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Original article

Phenotypic and genotypic characterization of Beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from patients in Kinshasa, Democratic Republic of Congo

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ABSTRACT

Background: The emergence and worldwide circulation of strains of beta-lactamase-producing *Enterobacterales* represent a major public health problem. The aim of this study was to determine the characterization of extended spectrum beta-lactamase (ESBL) and carbapenemase-producing strains of *Escherichia coli* and *Klebsiella pneumoniae* resistant strains isolated in Kinshasa hospitals. **Methods:** Urine samples were collected from hospitalized and non-hospitalized patients. Bacteria were identified by using conventional biochemical tests and MALDI-TOF. Antibiotic susceptibility testing was performed by disk diffusion method. ESBL and carbapenemase phenotypes were screened using standard microbiological tests. ESBL-encoding genes were tested by multiplex end-point PCR assays and carbapenemase enzymes were identified by immunochromatography assay. **Results:** Out of 286 urines, 200 (69.9%) Gram negative bacteria were isolated, among them 70 (35.0 %) *E. coli* and 23 (11.5%) *K. pneumoniae*. ESBL were identified in 62.9 % (44/70) and in 52.2% (12/23) of *E. coli* and *K. pneumoniae* strains respectively. The rate of ESBL-producing *Enterobacterales* was 60.2% (56/93). Among ESBL producers, the $bla_{\text{CTX-M1}}$ gene was identified in 88.6 % (39/44) isolates of *E. coli* and in 100% (12/12) isolates of *K. pneumoniae*. The *bla*-gene $bla_{\text{CTX-M-1}}$ was identified in 91.2% (51/56) of all ESBL producers' strains. Only 3.2% (3/93) of *Enterobacterales* strains produced the NDM enzyme. The Other genes encoding beta-lactamase were bla_{TEM} (64.3 %), $bla_{\text{OXA-1/-30}}$ (58.9 %), and bla_{SHV} (39.3%). **Conclusion:** A high rate of ESBL-producing *Enterobacterales* was observed. The results of the study report the first description of metallo-β-lactamase NDM producing *E. coli* and *K. pneumoniae* strains from patients in Democratic Republic of Congo.

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Introduction

The resistance of *Enterobacterales* to antibiotics is alarming and remains to this day a real global public health problem. The *Enterobacterales* family includes many Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae (K. pneumoniae*) [1]. The latter are multi-resistant to many antibiotics and cause hospital and community acquired infections [2, 3]. *Enterobacterales* have become resistant to β-lactam antibiotics due to the production of beta-lactamases such as extended-spectrum beta-lactamases (ESBL). *Escherichia coli* and *K. pneumoniae* are among the main ESBL producers [4]. Infections caused by ESBL-producing *Enterobacterales* are treated with carbapenems [5, 6]. However, their scale importance is threatened by the emergence and the spread of bacteria producing carbapenemase enzymes [7]. Bacteria expressing this resistance mechanism are highly and extremely difficult to treat [8]. The key to controlling the spread is through early detection and prompt isolation [9]. Studies on detection of beta-lactamase producers have been conducted in many African countries [10-13]. Few studies have been conducted on prevalence of ESBL-producing *Enterobacterales* in Democratic republic of Congo (DRC) [14-16]. However, we do not have sufficient data on the distribution of carbapenemase-producing *Enterobacterales* strains isolated from patients in DRC.

The aim of this study was the characterization of ESBL and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* resistant strains isolated from patients attending hospitals in the University Reference Center of Antimicrobial Resistance Surveillance (URC-AMRS) network.

Material and methods

Bacteria isolates

This study was conducted between May 2019 to April 2022 in the Centre Medical de Kinshasa (CMK) and Clinique Ngaliema, tertiary structures, in Kinshasa City. The samples were collected for diagnostic purposes by the microbiology laboratories of these hospitals and were from 286 hospitalized and non-hospitalized patients with urinary tract infections. Urine samples were cultured in CLED medium (Liofilchem, Roseto degli Abbruzzi, Italy) and were incubated at 37°C for 24 hours. The identification of Gram bacilli was performed with conventional methods including Gram staining, oxidase test, indole and urease production, citrate utilization, hydrogen sulphide, gas production and fermentation of sugars, and lysine decarboxylase (L.D.C.), ornithine decarboxylase (O.D.C.) and arginine dihydrolase (A.D.H.) tests. In National reference center for antimicrobial resistance in Gram negative bacilli, CHU-UCL NAMUR (Belgium), the identification of *E. coli* and *K. pneumoniae* strains were confirmed by MALDI-TOF mass spectrometry (Bruker, Leipzig, Germany) [17, 18].

