Advances in Microbiology, 2022, 12, 363-377
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Phenotypic and Genotypic Characterization of Metallo-Beta-Lactamase and Extended-Spectrum Beta-Lactamase among Enterobacteria Isolated at National Public Health Laboratory of Brazzaville

Nicole Prisca Makaya Dangui Nieko[1,2*], Cyr Jonas Morabandza[1,2,3], Moïse Doria Kaya-Ongoto[1,3], Duchel Jeanedvi Kinouani Kinavouidi[1,3], Haman Judel Mikia[2], Fabien Kangoula-Dia-Kikouidi-Kia-Louzala[1], Fabien Roch Niama[1,3]

[1]Service de Bactériologie, Laboratoire National de Santé Publique, Brazzaville, Congo
[2]Laboratoire de Microbiologie, Infectiologie et Immunologie, Ecole Normale Supérieure, Université Marien Ngouabi, Brazzaville, Congo
[3]Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Congo
Email: *nicolemakaya@gmail.com

How to cite this paper: Nieko, N.P.M.D., Morabandza, C.J., Kaya-Ongoto, M.D., Kinavouidi, D.J.K., Mikia, H.J., Kangoula-Dia-Kikouidi-Kia-Louzala, F. and Niama, F.R. (2022) Phenotypic and Genotypic Characterization of Metallo-Beta-Lactamase and Extended-Spectrum Beta-Lactamase among Enterobacteria Isolated at National Public Health Laboratory of Brazzaville. *Advances in Microbiology*, **12**, 363-377.
https://doi.org/10.4236/aim.2022.126026

Received: April 25, 2022
Accepted: June 20, 2022
Published: June 23, 2022

Abstract

The improper use of antimicrobials against infectious diseases has allowed microorganisms to develop defense mechanisms that give them insensitivity to these agents. All bacteria are concerned by this phenomenon. This work aimed to assess prevalence of beta-lactamase produced by enterobacterial isolates. Then, disc diffusion, double disc synergy test (DDST) and combined disc test (CDT) were respectively used for antimicrobial resistance, detection of Extended-Spectrum Beta-Lactamases (ESBL) and Metallo-Beta-Lactamases (MBL). *bla* genes were detected by PCR. A total of 132 enterobacterial strains were studied. Resistance to antibiotic families was observed with a greater frequency than 50%. Gentamicin was the least active beta-lactam antibiotic, with a resistance rate of 88%. 40.9% of strains show an ESBL phenotype and 16.6% were MBL. An overall prevalence of 74% (40/54) and respectively rates of 29.6%, 27.7% and 16.7% for *bla*SHV, *bla*CTX and *bla*TEM genes were observed. SHV, CTX, CTX/SHV/TEM, CTX/TEM, SHV/TEM and CTX/SHV were different ESBL genotypes observed. ESBL-producing enterobacteria isolation worried about the future of antimicrobial therapy in the Republic of Congo. This is a public health problem that requires careful monitoring and implementation of a policy of rational antibiotics use.

Keywords

Enterobacteria, Antibiotic Resistance, Extended-Spectrum Beta-Lactamases, Metallo-Beta-Lactamases, *bla* Genes

1. Introduction

The antimicrobials' introduction into the therapeutic arsenal of infectious diseases and their improper use has allowed microorganisms to develop defenses that give them insensitivity to these agents. All bacterial species are concerned by the phenomenon of antibiotic resistance, a real therapeutic problems [1]. Thus, the isolation in hospital and community environment of multidrug-resistant Gram-negative bacilli (GNB) in general and beta-lactamases producers in particular has become a global health problem [2]. This reality was recently recognized by the World Health Organization (WHO) in its report on antimicrobial resistance [3]. The last ten years have been marked by the emergence and dissemination of new resistance genes and the major challenges of this resistance have been encountered mainly in different species of enterobacteria [4]. Among multidrug-resistant bacteria (MRBs), enterobacteria producing β-lactamases have taken an important place due to their high frequency and high pathogenic potential. β-lactamases are the most inactivation enzymes frequently encountered and are the main mechanism of acquired resistance to β-lactam antibiotics. Based on the nature of their amino acids, there are four classes of beta-lactamases, A-D [5]. Classes A, C and D are β-lactamases comprising a serine with a hydrolytic mechanism, where we distinguish Extended Spectrum Beta-Lactamases (ESBLs). The first reported ESBLs concerned *Klebsiella pneumoniae*, but gradually other species such as *Escherichia coli*, *Enterobacter* sp. have been the cause of epidemics described in hospitals [6]. Currently, several ESBL groups are described including TEMs, SHVs, CTX-M and many others. All of these enzymes are not resistant to carbapenems. Carbapenems resistance appeared two decades later [7] and involves class B beta-lactamases, those that contain a zinc ion active site, hence their name, Metallo-Beta-Lactamases (MBL) containing subclasses B1, B2 and B3 [8]. They are characterized by a wide spectrum of activity towards β-lactams antibiotics, with the exception of monobactams, they are able to hydrolyze all classes of β-lactams, including carbapenems [9], antibiotics used in intensive therapy in clinical environment as a means of combating severe infections caused by MRBs, also thanks to their broad spectrum of action [10]. The emergence of MBLs is a serious problem in the community and clinical environment. MBLs are able to hydrolyze all classes of β-lactams, including carbapenems [9], antibiotics used in intensive therapy in clinical environment as a means of combating severe infections caused by MRBs, also thanks to their broad spectrum of action [10]. No effective inhibitor of these enzymes has yet been found. They are inactivated by metal chelators such as Ethylene Diamine Tetra-Acetic (EDTA), dipicolonic acid, phenantroline. It is also interesting to

note that the majority of genes encoding these enzymes are of plasmid origin and that they are easily transferable between bacterial strains by conjugation or transposition. MBLs can spread easily and allow the spread of resistance to carbapenems [7]. Bacteria producing carbapenemases have been reported in several countries, including Türkiye [11], China [12], Mexico [13] and Brazil [14].

