Phenotypic Distribution of Serine- and Zinc-Type Carbapenemases Among Clinical Bacterial Isolates in a Tertiary Hospital in Benin, Nigeria

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Abstract

Background: Serine and zinc type carbapenemases are distributed in many genera of bacteria and are typically associated with specific regions or countries.

Objectives: This study phenotypically determined the prevalence of serine and zinc-type carbapenemases among Gram-negative bacilli recovered from clinical specimens in Benin, Nigeria.

Materials and Methods: Totally, 158 consecutive non-duplicate bacterial isolates (gram-negative bacilli) recovered from clinical samples were screened for serine and zinc-type carbapenemases using the simplified carbapenemase inactivation (sCIM) and ethylenediaminetetraacetic acid -double-disc synergy test methods.

Results: The isolates recovered from clinical specimens included 126 Enterobacteriaceae (79.7%), 7 *Acinetobacter* spp (3.7%), and 28 oxidase positive gram negative bacilli (17.7%). Twenty-eight isolates (17.7%) out of the 158 tested samples were carbapenemase positive. There was no significant difference in the prevalence of serine- and zinc-type carbapenemases ($P=0.0748$). However, the prevalence of zinc-type carbapenemase was significantly higher in *Pseudomonas aeruginosa* compared with other isolates ($P=0.0028$) while that of serine-type carbapenemase was not affected by the type of clinical isolates ($P=0.7216$). Finally, the prevalence of both serine- and zinc-type carbapenemases were not affected ($P>0.05$) by clinical specimens and the source of isolates (in-patient vs. out-patient) respectively.

Conclusion: In general, the prevalence of zinc-type (12%) carbapenemases was insignificantly higher than that of serine-type (5.7%) carbapenemases. The measures to reduce infections caused by carbapenemase-producing organisms (CPOs) are advocated accordingly.

Background

The incidence of multidrug-resistant organisms, causing clinical infections as a major public health challenge, is on the rise and closely associated with morbidity and mortality.[1] Locally and internationally, carbapenem-resistant organisms are a growing concern in the clinical management of infections and pose a significant threat to currently available antibiotics.[2,3] The carbapenems are broad-spectrum β-lactams which are considered as the antibacterials of last-resort against MDR-Gram-negative bacteria like extended spectrum β-lactamase producing Enterobacteriaceae, *Acinetobacter* species, *Pseudomonas* species, and the like.[4-6] In recent years, however, their usefulness has been threatened by the emergence and spread of bacteria that produce carbapenemase enzymes.[7,8] The carbapenemases have been fittingly assigned to three of the four β-lactamases classes, namely, Ambler classes A, B, and D.[9,10] Interestingly, the aforementioned classes can be differentiated based on the hydrolytic mechanism at their active sites. Class A and D carbapenemases are referred to as "serine carbapenemases" because they have serine at their active sites whereas class B carbapenemases which are also referred to as "metallo-β-lactamases" have zinc at their active sites.[9]

Certain carbapenemase enzymes have been typically associated with specific regions or nations, though

carbapenemases have been recovered from many genera of bacteria globally. The era of international travel and medical tourism may have also influenced the spread of various carbapenemase types as per region thus necessitating an urgent need for routine local and national surveillance.[11]

The epidemiology of carbapenemase-producing organisms (CPOs) in North America, Europe, and Asia has been described in considerable detail,[10] though not much is known about their spread and clinical importance in Africa. For example, there are no data to determine whether serine- or zinc-type carbapenemase is the most prevalent in Nigeria. A systematic review in 2015 found only one study in this country that reported oxacillinase (OXA)-23 carbapenemase in *Acinetobacter baumannii.*[10] A worldwide map showing countries with reports on carbapenemases demonstrated no carbapenemase detected in Nigeria.[1] Similarly, another review with a worldwide map of *Klebsiella pneumoniae* carbapenemase, New Dehli metallo-β-lactamase (NDM), and OXA-48 carbapenemases showed Nigeria as one of the countries with unknown status.[11] Most studies from Nigeria targeted specific carbapenemases (either of serine- or zinc type)[12,13] perhaps due to the cost of molecular techniques. The findings of a study on the whole genomic sequence of 9 carbapenemase positive isolates represented that the serine-type of carbapenemase was most frequent while a phenotypic study showed that the zinc-type predominated the serine one.[14,15] Thus, it is important to identify the exact type so as to initiate an effective treatment and infection control program. Against this background, this study was conducted to phenotypically determine the most prevalent type of carbapenemases in our institution.

