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Phenotypic detection of extended-spectrum β-lactamase Enterobacterales isolated from people living with HIV/AIDS in Kinshasa, Democratic Republic of the Congo

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Abstract:

Background: People living with HIV/AIDS (PLWHA) are prone to opportunistic bacterial infections caused by multidrug-resistant organisms. The aim of this study was to determine the susceptibility of Enterobacterales isolated from urine and stool samples of PLWA attending the BOYAMBI Hospital Center, Kinshasa, Democratic Republic of the Congo to commonly used antibiotics and to detect extended-spectrum β-lactamases (ESBLs) producers among the isolates.
Methodology: A total of 163 HIV-infected patients attending the BOYAMBI Hospital, Kinshasa, DRC, were randomly selected for this study. Urine samples were collected from 108 patients, while stool samples were collected from 55 of them. Samples were cultured in MacConkey agar and identified using conventional microbiological methods. Antibiotic susceptibility on each isolate to selected antibiotics was performed by the disc diffusion method. Phenotypic detection of ESBL was done by the double-disc synergy test.
Results: A total of 120 Enterobacterales were isolated from the samples of the 163 HIV-infected patients with 65 of 108 (60.2%) urine and 55 of 55 (100.0%) stool samples. *Escherichia coli* was the most frequent bacterial species from both urine and stool with 84 (70.0%), followed by *Klebsiella*specieswith23 (19.2%). Other bacterial pathogens were *Citrobacter* (n=6, 5.0%), *Enterobacter* (n=4, 3.3%), *Proteus* (n=2, 1.7%) and *Morganella* (n=1, 0.8%) species. *Escherichia coli* isolates were resistant to amoxicillin (90.5%), sulfamethoxazole-trimethoprim (81.0%), ciprofloxacin (77.4%), ceftriaxone (77.4%), ceftazidime (73.8%), amoxicillin–clavulanic acid (61.9%), imipenem (60.7%), and cefotaxime (50.0%). *Klebsiella pneumoniae* isolates were resistant to ceftazidime (95.7%), ceftriaxone (91.3%), imipenem (91.3%), ciprofloxacin (87%), sulfamethoxazole-trimethoprim (78.3%), cefotaxime (56.5%), and amoxicillin–clavulanic acid (52.2%). *Citrobacter, Enterobacter, Morganella* and *Proteus* species were resistant to the majority of antibiotics. The rate of ESBL production was 23.0% (28/120) with *Citrobacter* spp being the most frequent ESBL-producer, followed *Klebsiella* spp, *Enterobacter* spp and *E. coli*.
Conclusion: The results obtained showed a high rate of ESBL-producing Enterobacterales isolates which were multi-drug resistant. Nitrofurantoin, gentamicin, chloramphenicol, cefixime and nalidixic acid were the most active antibiotics against the isolates.

Keywords: Extended-spectrum β-lactamase, Enterobacterales, HIV/AIDS, Democratic Republic of the Congo

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Détection phénotypique des β-lactamases à spectre etendu chezles Entérobactérales isolées des personnes vivant avec le VIH/SIDA à Kinshasa, République Démocratique du Congo

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Résumé:

Contexte: Les personnes vivant avec le VIH/SIDA (PLWHA) développent les infections bactériennes opportunistes causées par des organismes multi-résistants. L'objectif de cette étude était de déterminer la sensibilité aux antibiotiques des Entérobacterales isolées à des échantillons d'urine et de selles de personnes vivant avec le VIH/SIDA fréquentant le Centre Hospitalier BOYAMBI, à Kinshasa, en République démocratique du Congo, et de détecter les souches productrices de bêta-lactamases à spectre étendu (BLSE).
Méthodologie: Un total de 163 patients infectés par le VIH fréquentant l'hôpital BOYAMBI été sélectionnés au hasard pour cette étude. Des échantillons d'urine ont été prélevés sur 108 patients, tandis que des échantillons de selles ont été prélevés sur 55 d'entre eux. Les échantillons ont été mis en culture sur une gélose MacConkey et identifiés à l'aide de méthodes microbiologiques conventionnelles. La sensibilité de chaque isolat aux antibiotiques sélectionnés a été déterminée par la méthode de diffusion en milieu gélosé de Mueller Hinton. La détection phénotypique des BLSE a été effectuée par le test de double synergie de disque.
Résultats: Au total, 120 Entérobactérales ont été isolées à partir des échantillons des 163 patients infectés par le VIH, 65 des 108 (60,2%) échantillons d'urine et 55 des 55 (100,0%) échantillons de selles. *Escherichia coli* était l'espèce bactérienne la plus fréquente à la fois dans l'urine et dans les selles avec 84 souches (70,0%), suivie par les espèces de *Klebsiella* avec 23 souches (19,2%). Les autres bactéries pathogènes étaient *Citrobacter* (n=6, 5,0%), *Enterobacter* (n=4, 3,3%), *Proteus* (n=2, 1,7%) et *Morganella* (n=1, 0,8%). Les isolats d'*Escherichia coli* étaient résistants à l'amoxicilline (90,5%), au sulfaméthoxazole-triméthoprime (81,0%), à la ciprofloxacine (77,4%), à la ceftriaxone (77,4%), à la ceftazidime (73,8%), à l'amoxicilline-acide clavulanique (61,9%), à l'imipénème (60,7%) et à la céfotaxime (50,0%). Les isolats de *Klebsiella pneumoniae* étaient résistants à la ceftazidime (95,7%), à la ceftriaxone (91,3%), à l'imipénème (91,3%), à la ciprofloxacine (87.0%), au sulfaméthoxazole-triméthoprime (78,3%), à la céfotaxime (56,5%) et à l'amoxicilline-acide clavulanique (52,2%). Les espèces *Citrobacter*, *Enterobacter*, Morganella et *Proteus* étaient résistantes à la majorité des antibiotiques. Le taux de production de BLSE était de 23,0% (28/120), *Citrobacter* spp étant le producteur de BLSE le plus fréquent, suivi de *Klebsiella* spp, *Enterobacter* spp et *E. coli*.
Conclusion : Les résultats obtenus ont montré un taux élevé d'isolats d'Entérobactérales producteurs de BLSE qui étaient multi-résistants. La nitrofurantoïne, la gentamicine, le chloramphénicol, le céfixime et l'acide nalidixique étaient les antibiotiques les plus actifs contre les isolats.

Mots-clés: β-lactamase à spectre Etendu, Enterobacterales, VIH/SIDA, République Démocratique du Congo.

Introduction:

People living with HIV/AIDS (PLWHA) develop opportunistic infections which remain a cause of mortality and morbidity in the majority of patients. These infections are the result of severe immunosuppression very often due to the absence, failure or non-compliance with anti-HIV treatment. Majority of the opportunistic infections are caused by mycobacteria, parasites, fungi and bacteria. Among the bacteria involved in opportunistic infections, Enterobacterales play important role (1,2,3). Members of the order Enterobacterales have developed resistance to commonly prescribed antibiotics. One of the resistance mechanisms is the production of the extended spectrum β-lactamases (ESBLs), which are enzymes that confer resistance to penicillins, cephalosporins and monobactams.

The global prevalence of ESBL-producing Enterobacterales is increasing (4-8). Worldwide, the prevalence of ESBL differs between regions and varies between 0 and 100% (9), and it is estimated that 5% of urinary tract infections (UTIs) in pregnant women in Europe and 45% in Africa is caused by ESBL-producing pathogens (10). Among PLWHA, some studies have reported prevalence of ESBL among Enterobacterales in Nigeria (21.0%), in Ethiopia (99.0%), Zimbabwe (3.0%), and Germany (4.0%) (11-14). The production of these enzymes by bacteria leads to the hydrolysis of the beta-lactam nucleus and renders the treatment ineffective, which constitutes a real public health problem (6,11,15).