In Africa, several studies report that the continent is not on the margins of this reality [15]. Recently, a study reported the detection of MBL in *Pseudomonas aeruginosa* isolated from hospitalized patients in Abidjan, Côte d'Ivoire [16].

In the Republic of Congo, the study of MBL and ESBL enterobacterial is not sufficiently documented. To contribute to this problem, we proposed to conduct this study in order to explore the enterobacteria producing these enzymes giving them the resistance.

Nowadays, DNA is arguably one of the most widely used pathways in microorganisms identification with MALDI-TOF technology [17]; but these tools are very expensive and require equipment and consumable constraints for laboratories. In this study, the biochemical method was used for enterobacterial identification.

Different phenotypic tests including Modified Hodge, combined disc, and AmpC disc tests have been suggested to identify MBLs based on metal-chelating ability such as EDTA inhibiting MBL activity [18]. In the present study, the phenotypic experiment of combined disc test (CDT) was used for MBL detection. Similarly, double disc synergy test (DDST) was used for ESBLs phenotypic detection.

In recent years, molecular biology has revolutionized the scientific world with new technologies such as PCR. Thanks to it, specific regions of genomes can be detected and studied. Recently, multiplex PCR assays for the rapid detection of *bla* genes have been reported [19]. While these assays for rapid, sensitive, and specific detection appear to be promising. In this study, we use PCR to detect *bla* genes.

2. Methods

2.1. Enterobacterial Isolation

From august to November 2021, a total of 438 urine samples were aseptically collected at the Bacteriology department of National Public Health Laboratory of Brazzaville. Thus, by using conventional microbiology techniques, enterobacteria were isolated in Eosin Methylene Blue (EMB) agar medium after aerobic incubation at 37˚C for 24 h. Initially, the colonies were counted to confirm the sample positivity. After purification, Gram type of bacteria was determined by staining. Identification of strains was confirmed by using biochemical tests with "Integral System Enterobacteria" (Liofilchem).

2.2. Antimicrobial Susceptibility Testing

The antimicrobial resistance profile of all isolates was determined by using the

standard Kirby and Bauer method, based in antibiotics disc diffusion on Mueller-Hinton Agar (MHA). The antibiotics used were: Cefepime (30 µg), Ceftazidime (10 µg), Cefotaxime (5 µg), Aztreonam (30 µg), Amoxicillin + clavulanic acid (20 µg), Imipenem (10 µg), Norfloxacin (10 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg) and Amikacin (30 µg). The diameter of the inhibition zone for each antibiotics disc was measured and results were definided in accordance Clinical and Laboratory Standards Institute guidelines [20].

2.3. Phenotypic Screening of MBL

All strains previously resistant to imipenem were selected to perform imipenem/imipenem + EDTA combined disc test (CDT) in order to detect metallo-betalactamase (MBL) producing strains. Briefly, a bacterial inoculum with an optical density of 0.5 McFarland was prepared with 0.9% NaCl medium suspension. Two imipenem discs were placed 30 mm apart on MHA previously seeded with the bacterial inoculum. On one of disc, 4 µL of EDTA (0.5 M, pH = 8) were added. The diameter of inhibition zone around these discs are measured and compared after incubation of 18 h at 37˚C.

The result is considered as positive if inhibition diameter around imipenem + EDTA is greater than that obtained with the imipenem disc alone by at least 7 mm [21].

2.4. Determination of ESBL Producing Isolates

ESBL production was detected by Double Disc Synergy Test (DDST), which consists of placing discs of ceftazidime (10 µg), cefotaxime (5 µg), cefepime (30 µg), imipenem (10 µg) and aztreonam (30 µg) at a distance of 30 mm (center to center) from a disc of amoxicillin + clavulanic acid (30/10 µg). The increase of the inhibition zone between amoxicillin + clavulanic acid disc and aztreonam, cefotaxime indicates the production of ESBL [22]. The reference strain *E. coli* ATCC 25922 was used.

2.5. Detection of *bla* Genes by PCR

After DNA plasmid extraction using Nucleospin Plasmid Kit (Macherey-Nagel, Germany), different types of PCR were performed depending on the targeted gene using the primers shown in **Table 1**. A simplex PCR was performed for *bla*CTX gene detection. The PCR reaction was performed in a final volume of 50 µL containing 19 µL of sterile distilled water, 2 µL of DNA, 2 µL of each primer (forward and reverse), 25 µL of Promega PCR Master Mix. The amplification program used comprises: an initial denaturation at 95˚C for 5 min, followed by 30 cycles each comprising a denaturation at 95˚C for 1 min, hybridization at 55˚C for 45 s, an extension at 72˚C for 1 min and a final extension at 72˚C for 7 min.

Multiplex PCR using four primers was also performed to simultaneously detect *bla*SHV and *bla*TEM genes. PCR reaction was performed in a final volume of 50 µL

Table 1. Specific primers used for *bla* genes detection.

Genes	Primer name	Sequences (5'-3')	Product size (pb)	References
CTX	CTX-F	TCTTCCAGAATAAGGAATCCC	909	[23]
	CTX-R	CCGTTTCCGCTATTACAAAC		
SHV	os-5 (+)	TTTATGGCGTTACCTTTGACC	795	[24]
	os-6 (−)	ATTTGTCGCTTCTTTACTCGC		
TEM	a216 (+)	ATGAGTATTCAACATTTCCGTG	1079	[25]
	a217 (−)	TTACCAATGTCTTAATCAGTGAG		

containing 15 µL of sterile distilled water, 2 µL of DNA, 2 µL of each primer (forward and reverse), 25 µL of Promega PCR Master Mix. The used program for the amplifications comprises: an initial denaturation at 95˚C for 5 min, followed by 30 cycles each comprising a denaturation at 95˚C for 1 min, hybridization at 60˚C for 1 min, an extension at 72˚C for 1 min and a final extension at 72˚C for 7 min.