Antibiotic susceptibility testing

Antibiograms of each *E. coli* and *K. pneumoniae* strains using the diffusion method on Mueller Hinton agar (Bio-Rad, Marnes-la-Coquette, France) were realized with the following antibiotic disks (Bio-Rad, Marnes-la-Coquette, France): amoxicillin + clavulanic acid (AMC 30 µg), amikacin (AMK 30 µg), ampicillin (AMP 10 µg), aztreonam (ATM 30 µg), cefepime (FEP 30 µg), cefotaxime (CTX 5 µg), cefoxitin (FOX 30 µg), ceftazidime (CZD 10 µg), cefuroxime (CXM 30 µg), ciprofloxacin (CIP 5 µg), ertapenem (ETP 10 µg), gentamicin (GEN 10 µg), meropenem (MEM 10 µg) piperacillin-tazobactam (TZP 36 µg), sulfamethoxazole + trimethoprim (SXT 25 µg), temocillin (TEM 30 µg). The results were interpreted according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2017 [19]. The diameter of the inhibition zone was read by ADAGIO Automated System (Bio-Rad). *E. coli* ATCC 25912 was used for quality control [19].

Phenotypical detection of beta-lactamase presence

ESBL production was determined by a double synergetic test between amoxicillin-clavulanic acid and aztreonam, ceftazidime, and cefotaxime. Meropenem screening cut-off of zone diameter <28 mm was used as recommended for carbapenemase screening [20].

Genomic characterization of beta-lactamase-encoding genes

DNA extraction

DNA extraction was performed according to the protocol described by **Khaldi et al.** [21]. Five colonies from trypticase soy agar supplemented with 5% sheep blood (Bio-Rad, Marnes-la-Coquette, France) were suspended in 500 µL of DNase and RNase-free water (Invitrogen, Paisley, UK). The cells were then boiled for 10 min at 100° C in a

thermal block (Polystat 5; Bioblock Scientific, Cedex, France), then centrifuged at 19,000 g for 5 min. The obtained bacterial lysate containing DNA was used for the PCR amplification reaction. An aliquot of this DNA was stored at -20°C until use.

Detection of beta-lactamase-encoding genes

Detection of beta-lactamase resistance genes was done by three multiplex end-point PCR assays targeting *bla*$_{CTX-M}$ of group 1, 2 or 9 [22, 23] as described previously. Other resistance genes associated with ESBL such as *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA-1/-30}$ were searched [22,23]. This PCR was performed on a DNA extract using specific primers targeting the genes sought. An inhibition control (*Acinetobacter*-derived cephalosporinase gene of the ATCC 19606 strain) was amplified at the same time as the targets and makes it possible to control the smooth running of the extraction and the absence of inhibition during the amplification. The amplicons generated were analyzed by capillary electrophoresis with the QIAxcel system.

Identification of carbapenemase enzymes

The identification of carbapenemase enzymes was carried out by immunochromatographic essays RESIST-4 O.K.N.V. developed by Coris BioConcept for the detection of OXA-48-like and *Klebsiella pneumoniae* carbapenemase (KPC) carbapenemases on one cassette, and New Delhi metallo-beta-lactamase (NDM) and Verona imipenemase (VIM) carbapenemase on a second cassette [24-28].

Results

Bacteria isolates and antimicrobial susceptibility profile

Out of 286 urine samples collected from the hospitalized and non-hospitalized patients with urinary tract infections, 200 (69.9%) Gram negative bacilli were isolated during the period of the study. Among these strains, 70 strains were *E. coli* and 23 were *K. pneumoniae* (n=23). The selected 93 *E. coli* and *K. pneumoniae* strains were submitted to antibiotic susceptibility test in order to determine their antimicrobial susceptibility profiles. The results obtained are summarized in **table (1)** below.