Data on the prevalence of ESBL-producing Enterobacterales in the general population are very limited in Democratic Republic of the Congo. People living with HIV/AIDS have a high risk of developing multiple infections, some of which are due to multi-drug resistant ESBL-producing bacteria. The prevalence of ESBL in this category of patients is also not very well studied in the DRC. Thus,

this study aimed to assess the antibiotic susceptibility of Enterobacterales isolates and to determine the rate of ESBL-production among these isolates in HIV-infected patients at the BOYAMBI Hospital center in Kinshasa, DRC.

Materials and method:

Study setting, participants and samples:

This study was conducted between January and March 2022 at BOYAMBI Hospital Center in Kinshasa, Democratic Republic of the Congo. A total of 163 HIV/AIDS patients were randomly selected for the study and urine samples were collected from 108 and stool samples from 55 of the patients. The samples were analyzed at the University Reference Center of Antimicrobial Resistance Surveillance (URC-AMRS) of Faculty of Pharmaceutical Sciences, University of Kinshasa.

Isolation and identification of bacteria

The isolation of bacteria was done by culturing the stool samples on Hektoen and MacConkey agar plates (Liofilchem, Roseto Degli Abbruzzi, Italy). For urine samples, the enumeration of bacterial colonies was performed on Cysteine Lactose Electrolyte Deficient (CLED) agar plates (Liofilchem, Roseto Degli Abbruzzi, Italy) which were incubated for 24 hours at 37°C. Cultures were considered positive when 10^5 colony forming units (CFU)/ml of urine were counted.

Isolated Enterobacterales were identified by conventional microbiological identification methods including Gram staining, oxidase tests, indole and urease production, citrate utilization, hydrogen sulphide gas production and fermentation of sugars, as well as lysine decarboxylase (LDC), ornithine decarboxylase (ODC) and arginine dihydrolase (ADH) tests (17-19).

Antibiotic susceptibility tests:

Antimicrobial susceptibility test (AST) of each Enterobacterales isolate was done by the disc diffusion method on Mueller-Hinton (MH) agar using the following antibiotic disks (Liofilchem, Roseto Degli Abruzzi, Italy); cefotaxime (30µg), ceftriaxone (30µg), amoxicillin (30µg), amoxicillin-clavulanic acid (20/10µg), ceftazidime (20µg), cefixime (10µg), cefuroxime (15µg), co-trimoxazole (23.75/1.25µg), imipenem (10µg), nalidixic acid (10 µg), ciprofloxacin (5µg), nitrofurantoin (300 µg), aztreonam (30µg), gentamicin (20µg), and chloramphenicol (10µg). The interpretation of the results as sensitive, intermediate or resistant was done according to the criteria of the Clinical and Laboratory Standards Institute (16,20,21). *E. coli* ATCC 25922 was used for quality control.

Phenotypic screening and confirmation of ESBL production:

The screening for ESBLs production from each bacterial isolate was determined from the AST result of ceftazidime and cefotaxime. Thus, all isolates that were resistant to ceftazidime and/or cefotaxime were suspected to be ESBL producers (16,21). Phenotypic confirmation of ESBLs production was done by the 'double-disc' synergy test. Four antibiotic discs were used, with amoxicillin-clavulanic acid in the centre of the plate, surrounded by two discs of 3rd generation cephalosporins (cefotaxime and ceftazidime) and a disc of aztreonam. The discs were placed at a distance of 25 mm on MH agar plate that has been inoculated with bacterial suspension equivalent 0.5McFarland turbidity standard, and incubated for 18-24 hours at 37°C (16, 21). The characteristic image of 'champagne button' appearance confirms the presence ESBL. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Statistical analysis:

Statistical analysis of the data (descriptive analysis) was carried out using the Microsoft Excel 2010 and R software. Statistical comparisons of antibiotic resistance and prevalence of ESBL producing isolates in stool and urine samples was done using Chi-square test, with a significance threshold of 5% (95% CI, $p \leq 0.05$).