The 0.2 mL microtubes are then placed in the thermocycler (Biorad, Singapore). Amplification products was analysed by agarose gel electrophoresis at 1.5%.

2.6. Data Analysis

Data were entered in an Excel database (Microsoft Corporation, USA). This program was used for statistical analysis. The diameters have been analyzed; the percentages calculated statically. Experimental values were represented as mean and standard deviation. Graph Pad Prism (Version 7.0.0.159, USA) was also used for EBLS genotype.

3. Results

3.1. Enterobacterial Isolates

During the study, a total of 132 samples tested were positive, representing an isolation rate of 30%.

3.2. Isolates Identification

The use of conventional microbiology methods made possible the identification of the 132 enterobacterial isolates including, 33 strains of *Escherischia coli* which is the most representative species, followed by 23 strains of *Enterobacter aerogenes*. Finally, *Enterobacter cloacae* is the least representative species with 4 strains (Figure 1).

3.3. Antimicrobial Susceptibility Results

Different levels of resistance were observed to antibiotics tested in enterobacterial strains. For 132 strains studied, the results revealed good levofloxacin activity

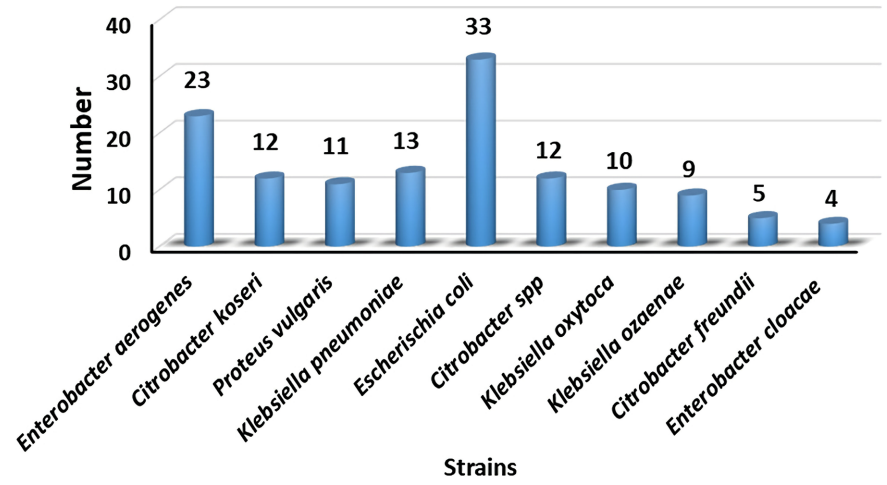

Figure 1. Distribution of identified enterobacterial strains.

with a sensitivity rate of 55%, followed by amikacin with a sensitivity rate of 45%. Gentamicin was the least active aminoglycoside antibiotic in the strains studied with a high resistance rate of 87%. However, cefepime was the least active antibiotic in the β-lactam family with a high resistance rate of 82% (**Figure 2**).

3.4. MBL-Producing Strains

Among the 132 studied strains, 78 were resistant to imipenem. These strains were selected for MBL screening, 22 were positive, a percentage of 16.6% taking into account the initial sample (**Figure 3**).

A predominance of MBLs was obtained with *E. coli* species, with 8 positive strains, 6% (**Table 2**).

3.5. ESBL-Producing Strains

The result of the synergy test for phenotypic detection of ESBLs is illustrated in **Figure 4**. Among 132 strains tested, 54 were positive, a rate of 40.9%. 12.8% of ESBL-producing strains are *E. coli* (**Table 2**). *K. pneumoniae* represents the least representative ESBL-producing species in this work, with a rate of 2.3%.

3.6. *bla* Gene Detection

Detection of CTX gene by simplex PCR

Fifty-four (54) ESBL-producing strains were selected for molecular analysis. After PCR, results showed the presence of CTX gene, around 909 bp (**Figure 5**). This gene was detected in 15 strains, a rate of 27.7% (**Table 3**).

Detection of SHV and TEM genes by multiplex PCR

Multiplex PCR was performed with the same samples (54 strains). The results of multiplex PCR showed after agarose electrophoretic analysis, the presence of SHV and TEM genes with respective bands around 800 and 1079 bp (**Figure 6**). SHV gene is observed in 16 (29.6%) strains tested. The TEM gene is detected in 9 strains (16.7%). These results are detailed in **Table 3**.

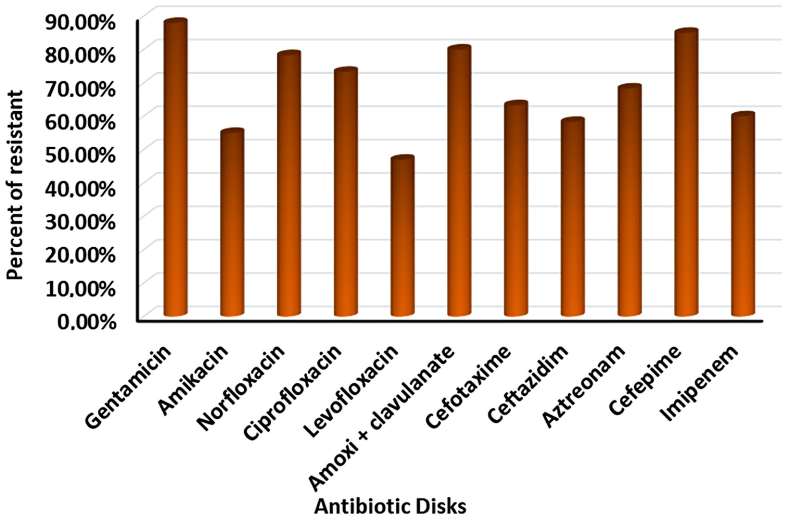

Figure 2. Antibiotics resistance profile.

