacteriales* isolated from both hospital-associated and community-acquired infections. As a result, they are a global public health concern (3). The standard treatment of infections caused by ESBL-secreting *Enterobacteriaceae* is based on the use of carbapenems. However, their usefulness is threatened by the emergence and spread of bacteria that produce carbapenemase enzymes (4-5).

Several reports indicated that ESBL producing *Enterobacteriaceae* are spreading and endemic in many African countries because of their capability to persist and to disperse in environmental hospital (6-8). Studies on detection of ESBL producers have also been conducted in DRC (9-11), but our bacteriology laboratories do not routinely detect ESBL secretion, despite the emergence and dissemination of antibiotic-resistant strains. When ESBLs are not identified in a timely manner, appropriate antimicrobial therapy is frequently delayed, resulting in poor clinical outcomes (3). Thus, detection and prevention of dissemination of ESBL producers in hospital constitute a veritable challenge. This study was undertaken to determine the antibiotic resistance profile of *Enterobacteriaceae* to β-lactams and the prevalence ESBL-producing *Enterobacteriaceae*.

Methods

Sample collection and bacteria identification

This study was conducted from January 2019 to December 2020 in the Clinique Ngaliema in Kinshasa. A total of 230 biological samples were collected from the patients who were hospitalized in surgery (n=134), internal medicine (n=41), pediatric (n=28), gynecology (n=09) and intensive care unit (n=18). The enterobacterial

strains were isolated from the following biological samples and invasive devices: urine (n=94), pus from surgical site infections (n=86), urinary catheter tubes (n=18), gastric juice samples (n=22), vaginal smears (n=03), blood (n=04), effusion fluid (n=02), and central venous catheters (n=01). Urinary samples were cultured on Hektoen agar medium (Liofilchem, Roseto degli Abruzzi, Italy). Colonies were counted onto Cystein Lactose Electrolyte Deficient (CLED) agar (Liofilchem, Roseto degli Abruzzi, Italy). Cultures were considered positive when 10^5 Colony Forming Units (CFU) were grow. The remaining samples were cultured on Mac Conkey Agar ((Liofilchem, Roseto degli Abruzzi, Italy). Inoculated plates were incubated at 37°C for 24 hours. *Enterobacteriaceae* isolated were identified using microbiological conventional methods including Gram staining, oxidase test, indole and urease production, citrate utilization, hydrogen sulphide and gas production, and fermentation of sugars.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the isolated enterobacterial strains carried out on Mueller-Hinton agar using the standard disk diffusion method to determine their antibiotic resistance patterns. The following antibiotic disks (Liofilchem, Roseto degli Abbruzzi, Italy) were used: amoxicillin(25µg) amoxicillin + clavulanic acid (20/10µg), piperacillin (100µg), piperacillin + tazobactam (100/10µg), cefazolin (30µg), cefuroxime (30µg), cefoxitin (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefixime (5µg), cefepime (30µg), imipenem (10µg), ertapenem (E10µg), meropenem (10µg). The Evaluation of the results was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (12). *E. coli* ATCC 25922 was used for quality control.

Screening for ESBLs production

The screening for ESBLs production from the isolated enterobacterial strains was performed by testing their susceptibility to ceftazidime and cefotaxime. Thus, all isolates that showed resistance for ceftazidime and/ or for cefotaxime were suspected for ESBL producer.

The confirmation of the production of ESBLs was confirmed by the combined disk test with cefotaxime and ceftazidime alone and in combination with clavulanic acid. The following combination disks were used: cefotaxime 30µg + clavulanic acid 10µg, and ceftazidime 30µg + clavulanic acid 10µg (Liofilchem, Roseto degli Abruzzi, Italy). Disks were placed at a distance of 25 mm on a Muller Hinton plate inoculated with bacterial suspension of 0.5 McFarland turbidity and incubated 18-24 hours at 37°C. An increase in the inhibition zone diameter of $\geq$ 5 mm for a combination disc versus ceftazidime or cefotaxime disk alone was confirmed as ESBL producing *Enterobacteriaceae* according to CLSI (2018) guidelines [13]. For ESBL testing, *K. pneumoniae* ATCC 700603 was used as a (positive control) and *E. coli* ATCC 25922 was used as a (negative control) strains.