Results:

Enterobacterales isolates:

Of the total of 163 HIV/AIDS patients from whom urine (n=108) and stool (n=55) samples were collected and analyzed, 120 Enterobacterales were isolated, with 65 from 108 (60.2%) urine and 55 from 55 (100.0%) stool samples. *Escherichia coli* was the most frequent bacterial species isolated from both urine and stool (Table 1). The isolated bacterial species belong to six bacterial genera; *Escherichia* (n=84, 70.0%), *Klebsiella* (n=23, 19.2%), *Citrobacter* (n=6, 5.0%), *Enterobacter* (n=4, 3.3%), *Proteus* (n=2, 1.7%) and *Morganella* (n=1, 0.8%).

Table 1: Frequency distribution of the Enterobacterales isolates

Enterobacterales isolates	**Biological specimen**		**Total**	**%**
	Urine	**Stool**		
Escherichia coli	40	44	84	70.0
Klebsiella spp	16	7	23	19.2
Citrobacter spp	3	3	6	5.0
Enterobacter spp	3	1	4	3.3
Morganella morganii	1	0	1	0.8
Proteus spp	2	0	2	1.7
Total (%)	**65 (54.2)**	**55 (45.8)**	**120**	**100.0**

Results of antibiotic susceptibility test:

The results in Tables 2 and 3 showed that the Enterobacterales isolates were resistant to most of the antibiotics tested. High resistance was observed to amoxicillin (92%), ceftriaxone (82.0%), sulfamethoxazole-trimethoprim (81.0%), ceftazidime (80.0%), ciprofloxacin (78.0%), imipenem (71.0%), cefuroxime (65.0%), amoxicillin-clavulanic acid (58.0%) and cefotaxime (53.0%). High sensitivity was obtained with nitrofurantoin (88%), gentamicin (77.0%), chloramphenicol (72%), cefixime (61.0%) and nalidixic acid (51.0%).

Escherichia coli isolates (n=84) exhibited high resistance to amoxicillin (90.5%), sulfamethoxazole-trimethoprim (81.0%), cipr rofloxacin (77.4%), ceftriaxone (77.4%), ceftazidime (73.8%), amoxicillin–clavulanic acid (61.9%), imipenem (60.7%) and cefotaxime (50.0%). The highest sensitivity of *E. coli* isolates was observed with nitrofurantoin (99.0%) followed by cefixime (61.0%).

Klebsiella isolates (n=23) were resistant to ceftazidime (95.7%), ceftriaxone (91.3%), imipenem (91.3%), ciprofloxacin (87%), sulfamethoxazole-trimethoprim (78.3%), cefotaxime (56.5%), and amoxicillin–clavulanic acid (52.2%) and were sensitive to cefixime (65.0%), nalidixic acid (60.0%) nitrofurantoin (60.0%), gentamicin (61.0%) and chloramphenicol (61.0%). The combination of amoxicillin and clavulanic acid showed average activity of 50.0% against *Klebsiella* isolates. *Citrobacter, Enterobacter, Morganella* and *Proteus* species were highly resistant and exhibited multiple resistance to the antibiotics tested.

Citrobacter isolates (n=6) were fully (100.0%) resistant to cefuroxime, ceftriaxone, ceftazidime, cefotaxime and imipenem, and 83.0% were resistant to nalidixic acid, ciprofloxacin, gentamicin and sulfamethoxazole-trimethoprim combination. High sensitivity was only observed with nitrofurantoin (83.0%). *Enterobacter* strains (n=4) exhibited high resistance to imipenem (75.0%), ceftazidime (75.0%) and sulfamethoxazole-trimethoprim (75.0%), but were 100.0% susceptible to nalidixic acid and 75.0% to cefixime, ciprofloxacin, gentamicin and cefotaxime.

The resistance rate of the urinary Enterobacterales isolates of 67.7% to amoxicillin-clavulanic acid was significantly higher than 47.3% for the stool isolates (p=0.0038), and the resistance rate of 40.0% for the urinary isolates to gentamicin was significantly higher than 25.5% for the stool isolates (p= 0.0283). Conversely, the resistance rate of 30.8% for urinary Enterobacterales isolates to chloramphenicol was significantly lower than 47.3% for the stool isolates (p=0.0167), and 75.4% resistance rate of the urinary isolates to sulfamethoxazole-trimethoprim was also significantly lower than 87.3% for the stool isolates (p=0.0309).