The results of antibiotic susceptibility tests presented in **"table 1"** showed that *E. coli* strains had resistance rates higher than 60 % to ampicillin (88.6%), sulfamethoxazole + trimethoprim (74.3 %), cefuroxime (71.4 %), cefotaxime and cefepime (68.6 %), ceftazidime (67.1 %), and aztreonam (61.5 %). Increasing resistance was observed against amoxicillin + clavulanic acid, gentamicin, piperacillin-tazobactam and temocillin. 60.9 % of *K. pneumoniae* were resistant to cefuroxime and the combination sulfamethoxazole + trimethoprim, 52.2 % to cefotaxime, ceftazidime, aztreonam and cefepime, and 47.8 % to ciprofloxacin. An increasing resistance was also observed against piperacillin-tazobactam, gentamicin, amoxicillin + clavulanic acid and temocillin. In contrast, the isolated strains of both *E. coli* and *K. pneumoniae* remained highly susceptible to meropenem, ertapenem, cefoxitin and amikacin, with susceptibility rates of more than 82 %.

Detection of resistance enzymes and molecular confirmation of *bla* genes

The investigation on the production of resistance enzymes was done in the 93 isolated *Enterobacterales* strains. We observed that ESBL were produced in 62. 9% (44/70) of *E. coli* and in in 52.2% (12/23) of *K. pneumoniae*. A rate of 60.2% (56 /93) of ESBL producers was observed. The majority of these enzyme-producing strains were resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, cefepime, temocillin, and aztreonam, as shown in **table (1)**.

The production of the beta-lactamases in the majority of the *Enterobacterales* strains led to the molecular detection of the *bla*-genes in these bacterial strains. The results of molecular *bla*-genes detection are presented in **tables (2, 3), (figures 1, 2)** below. Five types of beta-lactamase resistance genes were detected. These are the following genes: *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-9}$, *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{OXA-1/-30}$. Only one type of carbapenemase enzyme, NDM, were identified by immunochromatographic essay. The most isolated *bla*-genes in strains of *E. coli* and *K. pneumoniae* was *bla*$_{CTX-M-1}$ (91.2 %; 56/93). The *bla*$_{CTX-M1}$ gene was identified in 88.6 % (39/44), and 100% (12/12) strains of *E. coli* and of *K. pneumoniae* respectively. The gene *bla*$_{CTX-M-9}$ was detected in 9.8 % (5/44) strains of *E. coli*. The carbapenemase enzyme NDM was identified in only 3 strains, including 2 strains of *E. coli* and 1 strain of *K. pneumoniae*. The Other genes encoding beta-lactamase were *bla*$_{TEM}$ (64.3 %), *bla*$_{OXA-1/-30}$ (58.9 %), and *bla*$_{SHV}$ (39.3%). The *bla*$_{TEM}$ and the *bla*$_{SHV}$ genes were mostly produced in *K. pneumoniae* strains **(Table 2).** On the other hand, the carbapenemase enzyme NDM was identified in only 3.2 % (3/93) strains, among them 2 (2.9 %) *E. coli* and 1 (4. 3 %) *K. pneumoniae*.

A diversity of combinations of multiple genes was identified in different *Enterobacterales* strains in this study. The mostly detected combination of multiple genes was the genotype [$bla_{\text{CTX-M-1}}$ + bla_{TEM} + $bla_{\text{OXA-1/-30}}$] which was identified in 23.2 % (13/56) of strains, followed [$bla_{\text{CTX-M-1}}$ + $bla_{\text{OXA-1/-30}}$] in 10.7% (6/56) of strains, [$bla_{\text{CTX-M-1}}$ + bla_{TEM}] in 8.9% (5/56) of strains, and [$bla_{\text{CTX-M-1}}$-$bla_{\text{OXA-1/-30}}$- bla_{SHV}] and [$bla_{\text{CTX-M-1}}$-bla_{TEM} - $bla_{\text{OXA -1/-30}}$ - bla_{SHV}] in in 7.1% (4/56) of strains respectively, as shown in **table (4).** The *E. coli* strains positive for NDM enzyme were found to coproduce [bla_{TEM} + $bla_{\text{OXA-1/-30}}$ + bla_{SHV}] or [bla_{TEM} + $bla_{\text{OXA -1/-30}}$].