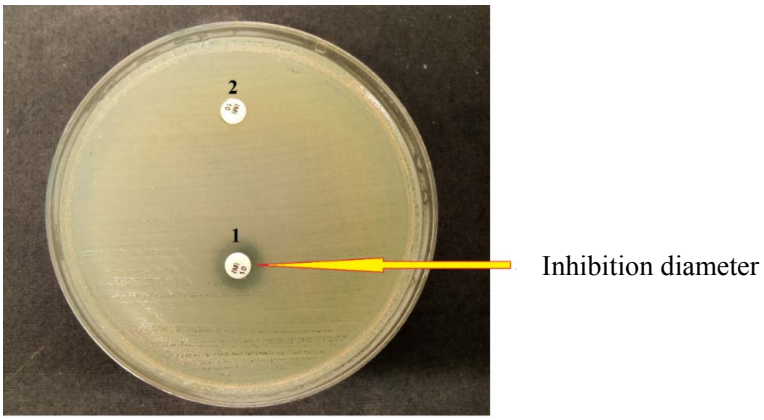

Figure 3. Double disc synergy test. 1: imipenem + EDTA, 2: imipenem. (EDTA: Ethylene Diamine Tetra-Acetic).

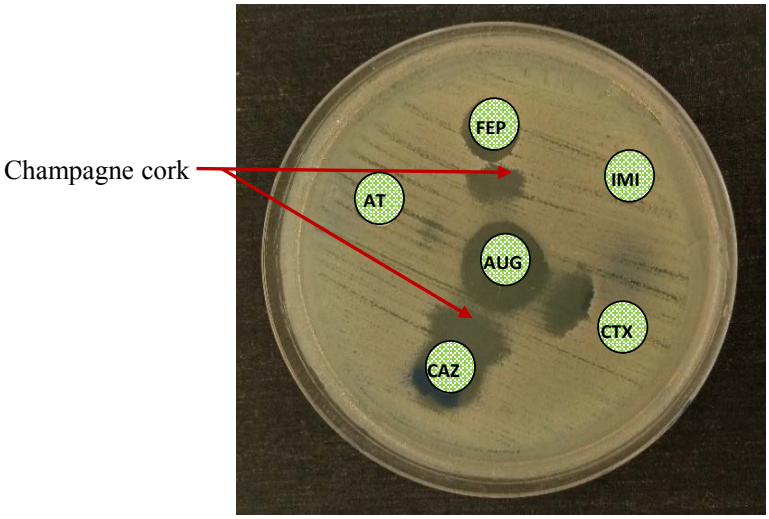

Figure 4. ESBL phenotypic test. CAZ: Ceftazidim, AT: Aztreonam, CTX: Cefotaxime, IMI: Imipenem, AUG: Amoxycilline + Cluvalanate, FEP: Cefepime.

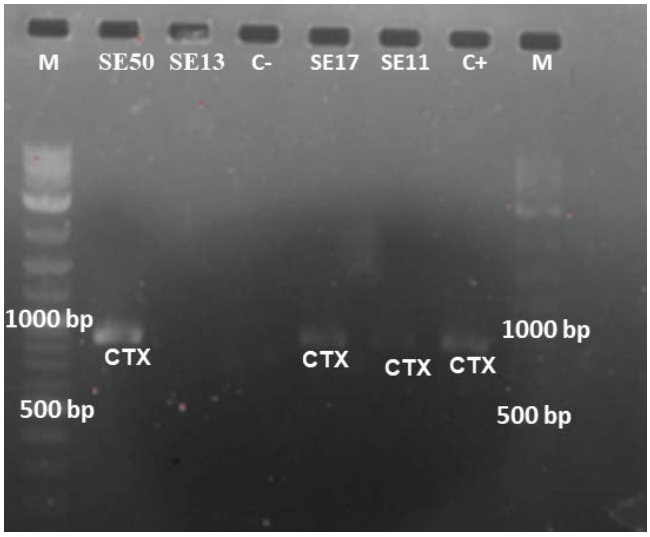

Figure 5. Amplification of the CTX gene. MP: Molecular size marker; C−: Negative control; C+: Positive control. SE50, SE13, SE17 and SE11: Enterobacterial isolates.

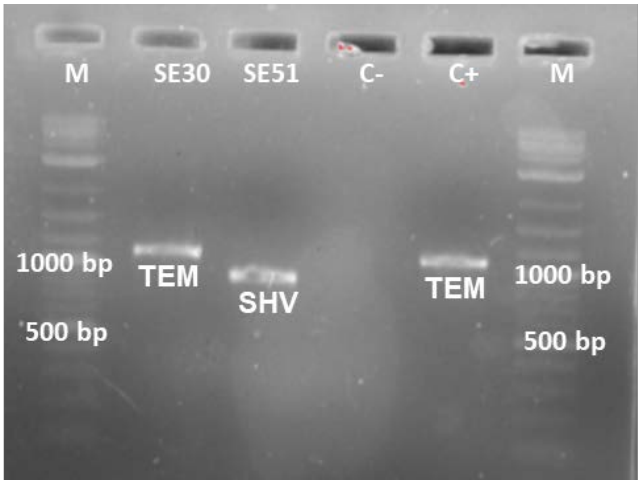

Figure 6. Multiplex PCR result for SHV and TEM genes detection. M: molecular size marker; C−: Negative control; C+: Positive control. SE30, SE51: Enterobacterial isolates.

Table 2. Prevalence of beta-lactamase-producing strains (n = 132).