Results of ESBL detection:

Out of the 120 Enterobacterales isolates, 98 showed resistance to at least one 3rd generation cephalosporin (ceftriaxone, ceftazidime and cefotaxime) and 112 isolates to penicillins (amoxicillin). Of the 120 isolates, 28 showed an ESBL phenotype after 'double disc synergy test' by the appearance of a 'champagne button' (Fig 1), giving an overall ESBL rate of 23.0%. The most frequent ESBL producing bacteria was *Citrobacter* spp (50%, 3/6) followed *Klebsiella* spp (26.1%, 6/23), *Enterobacter* spp (25.0%, 1/4) and *E. coli* (21.4%, 18/84) (Table 4). ESBL were detected in 15 of 65 (23.1%) Enterobacterales isolates from urine samples, while they were detected in 13 of 55 (23.6%) isolates from stool samples (x^2=0.0052, p=0.9424).

Table 2: Susceptibility of Enterobacterales isolates to selected antibiotics

Antibiotics	**Enterobacterales isolates**											
	***Citrobacter* spp (n=6)**		***Enterobacter* spp (n=4)**		***Escherichia coli* (n=84)**		***Klebsiella* spp (n=23)**		***Morganella* spp (n=1)**		***Proteus* spp (n=2)**	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
AMC	3 (50.0)	3 (50.0)	2 (50.0)	2 (50.0)	52 (61.9)	32 (38.1)	12 (52.2)	11 (47.8)	1 (100.0)	0	0	2 (100.0)
NA	5 (83.3)	1 (16.7)	0	4 (100.0)	44 (52.4)	40 (47.6)	9 (39.1)	14 (60.9)	0	1(100.0)	0	2 (100.0)
AML	ND	ND	ND	ND	76 (90.5)	8 (9.5)	ND	ND	ND	ND	ND	ND
CXM	6 (100.0)	0	2 (50.0)	2 (50.0)	49 (58.3)	35 (41.7)	19 (82.6)	4 (17.4)	1 (100.0)	0	1 (50.0)	1 (50.0)
CFM	4 (66.7)	2 (33.3)	1 (25.0)	3 (75.0)	33 (39.3)	51 (60.7)	8 (34.8)	15 (65.2)	0	1(100.0)	1 (50.0)	1 (50.0)
CRO	6 (100.0)	0	3 (75.0)	1 (25.0)	65 (77.4)	19 (22.6)	21 (91.3)	2 (8.7)	1 (100.0)	0	2 (100.0)	0
IMI	6 (100.0)	0	3 (75.0)	1 (25.0)	51 (60.7)	33 (39.3)	21 (91.3)	2 (8.7)	1 (100.0)	0	2 (100.0)	0
CIP	5 (83.3)	1 (16.7)	1 (25.0)	3 (75.0)	65 (77.4)	19 (22.6)	20 (87.0)	3 (13.0)	1 (100.0)	0	1 (50.0)	1 (50.0)
CN	5 (83.3)	1 (16.7)	1 (25.0)	3 (75.0)	25 (29.8)	59 (70.2)	9 (39.1)	14 (60.9)	0	1(100.0)	0	2 (100.0)
C	3 (50.0)	3 (50.0)	2 (50.0)	2 (50.0)	29 (34.5)	55 (65.5)	9 (39.1)	14 (60.9)	1 (100.0)	0	2 (100.0)	0
CAZ	6 (100.0)	0	3 (75.0)	1 (25.0)	62 (73.8)	22 (26.2)	22 (95.7)	1 (4.3)	1 (100.0)	0	2 (100.0)	0
CTX	6 (100.0)	0	1 (25.0)	3 (75.0)	42 (50.0)	50 (50.0)	13 (56.5)	10 (43.5)	0	1(100.0)	2 (100.0)	0
SXT	5 (83.3)	1 (16.7)	3 (75.0)	1 (25.0)	68 (81.0)	16 (19.0)	18 (78.3)	5 (21.7)	1 (100.0)	0	2 (100.0)	0
F	1 (16.7)	5 (83.3)	1 (25.0)	3 (75.0)	1 (1.2)	83 (98.8)	9 (39.1)	14 (60.9)	0	1(100.0)	2 (100.0)	0