Table 1. Antibiotic susceptibility profile of *E. coli* and *K. pneumoniae* strains.

Antibiotics	***Escherichia coli* (*n = 70*)**			***Klebsiella pneumoniae (n =23)***		
	R	**I**	**S**	**R**	**I**	**S**
AMP	62 (88.6)	0 (0.0)	8 (11.4)	-	-	-
AMC	31 (44.3)	0 (0.0)	39 (55.7)	6 (26.1)	0 (0.0)	17 (73.9)
TZP	27 (38.6)	0 (0.0)	43 (61.4)	8 (34.9)	0 (0.0)	15 (62.5)
TEM	21 (30.0)	49 (70.0)	0 (0.0)	4 (17.4)	19 (82.6)	0 (0.0)
CXM	50 (71.4)	20 (28.6)	0 (0.0)	14 (60.9)	9 (39.1)	0 (0.0)
FOX	7 (10.0)	0 (0.0)	63 (90.0)	1 (4.3)	0 (0.0)	22 (95.7)
CTX	48 (68.6)	0 (0.0)	22 (31.4)	12 (52.2)	0 (0.0)	11 (47.8)
CZD	47 (67.1)	1(1.4)	22 (31.5)	12 (52.2)	0 (0.0)	11 (47.8)
FEP	48 (68.6)	0 (0.0)	22 (31.4)	12 (52.2)	0 (0.0)	11 (47.8)
MEM	2 (2.9)	0 (0.0)	68 (97.1)	0 (0.0)	0 (0.0)	23 (100)
ETP	3 (4.3)	0 (0.0)	67 (95.7)	0 (0.0)	0 (0.0)	23 (100)
ATM	43 (61.5)	5 (7.1)	22 (31.4)	12 (52.2)	0 (0.0)	11 (47.8)
CIP	45 (64.3)	3 (4.2)	22 (31.5)	11 (47.8)	5 (21.7)	7 (30.5)
SXT	52 (74.3)	0 (0.0)	18 (25.7)	14 (60.9)	0 (0.0)	9 (39.1)
AMK	12 (17.1)	0 (0.0)	58 (82.9)	3 (13.0)	0 (0.0)	20 (87.0)
GEN	30 (42.9)	0 (0.0)	40 (57.1)	6 (26.1)	0 (0.0)	17 (73.9)

Amoxicillin+ clavulanic acid (AMC), amikacin (AMK), ampicillin (AMP), aztreonam (ATM), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CZD), cefuroxime (CXM), Ciprofloxacin (CIP), ertapenem (ETP), gentamicin (GEN), meropenem (MEM), piperacillin-tazobactam (TZP), Sulfamethoxazole + Trimethoprim (SXT), temocillin (TEM).

Table 2. Distribution of ESBL in *E. coli* and *K. pneumoniae* strains.

Resistance profile	***E. coli*** **N=44**	***K. pneumoniae*** **N=12**	**Total N=56**
ESBL	n (%)	n (%)	n (%)
$bla_{\text{CTX-M-1}}$	39 (88.6)	12(100)	51 (91.2)
$bla_{\text{CTX-M-9}}$	5 (11.4	0(0)	5 (9.8)

Table 3. Distribution of carbapenemase enzyme and other beta-lactamase genes.

Resistance profile	***E. coli*** **N=44**	***K. pneumoniae*** **N=12**	**Total N=56**
Carbapenemase	n (%)	n (%)	n (%)
NDM	2 (2.9)	1 (4.3)	3 (3.2)
Other genes encoding beta-lactamase	n (%)	n (%)	n (%)
bla_{TEM}	25 (56.8	11 (91.7)	36 (64.3)
$bla_{\text{OXA-1/-30}}$	28 (63.6)	5 (41.7)	33 (58.9)
bla_{SHV}	10 (22.7)	12 (100)	22 (39.3)

Table 4. Frequency and combinations of beta-lactamase resistance genes or carbapenemase enzymes in *E. coli* and *K. pneumoniae* isolates.