Screening for OXA-48 production

The screening for OXA-48 production was done on isolated enterobacterial strains resistant to imipenem, meropenem, and ertapemen which were considered as potential OXA-48 producers. Then, OXA-48 producing *Enterobacteriaceae* were screened on Chromatic™ OXA-48 chromogenic medium (Liofilchem, Roseto degli Abruzzi, Italy). After incubation at 37°C/24-48 hours, the color and the morphology of the colonies were observed and the results interpreted as follow: red colony (*E. coli*-producing OXA-48), blue-violet colony (*Klebsiella* spp producing OXA-48), blue-green (*Enterobacter* spp producing OXA-48), blue colony with red halo (*Citrobacter* spp producing OXA-48).

Ethical considerations

Not Applicable. Samples were collected for the diagnostic purpose.

Results

Isolated strains of Enterobacteriaceae

Among the 230 strains of *Enterobacteriaceae* isolated from various samples obtained from some hospitalized patients of the Clinique Ngaliema, 45.7 % (105/230) of them were *Escherichia coli*, followed by *Klebsiella pneumoniae* (14.8 %), *Citrobacter diversus* (10.4 %), *and Enterobacter agglomerans* (8.7%), as presented in Table 1. Other enterobacterial species were also identified: *Citrobacter freundii* (4.3 %), *Enterobacter cloaceae* (4.0 %), *Proteus mirabilis* (3.0 %), *Proteus vulgaris* (2.2 %), *Providencia rettgeri* (2.2 %), *Enterobacter gergoviae* (1.7 %), *Klebsiella oxytoca* (1.7 %), *Klebsiella ozaenae* (0.9 %), and *Salmonella* spp (0.4%). The majority of isolates were from surgery and internal medicine departments.

Table 1. Distribution of isolated strains of *Enterobacteriaceae*

Strains	Unit					Total
	Surgery N=135	Internal medicine N=41	Pediatric N=27	Resuscitation N=18	Gynecology N=9	N (%) N= 230
Escherichia coli	57	23	8	11	6	105 (45.7)
Klebsiella pneumoniae	18	4	11	1	0	34 (14.8)
Citrobacter diversus	13	4	4	1	2	24 (10.4)
Enterobacter agglomerans	11	3	3	2	1	20 (8.7)
Citrobacter freundii	7	2	0	1	0	10 (4.3)
Enterobacter cloacae	7	2	0	0	0	9 (4.0)
Proteus mirabilis	7	0	0	0	0	7 (3.0)
Proteus vulgaris	4	0	0	1	0	5 (2.2)
Providencia rettgeri	4	1	0	0	0	5 (2.2)
Enterobacter gergoviae	3	1	0	0	0	4 (1.7)
Klebsiella oxytoca	2	1	1	0	0	4 (1.7)
Klebsiella ozaenae	2	0	0	0	0	2 (0.9)
Salmonella spp	0	0	0	1	0	1 (0.4)

Antibiotic resistance pattern of Enterobacteriaceae isolates

High resistance rates were found against penicillins and cephalosporins as reported in Table 2. Out of 230 strains, 229/230 (99.6%) were resistant to amoxicillin, 220/230 (95.7 %) to piperacillin, 203/230 (88.3 %) resistant to amoxicillin-clavulanic acid, and 90 (39.1 %) resistant to the piperacillin-tazobactam combination. Enterobacteriaceae strains were also highly resistant to cephalosporins: 222/230 (96.5%) strains were resistant to cefazolin, 202/230 (87.8 %) to cefuroxime, 198/230 (86.1%) to cefotaxime, and 196/230 (85.5 %) resistant to cefixime. The resistance rate against cefoxitin was 62.2 % (143/230). Carbapenems were demonstrated to be very effective antibiotics by in vitro tests. The resistance rates of Enterobacteriaceae strains against imipenem, ertapenem, and meropenem were respectively 10.4 % (24/230), 6.5 % (15/230), and 4.5 % (11/230).