Materials and Methods
Sample Site and Bacterial Isolates
This study was conducted at the Medical Microbiology Laboratory, Medical Laboratory Services of the University of Benin Teaching Hospital, Benin, Nigeria. The hospital, a tertiary hospital located in the metropolitan city of Benin, is a referral centre with over 800 beds and tends to the specialist healthcare needs of about eight neighboring states. A total of 158 consecutive non-duplicate bacterial isolates (gram-negative bacilli) was recovered from clinical samples routinely and sent to the laboratory. The specimens included wound swabs (55), aspirates (9), catheter tip (6), ear swabs (2), eye swabs (10), sputum (11), blood (3), vaginal swabs (11), urine (49),) and throat swab (2). The isolates were recovered between 15th February 2019 and 30th April 2019. Eventually, the bacterial isolates were identified following using standard microbiological techniques.[9]

Simplified Carbapenemase Inactivation Method
A modification of simplified Carbapenemase Inactivation (sCIM) described by Jing et al was used in this study.[16]

Briefly, a 0.5 McFarland standard suspension of the indicator strain (*Escherichia coli* ATCC 25922) was swabbed in three directions on the Mueller-Hinton agar (MHA) plate and allowed to dry for 3-10 minutes. One to three colonies of the test bacillus grown on the blood agar was then smeared on one side of 10 μg meropenem and 10 μg imipenem discs (both from Oxoid, UK). The side of antibiotic discs smeared with the test organism was immediately placed on the already seeded MH plate and incubated at 35°C for 16-18 hours. Other meropenem and imipenem discs not smeared with the organism were placed on the MHA plate and served as controls. Discs with a zone diameter of ≤22 mm indicated that the isolate was capable of producing carbapenemase. Finally, inhibition zones of ≥26 mm and 23-25 mm were considered to be a negative result and carbapenemase indeterminate result, respectively.

Metallo-β-lactamase Method
A modification of the ethylenediaminetetraacetic acid (EDTA) double-disc synergy test described by Lee et al was used for the detection of Metallo-β-lactamase (MBL).[17] Briefly, each test organism (gram-negative bacillus) was seeded on the surface of the MHA plate. Meropenem and imipenem discs (10 μg each) were placed on either side of a 1900 μg EDTA disc, 10 mm apart from the EDTA disc (edge–to–edge) on the seeded plate. The plate was then incubated overnight at 37°C. A synergistic zone of inhibition between the EDTA disc and one disc or both was taken as positive for MBL.

Differentiation Between Serine and Zinc-Type Carbapenemases
The sCIM method detects both serine- and zinc-type carbapenemases.[16] Therefore, if both the sCIM and MBL methods were positive in an isolate, the isolate was inferred to be positive for the zinc-type carbapenemase (MBL). However, if it was positive as determined by the sCIM and negative as cleared by the MBL method, the isolate was considered to have serine-type carbapenemases.

Statistical Analysis
The obtained data were analyzed with the chi-square or Fischer exact tests as appropriate using INSTAT® software, version 2.05a. A *P* value of < 0.05 was deemed statistically significant.

Results
In general, 28 (17.7%) and 19 (12.0%) isolates were carbapenemase positive (serine- and zinc-types) and MBL positive (zinc-type only) out of 158 bacterial isolates that were screened for carbapenemase enzymes using MBL and sCIM methods for the detection of zinc-type carbapenemase-MBL and both serine- and zinc-type carbapenemase, respectively. Accordingly, the prevalence of serine-type carbapenemase was 9 (5.7%). In addition,

carbapenemase enzymes were more likely to be detected in both techniques by using a combination of imipenem and meropenem discs compared to either one alone (MBL vs. sCIM method: $P=0.0022$; vs. MBL method: $P<0.0001$). The prevalence of serine- and zinc-type carbapenemases did not differ significantly ($P=0.0748$) from each other (Table 1).