Beta-lactamase genes or carbapenemase enzymes alone or in combination	**Number of multiple genes or enzymes in combination**	***E. coli***	***K. pneumoniae***	**Total n (%) (N= 56)**
$\boldsymbol{bla_{\text{CTX-M-1}}}$	1	8	0	8 (14.3)
$\boldsymbol{bla_{\text{CTX-M-9}}}$	1	1	0	1 (1.8)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$	2	6	0	6 (10.7)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{TEM}}}$	2	5	0	5 (8.9)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{SHV}}}$	2	1	2	3 (5.4)
$\boldsymbol{bla_{\text{CTX-M-9}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$	2	1	0	1 (1.8)
$\boldsymbol{bla_{\text{CTX-M-9}}}$-$\boldsymbol{bla_{\text{SHV}}}$	2	1	0	1 (1.8)
$\boldsymbol{bla_{\text{CTX-M-9}}}$-$\boldsymbol{bla_{\text{TEM}}}$	2	1	0	1 (1.8)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$	3	13	0	13 (23.2)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$-$\boldsymbol{bla_{\text{SHV}}}$	3	4	0	4 (7.1)
$\boldsymbol{bla_{\text{CTX-M-9}}}$-$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$	3	1	0	1 (1.8
$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$ **-NDM**	3	1	0	1 (1.8)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$-$\boldsymbol{bla_{\text{SHV}}}$	4	0	4	4 (7.1)
$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$-$\boldsymbol{bla_{\text{SHV}}}$-**NDM**	4	1	1	2 (3.6)
$\boldsymbol{bla_{\text{CTX-M-1}}}$-$\boldsymbol{bla_{\text{CTX-M-9}}}$-$\boldsymbol{bla_{\text{TEM}}}$-$\boldsymbol{bla_{\text{OXA-1/-30}}}$-$\boldsymbol{bla_{\text{SHV}}}$	5	1	0	1 (1.8)

Figure 1. Amplification by multiplex end-point PCR of CTX-M gene groups. The amplicons generated were analyzed by capillary electrophoresis with the QIAxcel system. A01 CTX POS (*Acinetobacter*-derived cephalosporinase gene of the ATCC 19606 strain): St: 1059 bp DNA ladder; CTX-M-1 group :415 bp DNA ladder; CTX-M-2 group: 552 bp DNA ladder; CTX-M-9 group: 205 bp DNA ladder. A01-12; B01-B12; C01-C12; D01-D12; E01-E12, F01-F4: *E. coli* and *K. pneumoniae* isolate.