Using the commonly used definition of multidrug resistance (MDR) as an organism being resistant to three or more classes of antibiotics, the majority of isolated enterobacterial strains must be considered as MDR.

Table 2. The antibiotic resistance rate of *Enterobacteriaceae* strains isolated from clinical specimens

Species	N° of isolates	Penicillins				Cephalosporins								Carbapenems		
		AMX n (%)	AMC n (%)	PRL n (%)	TZP n (%)	CTX n (%)	CFM n (%)	CAZ n (%)	CRO n (%)	CXM n (%)	FOX n (%)	FEP n (%)	KZ n (%)	IMI n (%)	ERT n (%)	MRP n (%)
E. coli	105	104 (99)	86(81.9)	98(93.3)	39(37.1)	91 (86.6)	88(81.9)	91(86.7)	86(81.9)	91(86.)	63(60.0)	35(33.3)	101(96.2)	14(13.3)	5(4.8)	5(4.8)
K. pneumo	34	34 (100)	29(85.3)	33(97)	19(55.8)	26(76.5)	26(76.5)	26(76.5)	29(82,24)	29(82,24)	22(64.7)	17(50.0)	31(91.2)	2(5.9)	2(5.9)	2(5.9)
E. agglom	20	20 (100)	20(100)	20(100)	9(45)	19(95)	19(95)	17(85)	7(85)	7(85)	15(75)	10(45)	20(100)	2 (10)	1 (5)	
K. ozaenae	2	2 (100)	2(100)	2(100)	2(10)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	1(50)	2(100)			
C. diversus	24	24(100)	23 (95.8)	24(100)	9(37.5%)	21(87.5)	23(95.8)	22(91.6)	22(91.6)	23(95.8)	15(62.4)	7(29.1)	24(100)	1(4.1)	2(8.3)	1(4.1)
K. oxytoca	4	4(100)	3 (75)	4(100)	2(50)	4(100)	2(50)	4(100)	4(100)	4(100)	2(50)	0	4(100)			
C. freundii	10	10(100)	10(100)	10(100)	6(60)	10(100)	10(100)	10(100)	9(90)	10(100)	8(80)	7(70)	10(100)	4(40)	5(50)	2(20)
Salmonella ssp	1	1(100)	0	0	0	0	1(100)	0	0	1(100)	0	0	0			
P. vulgaris	5	5(100)	5(100)	5(100)	0	4(80)	5(100)	4(80)	5(100)	4(80)	2(40)	2(40)	5(100)			
P. mirabilis	7	7(100)	7(100)	7(100)	1(14.2)	6(85.7)	6(85.7)	5(71.4)	6(85.7)	6(85.7)	4(57.1)	4(57.1)	7(100)			
E. gergoviae	4	4(100)	4(100)	4(100)	1(25)	2(50)	2(50)	1(25)	2(50)	4(100)	1(25)	2(55)	4(100)	1(25)		
E. cloacea	9	9(100)	9(100)	8(88.8)	2(11.1)	8(88.9)	7(77.7)	5(55.5)	6(66.6)	7(77.7)	7(77.7)	4(44.4)	9(100)			1(11.1)
P. rettgeri	5	5(100)	5(100)	5(100)	2(40)	5(100)	5(100)	5(100)	5(100)	5(100)	2(40)	0	5(100)			
TOTAL	**230**	**229(99.6)**	**203(88.3)**	**220(95.7)**	**90(39.1)**	**198(86.1)**	**196(85.2)**	**192(83.5)**	**192(83.5)**	**202(87.8)**	**143(62.2)**	**87(37.8)**	**222(96.5)**	**24(10.4)**	**15(6.5)**	**11(4.8)**