On the other hand, the prevalence of zinc-type carbapenemase was significantly ($P=0.0028$) higher among the isolates of *Pseudomonas aeruginosa* compared with other isolates while the incidence of serine-type did not differ significantly ($P=0.0748$) among the tested isolates (Table 2).

Finally, the prevalence of serine- and zinc-type carbapenemases failed to differ significantly ($P>0.05$) in relation to clinical specimens (Table 3) and among the isolates that were recovered from in- and out-patients (Table 4)

Discussion

One of the most concerning forms of antimicrobial resistance is resistance to carbapenems.[11] The need for local and national surveillance on prevalent carbapenemase types among clinical isolates is necessary as widespread international travels may affect the local prevalence, along with antibiogram and therapeutic options. In Nigeria,

Table 1. Detection Rate for Carbapenemase for 158 Gram-Negative Bacilli

Antibiotics	MBL (%)	sCIM (%)
Imipenem	5 (3.2)	8 (5.1)
Meropenem	1 (0.6)	1 (0.6)
Imipenem + Meropenem	13 (8.2)	19 (11.4)
TOTAL	19 (12.0)	28 (17.7)

Note. MBL: Metallo-β-lactamase; sCIM: Simplified carbapenemase inactivation method MBL vs. sCIM method: $P=0.0022$; sCIM vs. MBL method: $P<0.0001$; Zinc vs. Serine (12% vs. 5.7%: $P=0.0748$).

Table 2. Distribution of Carbapenemase Enzymes Among Bacterial Isolates Causing Clinical Infections

Organism	Number of Tested Isolates	Type of Carbapenemase	
		Zinc (%)	Serine (%)
Citrobacter spp.	2	0*	0*
Enterobacter spp.	14	4 (28.6)	0*
Escherichia coli	42	1 (2.4)	5 (11.9)
Klebsiella spp.	42	0*	3 (7.1)
Providencia spp.	8	2 (25)	0*
Proteus spp.	14	1 (7.1)	1 (7.1)
Salmonella spp.	1	0*	0*
Alcaligenes spp.	3	1 (33.3)	0*
Pseudomonas aeruginosa	25	10 (40)	0*
Acinetobacter spp.	7	0*	0*

Note. * Not included for statistics; Zinc-type vs. isolates: $P=0.0028$; Serine-type vs. Isolates: $P=0.7216$.

Table 3. Prevalence of Carbapenemase Enzymes Among 158 Gram-Negative Bacilli in Relation to Clinical Specimen Type

Clinical Specimen	Number of Tested Isolates	Type of Carbapenemase	
		Zinc (%)	Serine (%)
Aspirates	6	1 (16.7)	0
Eye swab	10	-	1 (10.0)
Urine	49	9 (18.4)	6 (12.2)
Wound swab	55	9 (16.4)	2 (3.6)

Note. Zinc-type vs. specimen type: $P=0.5455$; Serine-type vs. specimen type: $P=0.3407$.

Table 4. Distribution of Carbapenemase Enzymes for 158 Gram-negative Bacilli in Relation to the Source of Patients

Source of Specimens	Number of Tested Isolates	Type of Carbapenemase	
		Zinc (%)	Serine (%)
In-patients	108	13 (12.0)	7 (6.5)
Out-patients	50	6 (12)	2 (4.0)

Note. Zinc-type vs. the source of patient: $P=0.9947$; Serine-type vs. the source of patient: $P=0.7792$.

several conflicting reports exist with no clear-cut answers as to whether serine- or zinc-type carbapenemase is most prevalent. Thus, the present study was conducted in this regard.

In this study, carbapenemase enzyme was more likely to be detected phenotypically by a combination of antibiotics (imipenem and meropenem) in both techniques (i.e., MBL and sCIM) compared to either antibiotic alone. This is an improvement of both methods and the use of more than one carbapenem is advocated accordingly. We had earlier reported the advantage of using more than one carbapenem in the MBL method.[18] Furthermore, the sCIM technique has been previously shown to have high concordance with the PCR technique (100%),[16] being able to detect both zinc- and serine-type enzymes of which an array of carbapenemase genes was implicated.