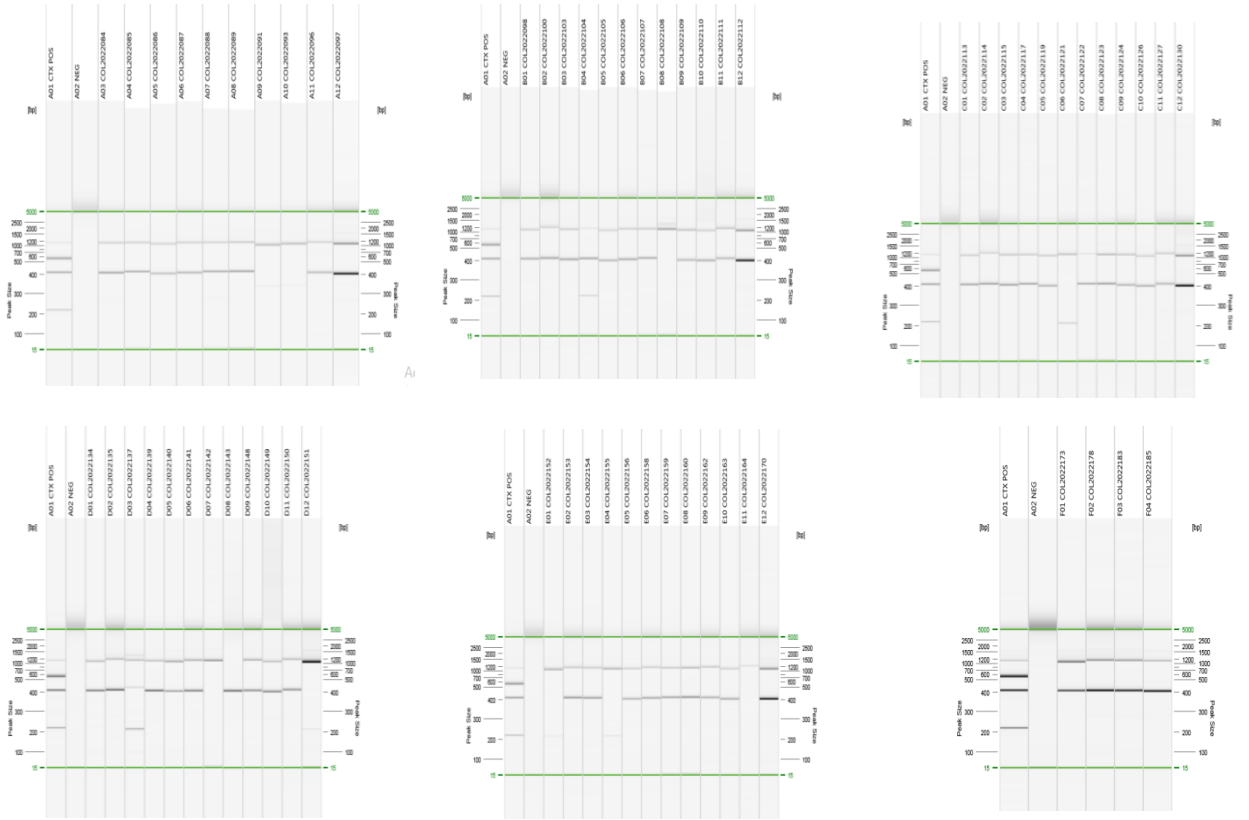
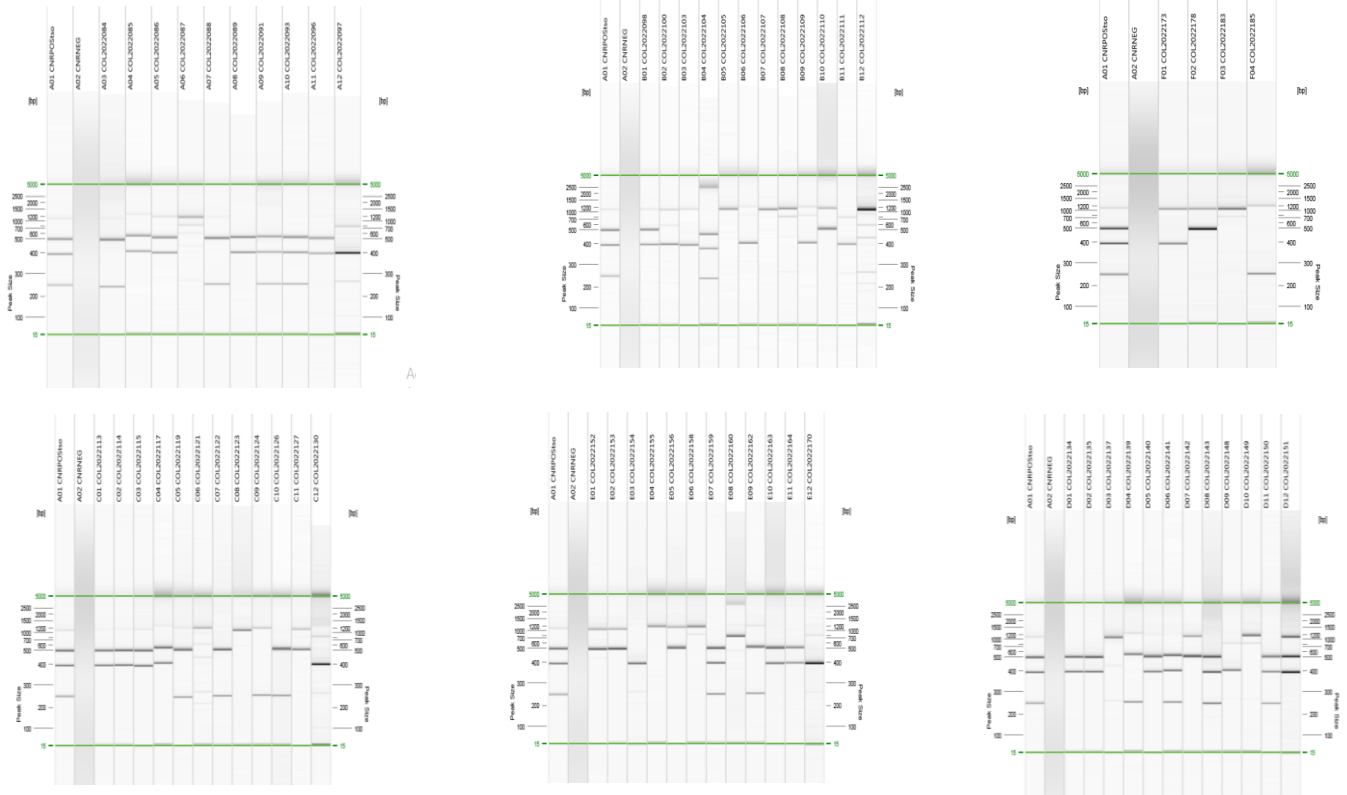