AMX: amoxicillin; AMC: amoxicillin clavulanic acid; PRL: Piperacillin; TZP: piperacillin + tazobactam; KZ: cefazolin; CFM: cefuroxime; FOX: cefoxitin; CTX: cefotaxime; CAZ: ceftazidime; CXM: Cefixim; CRO: ceftriaxone; FEP: cefepime; IPM: imipenem; ERT: ertapenem; MRP: Meropenem

ESBL and OXA-48 producing Enterobacteriaceae strains

ESBLs were detected in 157 (68.3 %) isolated strains of *Enterobacteriaceae* (Table 3), among them 76 strains of *Escherichia coli* (72.3 %), 17 strains of *Klebsiella pneumoniae* (50 %), 15 strains of *Enterobacter agglomerans* (75.0 %), 18 strains of *Citrobacter diversus* (75.0 %), 6 strains of *Proteus mirabilis* (85.7 %), 6 strains of *Enterobacter cloacae* (66.7 %), 5 strains of *Providencia rettgeri* (100.0 %), 5 strains of *Citrobacter freundii* (50.0%), 4 strains of *Proteus vulgaris* (80.0 %), 3 strains of *Klebsiella oxytoca* (75.0 %), 1 strain of *Enterobacter gergoviae* (25.0 %), and strain of 1 *Klebsiella ozaenae* (50.0 %).

Table 3. ESBL producers among different isolates

Species	N° of isolates N= 230	ESBL producers N° N=157 (%)	Non-ESBL producers N° (%) N= 73
Escherichia coli	105	76 (72.3)	29 (27.7)
Klebsiella pneumoniae	34	17 (50)	17 (50)
Enterobacter agglomerans	20	15 (75)	5 (25)
Klebsiella ozaenae	2	1 (50)	1 (50)
Citrobacter diversus	24	18 (75)	6 (25)
Klebsiella oxytoca	4	3 (75)	1 (25)
Citrobacter freundii	10	5 (50)	5 (50)
Salmonella ssp	1	0	1 (100)
Proteus vulgaris	5	4 (80)	1 (20)
Proteus mirabilis	7	6 (85.7)	1 (14.3)
Enterobacter gergoviae	4	1 (25)	3 (75)
Enterobacter cloacae	9	6 (66.7)	3 (33.3)
Providencia rettgeri	5	5 (100)	0

The majority of the ESBL-producing strains were isolated from urines, followed by them of pus, gastric fluid and urine catheter tubes (Table 4).

Table 4. Distribution of ESBL-producers in various clinical specimens

Samples	N° of isolates N= 230	ESBL producers n (%) N=157	Non- BLSE producers n (%) N= 73
Urines	94	67 (71.3)	27 (28.7)
Pus	86	55 (64)	31 (36)
Urine catheter tubes	18	14 (77.8)	4 (22.2)
Blood	4	0	4 (100)
Central venous catheters	1	1 (100)	0
Vaginal smears	3	0	3 (100)
Effusion fluids	2	2 (100)	0
Gastric juice	22	18 (81.8)	4 (18.2)

Most of the strains resistant to beta-lactams used in this study were ESBLs producers (Table 5).

High resistance rates were observed in ESBL-producing strains than in non-producers: amoxicillin 157/229 (78.7 %), amoxicillin + clavulanic acid 142/203 (69.9 %), piperacillin 154/220 (70.0 %), piperacillin + tazobactam 60/90 (66.6 %), cefotaxime 156/198 (78.7 %), cefuroxime 151/196 (77.0 %), ceftazidime 147/192 (76.5%), ceftriaxone (148/192(77.0 %), ceftriaxone 154/202 (67.8 %), cefepime 67/87 (77.0 %), and cefazolin 157/222 (70.7 %).

None of the ESBL-producing strains of *Enterobacteriaceae* which were resistant to carbapenems was identified as OXA-48-producer.