Strains	ESBL No (%)	MBL No (%)	Total No (%)
E. coli	17 (12.9)	8 (6)	25 (18.9)
K. pneumoniae	3 (2.3)	2 (1.5)	5 (3.8)
E. aerogenes	14 (10.6)	4 (3)	18 (13.6)
K. ozaenae	0 (0)	3 (2.3)	3 (2.3)
P. vulgaris	5 (3.8)	2 (1.5)	7 (5.35)
C. koseri	6 (4.5)	1 (0.8)	7 (5.35)
K. oxytoca	4 (3)	2 (1.5)	6 (4.5)
Citrobacter spp	5 (3.8)	0 (0)	5 (3.8)
Total No (%)	54 (40.9)	22 (16.6)	**76 (57.6)**

Table 3. Distribution of *bla* genes in enterobacterial isolates (n = 54).

bla gene	Strains					Total No (%)
	E. coli No (%)	*E. aerogenes* No (%)	*K. ozaenae* No (%)	*K. pneumoniae* No (%)	*Citrobacter spp* No (%)	
CTX	8 (14.8)	4 (7.4)	3 (5.5)	0 (0)	0 (0)	15 (27.7)
SHV	6 (11.1)	0 (0)	5 (9.3)	3 (5.5)	2 (3.7)	16 (29.6)
TEM	5 (9.3)	0 (0)	4 (7.4)	0 (0)	0 (0)	9 (16.7)
Total	19 (35.2)	4 (7.4)	12 (22.2)	3 (5.5)	2 (3.7)	**40 (74)**

3.7. Genotypic Profiles

Analysis of genotypic profiles shows that one or more types of ESBL genes are detected in enterobacterial strains (Figure 7). These genes are detected alone or in combination. No strains carrying only TEM gene were observed in this study. SHV and CTX genes detected alone were obtained at the respectively rates of 14.8% and 7.4%. CTX/SHV/TEM genotype was found at a rate of 3.7%. CTX/TEM, CTX/SHV and SHV/TEM genotypes were respectively observed at rates of 9.25%, 7.4% and 3.7%. There is a predominance of SHV genotype in this study.

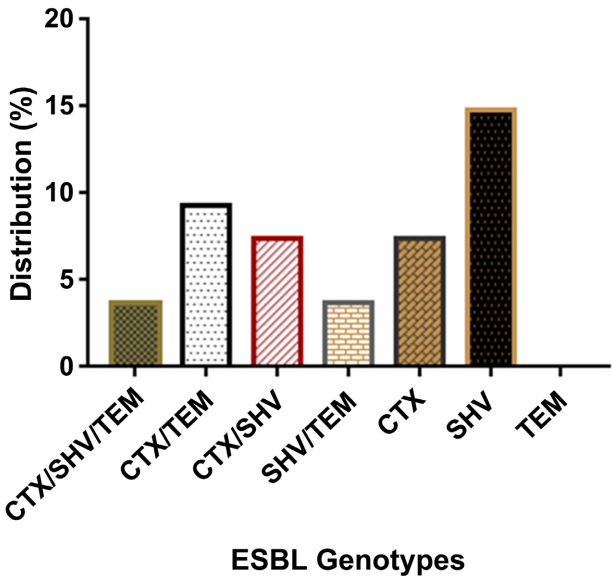

Figure 7. Genotypic profiles of ESBL associated genes.

4. Discussion

This study aimed to assess prevalence of beta-lactamase produced by enterobacteria isolated at the National Public Health Laboratory of in Brazzaville. Phenotypic and genotypic characterization of ESBL was done, as well as the phenotypic characterization of MBL. All isolates were from urine samples. 132 enterobacterial isolates have been identified and divided into 5 genera: *Escherischia*, *Enterobacter*, *Klebsiella*, *Citrobacter* and *Proteus*. These genera have been divided

into 10 species.

In this study, all strains were obtained from urine as biological material. These results are similar with those obtained by [26] in Iran, who isolated majority 67.6% of enterobacteria from urine samples.

Escherischia coli is the predominant species among studied strains, with a frequency of 25%. These results are similar to those obtained by [27] in Egypt and [28] in Morocco, who reported a predominance of *E. coli*. This predominance is explained by the fact that *E. coli* has adhesins capable of bacterium binding to the urinary epithelium and preventing its elimination by bladder emptying [29].

About strains susceptibility to antibiotics, there is significant resistance for each family of antibiotics tested. This resistance is greater than 50% for each disc, except for levofloxacin of the fluoroquinolone family.

For aminoglycoside, gentamicin with a high resistance rate of 88% was the least active antibiotic. The aminoglycoside resistance could be mainly due to the enzymes production, such as acetylases, adenylases and phosphorylases transferase. It can also be associated with active efflux mechanisms [30].

In β-lactam antibiotics, cefepime was the least active, bacteria showed resistance of 84.4% with this molecule. This could be attributed to cephalosporinase production [31].

In fluoroquinolones, norfloxacin was the antibiotic at which bacteria showed the most resistance with a rate of 78%. The high resistance to this molecule could be explained by the massive use in the treatment of urinary tract infections [32].

Globally, studied strains showed simultaneous resistance to all tested antibiotic families. In addition to being predominant in this work, *E. coli* has shown resistance to both fluoroquinolones, beta-lactams and aminoglycosides, which is worrying. The predominant and multidrug-resistant nature constitutes a public health threat to the success of the antibiotics arsenal currently available. It could be the consequence of selection pressure due to the improper use of broad-spectrum antibiotics in hospital and community settings, as well as the cross-transmission of resistance acquired to plasmid determinism [33].

In this study, 27.2% of the strains studied were resistant to imipenem. This rate is higher than that reported in Ivory Coast by [34] which obtained 10.4% of strains resistant to imipenem. However, the work of [35] reported an imipenem resistance rate of 97%. Resistance to imipenem can be explained by the selection of resistant bacteria favored by the frequent use of this antibiotic during the probabilistic treatment of severe nosocomial infections [36]. It could also be due to the loss of porin D (oprD) and/or the carbapenemase production [37].