R: Resistant; S: Susceptible; ND: Not determined; AMC: Amoxicillin-clavulanic acid; NA: Nalidixic acid; AML: Amoxicillin; CXM: Cefuroxime; CFM: Cefixime; CRO: Ceftriaxone; IMI: Imipenem; CIP: Ciprofloxacin; CN: Gentamicin; C: Chloramphenicol; CAZ: Ceftazidime; CTX: Cefotaxime; SXT: Sulfamethoxazole trimethoprim; F: Nitrofurantoin.

Table 3: Comparative resistance of Enterobacterales isolates from stool and urine samples

Antibiotics tested	**Resistance of Enterobacterales isolates to the tested antibiotics**				***p* value**
	Urine		**Stool**		
	No of isolates	**Number (%) of resistant isolates**	**No of isolates**	**Number (%) of resistant isolates**	
AMC	65	44 (67.7)	55	26 (47.3)	0.003814*
NA	65	30 (46.2	55	28 (50.9)	0.5007
AML	40	37 (92.5)	44	39 (88.6)	0.5469
CXM	65	42 (64.6)	55	36 (65.5)	0.902
CFM	65	25 (38.5)	55	22 (40.0)	0.8235
CRO	65	56 (86.2)	55	42 (76.4)	0.0761
IMI	65	46 (70.8)	55	39 (70.9)	0.9826
CIP	65	49 (75.4)	55	44 (80.0)	0.4326
CN	65	26 (40.0)	55	14 (25.5)	0.02833*
C	65	20 (30.8)	55	26 (47.3)	0.01676*
CAZ	65	54 (83.1)	55	42 (76.4)	0.2373
CTX	65	36 (55.4)	55	28 (50.9)	0.5265
SXT	65	49 (75.4)	55	48 (87.3)	0.03098*
F	65	10 (15.4)	55	4 (7.3)	0.07036

* = statistically significant at $p<0.05$; R: Resistant; S: Susceptible; ND: Not determined; AMC: Amoxicillin-clavulanic acid; NA: Nalidixic acid; AML: Amoxicillin; CXM: Cefuroxime; CFM: Cefixime; CRO: Ceftriaxone; IMI: Imipenem; CIP: Ciprofloxacin; CN: Gentamicin; C: Chloramphenicol; CAZ: Ceftazidime; CTX: Cefotaxime; SXT: Sulfamethoxazole trimethoprim; F: Nitrofurantoin.

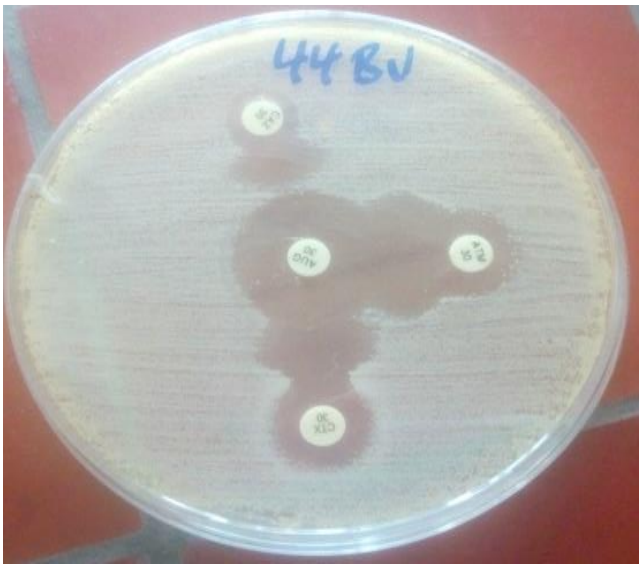

Fig 1: Champagne button appearance in a positive ESBL isolate by double-disc synergy test