Table 5. ESBL production and Resistance to penicillins and cephalosporins

ESBL	Resistance to penicillins			Resistance to cephalosporins								
	AMX n (%)	AMC n (%)	PRL n (%)	TZP n (%)	CTX n (%)	CFM n (%)	CAZ n (%)	CRO n (%)	CXM n (%)	FOX n (%)	FEP n (%)	KZ n (%)
Non-ESBL producers N° (%)	72 (31.5)	61(30.1)	66(30.0)	30(33.4)	42(21.3)	45(23.0)	45(23.5)	44(23.0)	48(23.8)	46(32.2)	20(23.0)	65(29.3)
ESBL producers N° (%)	157(78.7)	142(69.9)	154(70.0)	60(66.6)	156(78.7)	151(77.0)	147(76.5)	148(77.0)	154(76.2)	97(67.8)	67(77.0)	157(70.7)
Total	229(100)	203(100)	220(100)	90(100)	198(100)	196(100)	192(100)	192(100)	202(100)	143(100)	87(100)	222(100)
P value	0,142	0,002	0,008	0,677	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	0,489	0,018	<0,0001

AMX: amoxicillin; AMC: amoxicillin + clavulanic acid; PIP: piperacillin; TZP: piperacillin + tazobactam; KZ: cefazolin; CFM: cefuroxime; FOX: cefoxitin; CTX: cefotaxime; CAZ: ceftazidime; CXM: cefixime; CRO: ceftriaxone; FEP: cefepime

Discussion

The present study was undertaken in order to evaluate the resistance level to beta-lactams (penicillins, cephalosporins and carbapenems) of *Enterobacteriaceae* clinical strains isolated from hospitalized patients in the Clinique Ngaliema, a tertiary hospital in Kinshasa, and to detect the production of ESBLs. The results of the present study showed that *Escherichia coli* (45.7 %) was the predominant pathogen found in the patient's samples, followed by *Klebsiella pneumoniae* (14.8 %) and *Citrobacter diversus* (10.4 %) (**Table 1**). Our results were in consistence with recent studies conducted in Ethiopia and Gabon in which *E. coli* and *K. pneumoniae* were the major bacteria isolated from clinical samples (14,15). In this study, a very high resistance level was observed against penicillins (ampicillin, 99.6 %; amoxicillin + clavulanic acid, 88.3%; piperacillin, 95.7 %), in exception of the association piperacillin-tazobactam (39.1 %). Furthermore, highest resistance rates were also obtained against the first generation (cefazolin, 96.5 %), the second generation (cefuroxime, 85.2 %; cephamycin cefoxitin, 62.2 %), the third generation (cefotaxime, 86.1 %; cefixime, 87.8 %; ceftriaxone, 83.5 %; ceftazidime, 83.5 %), and the fourth generation cephalosporins (cefepime, 37.8 %). Comparable results were reported from studies performed in Tanzania (16), Ethiopia (14) and Sierra Leone (17). The majority of the isolated clinical strains of *Enterobacteriaceae* was multidrug bacteria. Thus, this high MDR level recorded in this study could be considered as alarming, because of the remaining few options for the treatment of such kind of infections. In contrast to the other beta-lactams, carbapenems were most active against *Enterobacteriaceae* isolates, accordingly to their lower resistance rates (meropenem, 4.5%; ertapenem, 6.5%; imipenem, 10.4%), as it was reported in previous studies (14, 18-20). Strains of *C. freundii* were the most resistant to cefoxitin (80 %), cefepime (70 %), piperacillin + tazobactam (60 %), ertapenem (50 %), imipenem (40 %) and meropenem (20 %).