Detection of MBL production was achieved by using combined disc test. 22 strains tested positive for combined discs, with a rate of 16.6%. This rate is higher than that obtained by [26] in Iran, who obtained a rate of 13% of MBLs.

A total of 54 strains among 132 tested produced the extended-spectrum

β-lactamases, a rate of 40.9%. This rate is higher than those obtained by [28] and [38] with respectively ESBL production rates of 3% and 12.2%.

In this study, the majority of the extended-spectrum β-lactamases-producing strains consist of *Escherichia coli* with a rate of 12.9% and *Enterobacter aerogenes* (10.6%). These results are similar to those reported in the literature [39], where *E. coli* was the predominant ESBL strain.

CTX, SHV and TEM genes were respectively detected at the rates of 27.7%, 29.6% and 16.7% in ESBL strains. These results are similar with those of [40], who worked in northern India hospitals and amplified CTX, SHV and TEM. However, they obtained rates of 28.8% for CTX, 13.7% for SHV and 10.9% for TEM. In addition, [41] obtained higher results in order of 84% for CTX, 81% for SHV and 73% for TEM in southern Chile.

The genotypic profile of the studied strains showed different genotypes. CTX/TEM and CTX/SHV are respectively observed at rates of 9.25% and 7.4%. These observed rates are higher than those reported by [42] whose work in Nigeria and showed CTX/TEM and CTX/SHV genotypes at rates of 3.7% each in *E. coli*. On the other hand, CTX/SHV/TEM genotype was observed with a rate of 3.7%. The presence and expression of these genes in the same strain could increase the spectrum of enterobacterial antibiotics resistance of and confer the phenomenon of multidrug resistance [37]. This could lead to therapeutic failures [43]. This co-resistance is the result of the dissemination of various resistance genes via conjugative plasmids or transposons between bacteria of the same species or different species [44].

5. Conclusion

This study shows a high level of resistance for most antibiotics usually prescribed in clinical environment. That calls into question conventional therapeutic strategies in community infections. The unregulated sale and non-rational and widespread consumption of antibiotics raise fears of a rapid spread of multi-resistant bacteria within all ecosystems. Isolation of beta-lactamase-producing enterobacteria worries for the future of antimicrobial therapy in the Republic of Congo. This is a public health problem that requires careful monitoring and the implementation of a policy of rational use of antibiotics both in community and hospital. Bacterial infections cannot be completely avoided, but strict compliance with hygiene rules reduces the risk. Similarly, the development of rapid tests for the detection of beta-lactamase-producing strains could help to prevent their spread.

Acknowledgements

The authors are grateful to Pr. Aimé Christian Kayath, for scientific discussions.

Data Availability

The Excel sheets including the data used to support the findings of this study are

available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

[1] Bryskier, A. (1999) Epidémiologie de la résistance aux antibactériens. In: *Antibiotiques, agents antibactériens et antifongiques*, Ellipses, Paris, 91.

[2] Meletis, G. (2015) Carbapenem Resistance: Overview of the Problem and Future Perpectives. *Therapeutic Advances in Infectious Disease*, **3**, 15-21. https://doi.org/10.1177/2049936115621709

[3] WHO (World Health Organization) (2014) RESIS. WHO Report, World Health Organization, Geneva.

[4] Bradford, P.A. (2001) Extended-Spectrum Beta-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistant Threat. *Clinical Microbiology Reviews*, **14**, 933-951. https://doi.org/10.1128/CMR.14.4.933-951.2001

[5] Ambler, R.P. (1980) The Structure of β-Lactamases. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, **289**, 321-331. https://doi.org/10.1098/rstb.1980.0049

[6] Kansaye, H. (2020) Phénotypes de résistance aux bêta-lactamines des souches d'entérobactéries isolées de pus, d'hémoculture et de selles au CHU du point G. Thèse de Doctorat, Université des sciences des techniques et des technologies de Bamako, 99 p.

[7] Bebrone, C. (2007) Metallo-Beta-Lactamases (Classification, Activity, Genetic Organization, Structure, Zinc Coordination) and Their Superfamily. *Biochemical Pharmacology*, **74**, 1686-1701. https://doi.org/10.1016/j.bcp.2007.05.021

[8] Walsh, T.R., Toleman, M.A., Poirel, L. and Nordmann, P. (2005) Metallo-β-Lactamases: The Quiet before the Storm? *Clinical Microbiology Reviews*, **18**, 306-325. https://doi.org/10.1128/CMR.18.2.306-325.2005

[9] Poirel, L., Pitout, J.D. and Nordmann, P. (2007) Carbapenemases: Molecular Diversity and Clinical Consequences. *Future Microbiology*, **2**, 501-512. https://doi.org/10.2217/17460913.2.5.501

[10] Nicolau, D.P. (2008) Carbapenems: A Potent Class of Antibiotics. *Expert Opinion on Pharmacotherapy*, **9**, 23-37. https://doi.org/10.1517/14656566.9.1.23

[11] Karabay, O., Altindis, M., Koroglu, M., Karatuna, O., Aydemir, O.A., and Erdem, A.F. (2016) The Carbapenem-Resistant Enterobacteriaceae Threat Is Growing: NDM-1 Epidemic at a Training Hospital in Turkey. *Annals of Clinical Microbiology and Antimicrobials*, **15**, Article No. 6. https://doi.org/10.1186/s12941-016-0118-4

[12] Wang, J., Yao, X., Luo, J., Zeng, L., Lv, Z. and Liu, J.H. (2018) Emergence of *Escherichia coli* coproducing NDM-1 and KPC-2 Carbapenemases from a Retail Vegetable, China. *Journal of Antimicrobial Chemotherapy*, **73**, 252-254. https://doi.org/10.1093/jac/dkx335

