

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

Jean-Robert Tundru Angho ^{1,2}, Jean-Francois Baleka Fefe ², Irénée Abibi Amegiede ³, Jean-Marie Liesse Iyamba ¹, NB Takaisi-Kikuni ¹

Corresponding author

Jean-Robert Tundru Angho Courriel: <u>biotundru@gmail.com</u>

University of Kinshasa

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, pathogènes prédominants les 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.

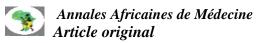
Results: In a total of 230 enterobacterial strains isolated, the predominant pathogens were Escherichia coli 105/230 (45.7 %), followed by Klebsiella pneumoniae 34/230 (14.8 %), and Citrobacter diversus 24/230 (10.4 %). Among these 230 strains, 157 (68.3 %) had an ESBL phenotype. Escherichia coli 72 (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-lactamas, *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



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

- University Reference Center of Antimicrobial Resistance Surveillance (URC-AMRS), Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo.
- Laboratory of Microbiology, Clinique Ngaliema, Kinshasa, Democratic Republic of Congo
- University Clinics of Kisangani, Faculty of Medicine and Pharmacy, Kisangani, Democratic Republic of Congo

Introduction

The Family of *Enterobacteriaceae* encompasses Gram-negative bacteria, such 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 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 expandedspectrum β-lactam antibiotics. Since their discovery in the early 1980s, they have spread worldwide now endemic and are Enterobacteriales isolated from both hospitalassociated 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 environmental hospital (6-8).Studies 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 **ESBL-producing** the prevalence 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 10⁵ 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 $10\mu g$, and ceftazidime $30\mu g$ + clavulanic acid $10\mu g$ (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 ≥ 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 screened on ChromaticTM 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. coliproducing 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

			Unit		Total	
Strains	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.

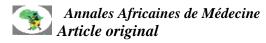
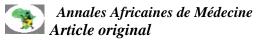


Table 2. The antibiotic resistance rate of *Enterobacteriaceae* strains isolated from clinical specimens

Species		Penicillins					Cephalosporins								Carbapenems		
	N° of isolates	AMX	AMC	PRL	TZP n (%)	CTX n (%)	CFM	CAZ	CRO	CXM	FOX	FEP	KZ	IMI	ERT	MRP	
		n (%)	n (%)	n (%)	, ,	` ′	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	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(10 0)	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(8 6.1)	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	ESBL producers N°	Non-ESBL producers N° (%)				
-	N=230	N=157 (%)	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 (%)	Non- BLSE producers n (%)			
		N=157	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.

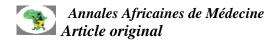
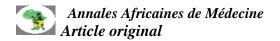


Table 5. ESBL production and Resistance to penicillins and cephalosporins

	Resistance	to penicillins	1									
ESBL	Resistance to cephalosporins											
	AMX	AMC	PRL	TZP	CTX	CFM	CAZ	CRO	CXM	FOX	FEP	KZ
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	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 betalactams, 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 %).

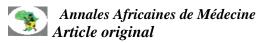
Concerning the prevalence of ESBL production among the isolated clinical strains of *Enterobacteriaceae*, a high global rate of the ESBL-producing strains (68.3 %) was observed in the present study. Our observation was in accord with those reported in earlier studies from Uganda (89 %)(21), Ghana (61 %) (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 ESBLproducing 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 that ESBL-producing 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 nonproduction 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.

References

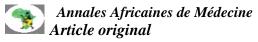
- 1. Van Duin D, Paterson DL. Multidrugresistant bacteria in the community: Trends and lessons learned. *Infect Dis Clin North Am.* 2016; **30**: 377-390.
- 2. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother*. 2010; **54**:969-976.
- Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC Antimicrob Resist*. 2021; 3: dlab092.
- 4. Kohlenberg A, Weitzel-Kage D, van der Linden P, Sohr D, Vögeler S, Kola A, Halle E, Rüden H, Weist K. Outbreak of carbapenem-resistant Pseudomonas aeruginosa infection in a surgical intensive care unit. *J Hosp Infect*. 2010; **74**: 350–357.

- 5. Cezário RC, Duarte De Morais L, Ferreira JC, Costa-Pinto RM, da Costa Darini AL, Gontijo-Filho PP. Nosocomial outbreak by imipenem-resistant metallo-b-lactamase-producing Pseudomonas aeruginosa in an adult intensive care unit in a Brazilian teaching hospital. *Enferm Infect Microbiol Clin.* 2009; 27: 269–274.
- Saravanan M, Ramachandran B, Barabadi H. The prevalence and drug resistance pattern of extended spectrum β-lactamases (ESBLs) producing Enterobacteriaceae in Africa. *Microb Pathog.* 2018; 114:180-192.
- 7. Sanke-Waïgana H, Mbecko JR, Ngaya G, Manirakiza A, Alain BA. 2003-2019: explosive spread of enterobacteria producing extended-spectrum betalactamases in Bangui Central African Republic. *Pan Afr Med J.* 2021; **39**:22.
- 8. Ouchar Mahamat O, Kempf M, Lounnas M, Tidjani A, Hide M, Benavides JA, *et al*. Epidemiology and prevalence of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae in humans, animals and the environment in West and Central Africa. *Int J Antimicrob Agents*. 2021; **57**:106203.
- 9. Phoba MF, Barbé B, Lunguya O, Masendu L, Lulengwa D, Dougan G, *et al.* Salmonella enterica serovar Typhi Producing CTX-M-15 Extended Spectrum β-Lactamase in the Democratic Republic of the Congo. *Clin Infect Dis.* 2017; **65**: 1229-1231.
- 10. Lunguya O, Lejon V, Phoba MF, Bertrand S, Vanhoof R, Glupczynski Y, *et al.* Antimicrobial resistance in invasive nontyphoid Salmonella from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta lactamases. *PLoS Negl Trop Dis.* 2013; 7: e2103.
- 11. Kalonji LM, Post A, Phoba MF, Falay D, Ngbonda D, Muyembe JJ, *et al.* Invasive Salmonella Infections at Multiple Surveillance Sites in the Democratic Republic of the Congo, 2011-2014. *Clin Infect Dis.* 2015; **61**: S346-S353.
- 12. Clinical & Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement. CLSI document. 2012; M 100-S22.
- 13. Clinical & Laboratory Standard Institute (CLSI). Performance standards for