Figure 2. Amplification by multiplex end-point PCR of TEM, SHV, and OXA-1/-30 genes. The amplicons generated were analyzed by capillary electrophoresis with the QIAxcel system. A01CNRPOStso (*Acinetobacter*-derived cephalosporinase gene of the ATCC 19606 strain): St: 1059 bp DNA ladder; TEM :503 bp DNA ladder; SHV: 234 bp DNA ladder; OXA-1/-30: 391 bp DNA ladder. A01-12; B01-B12; C01-C12; D01-D12; E01-E12, F01-F4 : *E. coli* and *K. pneumoniae* isolates.



Discussion

The present study focused on the phenotypic and genotypic characterization of bacterial strains of *E. coli* and *K. pneumoniae* which were isolated from patients with urinary tract infections. High resistance to 16 antibiotics tested was observed in the most of strains of *E. coli* and *K. pneumoniae*. These strains exhibited high resistance rates to penicillins (aminopenicillins, carboxypenicillins and ureidopenicillins), to second-, third- and fourth-generation cephalosporins (in exception of the cephamycin, cefoxitin), and to monobactams. Furthermore, most of the *Enterobacterales* strains were resistant to the fluoroquinolone, ciprofloxacin and to the combination of sulfamethoxazole + trimethoprim. The combination of penicillins with a beta-lactamase inhibitor (clavulanate or tazobactam) led only to a minor decrease of the resistance rate. In the other side, the carbapenems, meropenem and ertapenem, and the cephamycin, cefoxitin, were the most effective antibiotics with susceptibility rates of ≥ 90.0 % **(Table 1).** This could be due to the non-dissemination of the resistance carbapenemase genes in the isolated strains in Kinshasa hospitals and to the lack of sensitivity of cephamycins to beta-lactamases [29, 30]. Carbapenems and TZP are effective antibiotics against ESBL-producing *Enterobacterales*, and are used to treat serious infections [31].

In DRC as in other African countries, the increase of resistance could be due to many factors such as the overuse of antibiotics in the hospitals and in the community (self-medication, breeding), the poor quality of antibiotics (counterfeit, substandard), poor hospital hygiene (dissemination of resistant bacteria), insufficient qualified human resources in laboratories, poorly effective diagnostic tools, and the nonexistence of a national antimicrobial resistance surveillance system [32]. Therefore, the use of these antibiotics should be under surveillance and the World Health Organization (WHO) Access, Watch, and Reserve (AWaRe) classification of antibiotics must be promoted [33-35].

ESBLs are enzymes which are able to hydrolyze all cephalosporins and monobactams. The current study identified a rate of 60.2 % (56/93) of ESBL producers with 44 (62.9 %) strains of *E. coli* were ESBL producers compared to 12(52.2 %) strains of *K. pneumoniae*. Only 3, 2 % (3/93 strains) of carbapenemase producers. These results corroborated many others studies which have demonstrated high rates of ESBL compared to carbapenemases [36, 37]. The rate of ESBL producers observed in study were much higher than those found in other similar studies conducted in Mali (61.8 %) [38] in Tchad (47.7%) [39], in Tanzania (34.3%) [40], in Ethiopia (44%) [36], and in Algeria (11.42%) [21].