Table 4: Frequency of ESBL phenotypes in Enterobacterales isolates

Enterobacterales isolates	Total number of isolates	Number (%) with ESBL phenotype
Escherichia coli	84	18 (21.4)
Klebsiella spp	23	6 (26.1)
Citrobacter spp	6	3 (50.0)
Enterobacter spp	4	1 (25.0)
Proteus spp	2	0
Morganella spp	1	0
Total	**120**	**28 (23.3)**

Discussion:

In this study, microbiological analysis of urine (n=108) and stool (n=55) samples obtained from 163 PLWHA in BOYAMBI Hospital center in Kinshasa resulted in isolation of 120 Enterobacterales isolates, with 65 from 108 (60.2%) urine and 55 from 55 (100.0%) stool samples. These isolates belong to six major genera of Enterobacterales; *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus* and *Morganella*. *Escherichia coli* was the most frequently isolated bacterial species in both stool (80%, n=44) and urine (61%, n=40) samples, followed by *Klebsiella* spp with 13.0% and 25.0% respectively. These results agree with that of Iyamba et al., (1) in 2013 in Democratic Republic of the Congo, which reported the predominance of *E. coli* in both stool (87.0%) and urine (62.5%) samples. Our results also agree with that of Bayleyegn et al., (12) in Ethiopia in 2021 that reported 60.0% *E. coli* and 16.1% *Klebsiella*, Olaru (13) in 2021 in Zimbabwe with 75.2% *E. coli*, and Salah et al., (23) in Lome Togo. Our results are also consistent with studies from Nigeria in 2021 by Oluwaseun et al., (22) with 23.0% *E. coli* and 11.8% *Klebsiella* spp, and Mofolorunsho et al., (15) with 69.0% *E. coli* and 31.0% *Klebsiella pneumoniae*.

The susceptibility test of the Enterobacterales isolates to 14 antibiotics showed high resistance to many antibiotics tested such as the 3rd cephalosporins, sulfamethoxazole-trimethoprim, ciprofloxacin, imipenem, cefuroxime, and amoxicillin-clavulanic acid. But they showed high sensitivity to nitrofurantoin, gentamicin, chloramphenicol, cefixime, and nalidixic acid. These results disagree with those of Liesse et al., (1) in 2014 which reported that cefotaxime, ceftriaxone and gentamicin were still the most effective antibiotics with *in vitro* sensitivity of over 74.1% by the bacterial isolates. These antibiotic susceptibility results showed that Enterobacte-

rales in general show high rate of resistance to the antibiotics commonly used in human health. In Nigeria, Ifeoma et al., (24) reported resistance rates of 99.0%, 97.1%, 88.2%, 82.4%, 81.4%, 65.7%, 54.9%, 46.1%, 46.1%, and 23.5% for ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftriaxone, ceftazidime, amoxicillin-clavulanate, gentamicin, cefoxitin, nitrofurantoin and imipenem respectively among Enterobacterales isolates (24). Our results also disagree with those obtained in Ethiopia by Bayleyegn et al., (12) in 2021 where bacterial isolates were highly susceptible to chloramphenicol, cefotaxime and ceftazidime. Our results confirm the high level of resistance among Enterobacterales reported in many African countries (9,11), although the study by Ali et al., (11) in Nigeria in 2020 reported that the addition of clavulanic acid has allowed the restoration of amoxicillin activity on some bacterial strains such as *Klebsiella* species.