[13] Torres-González, P., Bobadilla-del Valle, M., Tovar-Calderón, E., Leal-Vega, F., Hernández-Cruz, A., Martínez-Gamboa, A., *et al.* (2015) Outbreak Caused by Enterobacteriaceae Harboring NDM-1 Metallo-β-Lactamase Carried in an IncFII Plasmid in a Tertiary Care Hospital in Mexico City. *Antimicrobial Agents and Chemotherapy*, **59**, 7080-7083. https://doi.org/10.1128/AAC.00055-15

[14] Rossi Gonçalves, I., Ferreira, M.L., Araujo, B.F., Campos, P.A., Royer, S., Batistão, D.W., *et al.* (2016) Outbreaks of Colistin-Resistant and Colistin-Susceptible KPC-Producing *Klebsiella pneumoniae* in a Brazilian Intensive Care Unit. *Journal of Hospital Infection*, **94**, 322-329. https://doi.org/10.1016/j.jhin.2016.08.019

[15] Elouennas, M., Sahnoum, I., Zrara, A. and Bajjou, T. (2008) Epidemiology and susceptibility profile of blood Culture Isolates in an Intensive Care Unit (2002-2005). *Médecine et Maladies Infectieuses*, **38**, 18-24. https://doi.org/10.1016/j.medmal.2007.10.006

[16] Gba Karine, M.K., Guessennd Nathalie, K., Makaya Dangui Nieko Nicole, P., Tahou Eric, J., Konan, F., *et al.* (2018) Detection of Metallo-beta-lactamase Producing *Pseudomonas aeruginosa* in an Abidjan Hospital, Côte d'Ivoire. *Journal of Advances in Microbiology*, **8**, 1-7. https://doi.org/10.9734/JAMB/2018/39343

[17] Kaya-Ongoto, M.D., Kayath, C.A., Nguimbi, E., Lebonguy, A.A., Nzaou, S.A.E., Elenga Wilson, P.S., *et al.* (2019) Genetic clearness novel strategy of group I *Bacillus* Species Isolated from Fermented Food and Beverages by Using Fibrinolytic Enzyme Gene Encoding a Serine-Like Enzyme. *Journal of Nucleic Acids*, **2019**, Article ID: 5484896. https://doi.org/10.1155/2019/5484896

[18] Saderi, H., Karimi, Z., Owlia, P., Bahar, A. and Akhavi, R.S.M. (2008) Phenotypic Detection of Metallobeta-Lactamase-Producing *Pseudomonas aeruginosa* Strain Isolated from Burned Patients. *Iranian Journal of Pathology,* **3**, 20-24.

[19] Sedighi, M., Halajzadeh, M., Ramazanzadeh, R., Amirmozafari, N., Heidary, M. and Pirouzi, S. (2017) Molecular Detection of β-Lactamase and Integron Genes in clinical Strains of *Klebsiella pneumoniae* by Multiplex Polymerase Chain Reaction. *Revista da Sociedade Brasileira de Medicina Tropica*, **50**, 321-328. https://doi.org/10.1590/0037-8682-0001-2017

[20] CLSI (Clinical and Laboratory Standards Institute) (2021) Performance standards for antimicrobial susceptibility testing. 25th informational supplement, Clinical and Laboratory Standards Institute, Wayne, M100-S25.

[21] Yong, D., Lee, K., Yum, J., Shin, H., Rossolini, G. and Chong, Y. (2002) Imipenem-EDTA Disk Method for Differentiation of Metallo-Beta-Lactamase-Producing Clinical Isolates of *Pseudomonas spp.* and *Acinetobacter spp. Journal of Clinical Microbiology*, **40**, 3798-3801. https://doi.org/10.1128/JCM.40.10.3798-3801.2002

[22] Frascas, D., Dahyot Fizelier, C. and Mimoz, O. (2008) La colistine en reanimation. *Réanimation*, **17**, 251-258. https://doi.org/10.1016/j.reaurg.2008.01.005

[23] Sturenburg, E., Kuhn, A., Mack, D. andLaufs, R. (2004) A Novel Extended-Spectrum Beta-Lactamase CTX-M-23 with a P167T Substitution in the Activesite Omega Loop Associated with Ceftazidime Resistance. *Journal of Antimicrobial Chemotherapy*, **54**, 406-409. https://doi.org/10.1093/jac/dkh334

[24] Yagi, T., Kurokawa, H., Shibata, N., Shibayama K and Arakawa, Y. (2000) A Preliminary Survey of Extended-Spectrum Beta-Lactamases (ESBLs) in Clinical Isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. *FEMS Microbiology Letters*, **184**, 53-56. https://doi.org/10.1111/j.1574-6968.2000.tb08989.x

[25] Essack, S.Y., Hall, L.M. and Livermore, D.M. (2004) *Klebsiella pneumoniae* Isolate from South Africa with Multiple TEM, SHV and AmpC Beta-Lactamases. *International Journal of Antimicrobial Agents*, **23**, 398-400. https://doi.org/10.1016/j.ijantimicag.2003.08.010

[26] Bahmani, N. (2019) Detection of VIM-1, VIM-2 and IMP-1 Metallo-β-lactamase genes in *Klebsiella pneumoniae* Isolated from Clinical Samples in Sanandaj, Kurdistan, West of Iran. *Iranian Journal of Microbiology*, **11**, 225-231.

https://doi.org/10.18502/ijm.v11i3.1325

[27] Abo-State, M., Saleh, Y. and Ghareeb, M. (2018) Prevalence and Sequence of Aminoglycosides Modifying Enzymes Genes among *E. coli* and *Klebsiella* Species Isolated from Egyptian Hospitals. *Journal of Radiation Research and Applied Sciences*, **11**, 408-415. https://doi.org/10.1016/j.jrras.2018.08.005

[28] Jamali, L., Haouzane, F., Bouchakour, M., Oufrid, S., Ghazlane, Z., El Mdaghri, N., Nadifi, S. and Timinouni, M. (2014) Prevalence of Plasmid Mediated Quinolone Resistance Genes among Enterobacteria Isolates in Moroccan Community. *International Journal of Innovation and Scientific Research*, **11**, 387-399.