In the ESBL-producers collected in this study, the dominant resistance gene was $bla_{\text{CTX-M}}$. The genetic analysis *Enterobacterales* isolated from Egyptian patients with suspected bloodstream infections showed that CTX-M was present in 89.13% (41/46) of the ESBL-producing *Enterobacterales*, whereas TEM and SHV were detected in 56.52% (26/46) and 21.74% (10/46) respectively [41]. The most identified resistance gene was $bla_{\text{CTX-M-1}}$ with a rate of 91.1 % (51/56) as well as in *E. coli* (88.6%) and in *K. pneumoniae* (100%). However, CTX-M-9 was only present in *E. coli* isolates (11.4%). The study on *Enterobacterales* isolated from patients with urinary tract infections in Gaza strip showed that ESBL-CTX-M-1 group was confirmed in 93.3%, and the remaining carried CTX-M-9 group [42]. In contrast, the data from Libya and Tunisia showed that the antibiotic resistance-encoding genes detected were $bla_{\text{CTX-M-15}}$ (51.7%), $bla_{\text{TEM-1}}$ (35.6%), several variants of bla_{SHV} (21.8%), and $bla_{\text{OXA-48}}$ (11.4%) [43]. The results of the current study confirmed that CTX-M-type enzymes are the most commonly found [44]. This study is the first one which reported the presence of CTX-M-1, CTX-M-9, TEM, and OXA-1/-30 from a large number of strains of *E. coli* and *K. pneumoniae* isolated in urine samples collected from hospitalized patients and outpatients attending Congolese hospitals.

A diversity of combinations of resistance genes were observed in this work. These results were in accord with those reported in previous studies in which isolates expressed co-carriage of two to six different resistance genes [21].

The NDM enzyme was found to be combined with the genes bla_{TEM}, $bla_{\text{OXA-1/-30}}$ and bla_{SHV}, but never with the gene $bla_{\text{CTX-M-1}}$. Its presence in an enterobacterial strain was associated with the resistance to ertapenem and meropenem. Thus, the bacterial strains that expressed the combination of NDM with other resistance factors were resistant to all commonly used antibiotics. This is the first time that a strain of carbapenemase-

producing *Enterobacterales* isolated from a patient has been identified in the DRC.

In the present study, we observed a low rate of carbapenemase-producing *Enterobacterales* (3.2 %). Our results were in consistence with those from Algeria in which the prevalence of carbapenemase-producing *Enterobacterales* was 2.85 % [21], but in contrast with those of studies conducted in Tunisian and Libyan hospitals which revealed that 11.4 % of *K. pneumoniae strains* were carbapenemase producers [43].

The emergence of carbapenemases among *Enterobacterales* is now a major public health problem in Africa [45-48].

Conclusion

High rates of ESBL-producing *E. coli* and *K. pneumoniae* were observed, in contrast to low rate of NDM-producing strains. This study reported the first description of NDM-producing *E. coli* and *K. pneumoniae* isolated from clinical samples in Democratic Republic of Congo. All these findings attest that beta-lactamase-producing *Enterobacterales* are now disseminated in the community and in hospital settings in Kinshasa. Antibiotic surveillance system, antibiotic stewardship and an infection prevention programs should be implemented in order to detect, to control the spread of Multidrug resistant bacteria, and to reduce the risk for hospital-acquired infections and in Kinshasa health care settings.

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Ethical considerations

The study ethical approval was obtained from Ethical comity of Ecole de Santé Publique, University of Kinshasa, Approbation N° ESP/CE/159/2024.

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Conflict of interest

The authors declare that no competing interests.

Availability of data and material

The data for this study are available from the corresponding author after request.

Author contributions

Investigation and conceptualization: LI, TK, DO. Laboratory processing: ML, MV, TA, BW, WI, HM, BC. Analyze of the data: DO, BP, HTD. Writing original draft: LI, TK. All authors made an outstanding contribution to the work, and read and approved the final version of the manuscript.

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