Concerning the prevalence of ESBL production among the isolated clinical strains of *Enterobacteriaceae*, a high global rate of the ESBL-producing strains (68.3 %) was observed in the present study. Our observation was in accord with those reported in earlier studies from Uganda (89 %)(21), Ghana (61 %) (22), and Mali (61.8 %) (23). However, this prevalence of ESBLs producers was higher than those found in other studies done in Nigeria (58.0 %) (24), Ethiopia (55.6%) (25), Cameroon (55.3 %) (26), and Chad (47.7 %) (27). This wide variation could be due to differences in study population, type of specimen, sample size and the extent of antibiotic use (14). In exception of *Salmonella spp* and *Enterobacter gergoviae*, all enterobacterial species isolated in this work had very high rates of ESBL production ranged from 50 % to 100 %, as reported in Table 3. In term of production prevalence, the predominant ESBL-producing species were *P. rettgeri* (100 %), *P. mirabilis* (85 %) and *P. vulgaris* (80 %), followed by *C. diversus* (75 %), *E. agglomerans* (75 %), *K. oxytoca* (75 %), *E. coli* (72 %), *E. cloacae* (66.7 %), *C. freundii* (50 %), *K. pneumoniae* (50 %) and *K. ozaenae* (50 %). Results obtained in previous studies in other African countries reported *E. coli* and *K. pneumoniae* as predominant ESBLs [23, 28]. In the other hand, the predominant ESBL-producing genus were *Providencia* (100 %) and *Proteus* (83,3 %), followed by *Escherichia* (72 %), *Citrobacter* (67.6 %), *Enterobacter* (66.7 %) and *Klebsiella* (58.3 %). Furthermore, highest prevalence of ESBL-producing strains was found among clinical enterobacterial strains isolated in gastric juice (81.8 %), urine catheter tubes (77.8 %), urine (71.3 %) and pus samples (64 %).

Comparing the ESBL-production with the resistance level against beta-lactams used in this study, we found that ESBL-producing strains of *Enterobacteriaceae* presented more than 2 times higher rates of resistance to penicillins, cephalosporins and carbapenems than non ESBL producers. Our findings were in accordance with those reported by previous African studies done in Ethiopia, Uganda and Ghana (14, 29-31). However, the resistance levels to carbapenems observed in the present work were lesser than those reported in a study from Ghana where bacteria strains displayed susceptibility rates of 99.2% to imipenem, 97.7 % to meropenem, and 98.5% to ertapenem (14). The non-production of OXA-48 carbapenemase suggested that the isolated enterobacterial strains resistant to carbapenems would use other resistance mechanisms.

Conclusion

In this study, we detected multidrug-resistant ESBLs-producing *Enterobacteriaceae* strains isolated from clinical samples of hospitalized patients in the Clinique Ngaliema. The most effective antibiotics for treatment of the identified

gram negative ESBLs producers were the association piperacillin-tazobactam, imipenem, meropenem and ertapenem. Resistant strains of enterobacteria harboring ESBLs are potentially in circulation in this hospital and must be considered as a source of community- or hospital-acquired infections. A programm of surveillance of bacterial antibiotic resistance must be instituted in this hospital.

Source of funding
This work was supported by a grant from Académie de Recherche et d'Enseignement Supérieur (ARES) of Belgium. CURS-RAM/ (URC-AMRS) is a project supported by a grant from ARES.

Conflict of Interest: Authors declare no conflicts of interest

Author's contribution:
Jean-Robert Tundru Angho, Jean-Francois Baleka Fefe, and Irénée Abibi Amegiede contributed to the processing of samples and antibiotic susceptibility tests. Jean-Marie Liesse Iyamba and Ntondo za Balega Takaisi Kikuni contributed to the conception of the study, the analysis of the results and the preparation of the manuscript. All the authors have read and approved the final version of the manuscript

Acknowledgements
We thank Microbiology Laboratory staff members of Clinique Ngaliema, Kinshasa, for their collaboration and assistance during the study.

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Cite this article as: Angho JRT, Fefe JFB, Amegiede IA, Iyamba JML, Takaisi-Kikuni NB. Phenotypic detection of Extended-spectrum beta-lactamases and antibiotic resistance of Enterobacteriaceae isolated from hospitalized patients in the Ngaliema Clinic of Kinshasa, the Democratic Republic of the Congo. *Ann Afr Med* 2024; **17** (2): e5437-e5447. https://dx.doi.org/10.4314/aamed.v17i2.2