[29] Bakiri, N. and Amamra, I. (2009) Etude de l'antibiorésistance de souche d'entérobactéries isolées des eaux polluées et en milieu hospitalier, Microbiologie général et biologie moléculaire des micro-organismes. Université des frères Mentouri Constantine, Constantine, 1 p.

[30] Decré, D. (2012) *Acinetobacter baumannii* et résistance aux antibiotiques: Un modèle d'adaptation. *Revue francophone des laboratories*, **441**, 43-52. https://doi.org/10.1016/S1773-035X(12)71412-0

[31] Bradford, P.A. (2001) Extended-Spectrum Beta-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. *Clinical Microbiology Reviews*, **14**, 933-951. https://doi.org/10.1128/CMR.14.4.933-951.2001

[32] Thabet, L., Memmi, M., Turki, A. and Messadi, A.A. (2010) The Impact of Use an Antibiotic Resistance in an Intensive Care Burn Department. *Laboratory of Biology*, **88**, 296-300.

[33] Ferjani, A., Mkaddemi, H., Tilouche, S., Marzouk, M., Hannechi, N., Boughammoura, L. and Boukadida, J. (2011) Caractéristiques épidémiologiques et bactériologiques des bactéries uropathogènes isolées dans un milieu pédiatrique. *Archives de Pédiatrie*, **18**, 230-234. https://doi.org/10.1016/j.arcped.2010.09.024

[34] Guessennd, N.K., Ouattara, M.B., Ouattara, N.D., Nevry, R.K., Gbonon, V., Tiekoura, K.B., *et al.* (2013) Étude des bactéries multirésistantes des effluents hospitaliers d'un centre hospitalier et universitaire (CHU) de la ville d'Abidjan (Côte d'Ivoire). *Journal of Applied Biosciences*, **69**, 5456-5464. https://doi.org/10.4314/jab.v69i0.95071

[35] Asadolah-Malayeri, H.O., Hakemi-Vala, M. and Davari, K. (2016) Role of *Aders* and *OXA23* genes among Imipenem resistant *Acinetobacter baumannii* isolates from two hospitals of Tehran, Iran. *Iranian Journal of Pathology*, **11**, 345-353. https://doi.org/10.1016/j.antinf.2011.03.005

[36] Grall, N., Andremont, A. and Armand-Lefèvre, L. (2011) Résistance aux carbapénèmes: Vers une nouvelle impasse? *Journal des Anti-Infectieux*, **13**, 87-102.

[37] Mesaros, N., Van Bambeke, F.J., Glupczynski, Y. and Tulkens, P.M. (2005) L'efflux des Antibiotiques: Un Mécanise Ubiquitaire Conduisant à la Résistance. Etat de la Question et Implications microbiologiques et Cliniques. *Louvain Médical*, **125**, 308-320.

[38] Sbiti, M., Lahmadi, K. and louzi, L. (2017) Profil épidémiologique des entérobactéries uropathogènes productrices de bêta-lactamases à spectre élargi. *Pan African Medical Journal*, **28**, Article No. 29, 8 p. https://doi.org/10.11604/pamj.2017.28.29.11402

[39] Henniche, F.Z., Yamouni, F., Bensersa, D., Sebahi, H., Aggoune, N., Chabani, A. and Zerouki, A. (2021) Infection urinaire à entérobactéries productrices des β-lactamases à spectre élargi chez l'enfant: Fréquence, facteurs de risque et alternatives thérapeutiques". *Journal de Pédiatrie et de Puériculture*, **34**, 223-228.

https://doi.org/10.1016/j.jpp.2021.05.005

[40] Shahid, M., Singh, A., Sobia, F., Rashid, M., Malik, A., Shukla, I. and Khan, H. (2011) bla_{CTX-M}, bla_{TEM}, and bla_{SHV} in Enterobacteriaceae from North-Indian Tertiary Hospital: High Occurrence of Combination Genes. *Asian Pacific Journal of Tropical Medicine*, **4**, 101-105. https://doi.org/10.1016/S1995-7645(11)60046-1

[41] Paveza, M., Troncosoa, C., Ossesa, I., Salazara, R., Illesca, V., Reydetc, P., Rodríguezc, C., Chahind, C., Conchad, C. and Barrientos, L. (2019) High Prevalence of CTX-M-1 Group in ESBL-Producing Enterobacteriaceae Infection in Intensive Care Units in Southern Chile. *The Brazilian Journal of Infectious Diseases*, **23**, 102-110. https://doi.org/10.1016/j.bjid.2019.03.002

[42] Raji, M.A., Jamal, W., Ojemeh, O. and Rotimi, V.O. (2015) Sequence Analysis of Genes Mediating Extended-Spectrum Beta-Lactamase (ESBL) Production in Isolates of *Enterobacteriaceae* in a Lagos Teaching Hospital, Nigeria. *BMC Infectious Diseases*, **15**, Article No. 259. https://doi.org/10.1186/s12879-015-1005-x

[43] Hagel, S., Stallmach, A., Keller, P. and Pletz, M. (2015) Multiresistant Organisms. *Zentralblatt fur Chirurgie*, **140**, 417-425. https://doi.org/10.1055/s-0032-1328343

[44] Skurnik, D. and Andremont, A. (2006) Antibiothérapie sélectionnante: De la théorie à la pratique. *Réanimation*, **15**, 198-204. https://doi.org/10.1016/j.reaurg.2006.03.002