Our study also showed several multidrug-resistant isolates, which can be explained by the fact that the genes carrying these resistance traits are found on the same plasmids, coexistence of several resistance mechanisms, and by multiplicity of enzyme types (42). The rate of ESBL producers in our study was 23.3% (28/120). *Citrobacter* spp was the most predominant ESBL producer (50.0%, 3/6) followed *Klebsiella* spp (26.1%, 6/23), *Enterobacter* spp (25.0%, 1/4) and *E. coli* (21.4%, 18/84). Sami et al., (40) reported a prevalence of 24.7% (61/246) of ESBL-producing *Citrobacter* spp, which is lower than the rate in our study. The distribution of ESBL-producing Enterobacterales in our study is also at odd with the results of Choi et al., (41), which reported ESBL rates of 12.8%, 12.4%, 4.9%, and 0% in *Enterobacter* spp., *S. marcescens*, *C. freundii*, and *M. morganii* respectively. The ESBL rates in our study are higher than that reported by Tshilumbu (25) in Democratic Republic of the Congo in 2012, with 19.2% and predominance among hospitalized patients (10.1%) than those living in the community (9.1%). Dikoumba et al., (26) in Gabon reported ESBL prevalence among *Enterobacteriaceae* of 11.8% with predominance of *E. coli* and *K. pneumoniae*. Olaru et al., (13) in 2021 reported a prevalence of 19.3% in people living with and without HIV/AIDS in primary clinics in Zimbabwe. Utulo et al., (27) in 2021 reported 17% ESBL prevalence in some health facilities in Nigeria.

In a systematic review by Mansouri (10) in 2019 on the prevalence of ESBL in *Enterobacteriaceae* from urine of pregnant and postpartum women, the pooled prevalence of 25.0% was reported in all the studies (6 in Africa, 2 in North America, 12 in Asia, 1 in South America and 2 in Europe). The estimated prevalence rates were 45.0% in Africa, 33.0% in India, 5.0% in Europe, 4.0% in South America and 3.0% in North America. The rate of ESBL obtained in our study is lower than that reported in some countries around the world. This is particularly true in certain countries such as Nigeria (with 31.2 - 51.1% rate), Central African Republic (50.8%), Nepal (37.8% in PLWHA compared to 68.9% in non-PLWHA); Tanzania (50.4% in hospitalized children compared to 11.6% among children in the community, 89.7% in PLWHA children versus 16.9% in non-PLWHA), Ethiopia (73.9%) and 44.0% in Chad (72.0%) (4,5,14,22,28-35). It is also slightly lower than that reported by Bajpai et al., (36) in 2017 and Braide et al., (37) in 2018, with ESBL prevalence of 45.0% among urinary isolates.

The ESBL rate in Enterobacterales isolates from urine samples in our study was 23.1% (15/65) and 23.6% (13/55) for the stool samples. These prevalence rates are in agreement with those of Iliyasu et al., (6) who reported 28.2% for urine samples in UTIs and 27.1% in stool samples, but contradicted those of Utulo et al., (27) who reported higher ESBL prevalence in health facilities in Nigeria of 22.0% from stool samples than from blood (21.4%) and urine (13.8%) samples. In our study however, there was no significant difference in the ESBL rate between Enterobacterales isolates from urine and stool samples (x^2=0.0052, p=0.9424).

Conclusion:

The results of our study showed a relatively high rate of ESBL-producing Enterobacterales isolates with high *in vitro* and multi-drug resistance to commonly used antimicrobials. Certain β-lactam agents (penicillins, cefuroxime, ceftriaxone, cefotaxime, ceftazidime) should no longer be used as first-line empirical antibiotic therapy of infections of the urinary and gastrointestinal systems, where Enterobacterales are involved in the Democratic Republic of the Congo. Nitrofurantoin, gentamicin and chloramphenicol are the most active antibiotics *in vitro* against the isolates, and should be considered as firstline drugs in the empirical treatment of infections caused by these organisms in DRC.

Our study was limited by the relatively small sample which may not allow us to give accurate estimate of the true prevalence of ESBL-producing Enterobacterales in our environment.

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Contributions of authors:

MVG was involved in the study conceptualization, methodology, laboratory analysis, interpretation of the results, and original drafting of the manuscript. ILJ and MML were involved in sample collection. MLC and NNJ were involved in the processing of the samples. NPO and BMT were involved in the statistical analysis of the data. LIJM and MWJ corrected the study protocol and revised and finalized the manuscript. TK and OD participated in the critical review of the manuscript. All authors have read and agreed to the final version of this manuscript.

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Conflict of interest:

Authors declare no conflict of interest

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