The prevalence of zinc-type carbapenemase in this study was 12.0% while that of the serine-type was 5.7%, indicating the higher prevalence of zinc-type carbapenemase in our setting. This is in agreement with a report from Lagos, Nigeria,[15] which also used phenotypic methods and demonstrated the higher prevalence of zinc-type carbapenemase. A study in Benin, Nigeria[14] utilized the whole genome sequencing on 9 carbapenemase-producing isolates that were recovered from clinical specimens. Based on the results, genes encoding serine-type enzymes were detected in 7 isolates (i.e., one OXA-181 and 6 OXA-48) while the other two isolates harbored a zinc-type enzyme (NDM-1), indicating the higher preponderance of the serine-type in Benin and contradicting the finding of this study. However, as Jesumirhewe et al used 9 isolates from 3 genera of the Enterobacteriaceae as against 158 isolates consisting of the members of the Enterobacteriaceae, *Acinetobacter, Alcaligenes,* and *Pseudomonas* in this study.[14] The presence

of the zinc-type carbapenemase, especially NDM-1 in Nigeria has been suggested to originate from the Indian subcontinent where the gene is prevalent.[19] Nigerians frequently visit India for medical tourism. It has been reported that one in four patients admitted to an Indian hospital will acquire a nosocomial infection.[20] This may explain the higher prevalence observed in this study. However, there was no significant difference ($P = 0.0748$) in the prevalence of both serine-type (5.7%) and zinc-type (12%) carbapenemases in this study.

Conversely, the incidence of zinc-type carbapenemase was significantly higher ($P = 0.0028$) among the isolates of *P. aeruginosa* compared with other isolates. Recent studies worldwide have shown the increased isolation of MBL-producing (zinc-type) *P. aeruginosa* strains causing clinical infections.[16,21,22] Although this organism has intrinsic resistance to certain antibiotics through some mechanisms (e.g., multidrug resistance efflux pumps, decreased permeability, and the loss of the outer membrane porin protein),[23] the detection of MBL among the majority of strains causing infections in this study is the cause of concern as far as patient management and infection control is concerned. There was no significant difference in the prevalence of serine-type carbapenemase in relation to isolates perhaps due to the small number recorded in this study.

The finding regarding the prevalence of carbapenemase (serine- and zinc-types) did not differ significantly ($P > 0.05$) in relation to clinical specimens, which agrees with a previous report, with respect to zinc-type carbapenemases.[18] Similarly, there was no significant difference ($P > 0.05$) in the prevalence of both serine- and zinc-type carbapenemases among inpatients and outpatients. Antibiotics use in Nigeria is unregulated and over the counter sales of antibiotics without prescriptions are rife.[24-26] This may explain the finding of this study especially as CPOs were implicated in community infections among outpatients.

The isolates recovered from the urine samples had the highest prevalence of CPOs in comparison with other samples. This finding concurs with the results of several previous studies in Nigeria that reported the source of the CPOs namely Benin, Kano, and Lagos states, respectively.[6,11,12,15] As the global prevalence of urinary tract infection is on the rise, it is likely that in settings such as Nigeria where antibiotic use is unregulated, β-lactam antibiotics (i.e., penicillins, cephalosporins, and carbapenems) may be easily relied upon when such infections are under suspicion. This may inadvertently lead to selective pressure and thus the survival and spread of MDR organisms. The increased use of carbapenem antibiotics owing to the rising incidence of MDR organisms may have also led to the proliferation of these carbapenemase-producing superbugs in our locality.[6,11,15] Although carbapenem antibiotics are considered as the drugs of last resort in our setting, it is also very likely that

the frequent travels for medical tourism by Nigerians to other nations may have increased the local prevalence of CPO causing infections. It has been estimated that 30 000 Nigerians spend $1 billion on medical tourism to other nations and India is the most visited country.[17] There is, therefore, a high index of suspicion that this has contributed to the local burden and global dissemination of CPO.

Conclusion
The overall prevalence of CPOs in this study was 17.7%, with zinc-type predominating, albeit, insignificantly. The clinical specimen that the isolates were recovered from, as well as the source of patients (inpatient or outpatient) did not affect the prevalence of both types of carbapenemases. Accordingly, there is need to increase local and national surveillance in order to reduce the incidence and spread of CPOs in our hospital and community setting.

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