- antimicrobial susceptibility testing. CLSI supplement. 2018; Vol. 26th ed., M100S.
- 14. Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH. Extended-spectrum Betalactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. *PLoS One*. 2020; **15**: e0241984.
- 15. Dikoumba AC, Onanga R, Boundenga L, Bignoumba M, Ngoungou EB, Godreuil S. Prevalence and Characterization of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae in Major Hospitals in Gabon. *Microb Drug Resist*. 2021; 27:1525-1534.
- 16. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram-negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *Biomed Res Int*; 2014:303104.
- 17. Leski TA, Taitt CR, Bangura U, Stockelman MG, Ansumana R, Cooper WH 3rd, Stenger DA, Vora GJ. (High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. *BMC Infect Dis.* 2016; **16**:167.
- 18. Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA, Newman MJ. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. *Am J Trop Med Hyg.* 2013; **89**: 960-964.
- 19. Irenge LM, Kabego L, Vandenberg O, Chirimwami RB, Gala JL. Antimicrobial resistance in urinary isolates from inpatients and outpatients at a tertiary care hospital in South-Kivu Province (Democratic Republic of Congo). *BMC Res Notes*. 2014; 7: 374.
- 20. Shashwati N, Kiran T, Dhanvijay AG. Study of extended spectrum β-lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital. *J Nat Sci Biol Med.* 2014; **51**:30-35.
- 21. Andrew B, Kagirita A, Bazira J. Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, Southwestern Uganda. *Int J Microbiol.* 2017:3183076.
- 22. Falgenhauer L, Imirzalioglu C, Oppong K, Akenten CW, Hogan B, Krumkamp R, et al.

- Detection and Characterization of ESBL-Producing Escherichia coli From Humans and Poultry in Ghana. *Front Microbiol.* 2019; **9**: 3358.
- 23. Sangare SA, Maiga AI, Guindo I, Maiga A, Camara N, Dicko OA, *et al.* Prevalence of ESBL-producing Enterobacteriaceae isolated from blood cultures in Mali. *J Infect Dev Ctries*. 2016; **10**:1059-1064.
- 24. Ibrahim Y, Sani Y, Saleh Q, Saleh A, Hakeem G. Phenotypic Detection of Extended Spectrum Beta-lactamase and Carbapenemase co-producing clinical isolates from Two Tertiary Hospitals in Kano, North West Nigeria. *Ethiop J Health Sci.* 2017; 27:3010.
- 25. Moges F, Gizachew M, Dagnew M, Amare A, Sharew B, Eshetie S, *et al.* Multidrug resistance and extended-spectrum betalactamase producing Gram-negative bacteria from three Referral Hospitals of Amhara region, Ethiopia. *Ann Clin Microbiol Antimicrob.* 2021; **20**:16.
- 26. Lonchel CM, Melin P, Gangoué-Piéboji J, Assoumou MC, Boreux R, De Mol P. Extended-spectrum β-lactamase-producing enterobacteriaceae in Cameroonian hospitals. *Eur J Clin Microbiol Infect Dis.* 2013; **32**: 79-87.
- 27. Ouchar Mahamat O, Lounnas M, Hide M, Dumont Y, Tidjani A, Kamougam K, *et al*. High prevalence and characterization of extended-spectrum β-lactamase producing Enterobacteriaceae in Chadian hospitals. *BMC Infectious Diseases*. 2019; **19**: 205.
- 28. Magale HI, Kassim IA, Odera SA, Omolo MJ, Jaoko WG, Jolly PE. Antibiotic susceptibility of organisms causing urinary tract infection in patients presenting at Kenyatta National Hospital, Nairobi. *East Afr Med J.* 2016; **2**:5.
- 29. Mulisa G, Selassie LG, Jarso G, Shiferew T, Zewdu A, Abebe W, *et al.*Prevalence of Extended Spectrum Betalactamase Producing Enterobacteriaceae: A cross sectional study at Adama Hospital, Adama, Ethiopia. *J Emerg Infect Dis.* 2016; 1:2–6.
- 30. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBLproducing Enterobacteriaceae in samples collected from patients in various wards of



Mulago Hospital, Uganda. *BMC Pharma and Toxic*. 2015; **16**:14.

31. Nkrumah NO, Danso KT, Krogfelt KA, Newman MJ. High Levels of Extended-Spectrum Beta-Lactamases in a Major Teaching Hospital in Ghana: The Need for Regular Monitoring and Evaluation of Antibiotic Resistance. *Am J Trop Med Hyg.* 2013; **89**: 960–964.

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