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Detection of Metallo-β-Lactamase-Producing Gram-Negative Bacterial Isolates from Patients with Lower Respiratory Tract Infections in a Tertiary Hospital in Benin City, Nigeria

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ABSTRACT

Objective: Lower respiratory infections (LRTIs) are a persistent threat to quality of life and resistant bacterial strains serve to worsen treatment outcomes. This study aimed at investigating the prevalence of bacteria-producing metallo-β-lactamase (MBL) among patients with lower respiratory tract infections in a tertiary hospital in Benin City, Nigeria.

Methodology: A cross-sectional study was conducted at the University of Benin Teaching Hospital, Benin City. Questionnaires were filled for each study participant presenting with signs and symptoms of LRTI. Sputum specimens were collected from each patient into sterile wide-mouth containers and sent to the Medical Microbiology Laboratory for microbiological processing. Emergent bacterial colonies were identified and antimicrobial susceptibility tests carried out using standard techniques.

Result: A total of 185 Gram negative bacterial isolates were recovered from the sputum specimens of patients. These included Enterobacteriaceae (160), *Acinetobacter* spp (7), *Alkaligenes* spp (4) and *P.aeruginosa* (14). A total of 9 (4.7%) isolates were MBL-producing; among them *Hafnia alvei* was most likely MBL-producing when compared with other isolates (0.0191). Very poor susceptibility was observed for MBL-producing bacteria as 100% multi-drug resistance was observed, non-MBL-producing isolates showed 63.8% MDR.

Conclusion: The prevalence of MBL-producing bacteria causing LRTI in this study was 4.7%. The study harps on prudence in the use of antibiotics in order to minimize the emergence and spread of resistant bacterial strains in our setting.

Keywords: Metallo-beta-lactamase, bacteria, lower respiratory tract infection, resistance.

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INTRODUCTION

Lower respiratory infections (LRTIs) are a persistent threat to quality of life and alongside chronic obstructive pulmonary disease (COPD), ranks among the commonest causes of death globally after ischemic heart disease and cerebrovascular disease (1). Expectedly, LRTIs have worse outcomes in low and middle-income countries as recent data suggest that the highest morbidity and mortality figures are from such nations (2). The etiologic agents of LRTI are bacterial, fungal, viral, parasitic and protozoan with bacterial agents accounting for a large majority of documented cases (1, 2).

Beta-lactam antibiotics are the drugs of choice in managing infections of bacterial etiology (3). They comprise the penicillins, cephalosporins, cephamycins and carbapenems. The carbapenems are broad spectrum in action and resistant to several β-lactamases notably extended spectrum β-lactamase (ESBL), making them drugs of choice (4, 5). They are drugs of last resort in managing systemic infections caused by bacterial strains that are resistant to several antibacterial agents. However in recent times, resistance has been demonstrated to this class of antibacterials. Resistance to the carbapenems is due to impermeability of the outer membrane of bacterial strains due to loss of outer membrane porin (4), active expulsion of carbapenems out of the periplasmic space after their entrance by efflux pumps (4), production of non-metallo carbapenemases, such as the GES (Guiana extended-spectrum β-lactamase) and KPC (*Klebsiella pneumoniae* carbapenemase) (types and the production of metallo-β-lactamases (MBLs) (4, 6).

Carbapenem resistance among *Enterobacteriaceae* can be conferred by several genetic mechanisms, but epidemiologically the most important of them results in the production of beta-lactamases (carbapenemases), which hydrolyze and inactivates carbapenems and most other beta-lactams (7). Carbapenemases either have serine or zinc in their active sites (4). The carbapenemases in *Enterobacteriaceae* belong to 3 classes of β-lactameses – the Ambler class A, B and D (4, 7). The carbapenemases with zinc in their active sites are in Ambler class B and are mostly referred to as metallo-β-lactamases.

Several genes have been implicated in MBL production, namely; imipenemase (IMP), verona integrase metallo-β-lactamase (VIM) and New Dehli metallo-β-lactamase (NDM) enzymes. These genes are easily transferred between bacteria as they are harbored by highly mobile genetic elements (4, 6).

We had previously showed the presence of ESBL among bacterial agents of LRTI in Benin City (5). As dissemination of multi-drug resistant bacteria is increasingly recognized as a threat to quality healthcare, this study aims to determine the prevalence of bacteria-producing metallo-beta-lactamase among patients with lower respiratory tract infections in a tertiary hospital in Benin City, Nigeria.

MATERIALS AND METHODS

Study Population:

A cross-sectional study was adopted for this research. The study was conducted at the University of Benin Teaching Hospital, Benin City between 3rd July 2017 and 4th March, 2018. A total of 489 patients presenting with signs and symptoms of LRTI were recruited for the study. The study participants included inpatients (admitted in wards) and outpatients (attending outpatient

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clinics). Informed consent was obtained from all patients or parents/guardians in case of children. The study was reviewed and approved by the Ethics Committee of the hospital with number: ADM/E 22/A/VOL. VII/1489.

Specimen Collection and Sample Processing:

Sputum specimens were collected from each patient into sterile wide-mouth containers and sent to the Medical Microbiology Laboratory, University of Benin Teaching Hospital for microbiological processing. Films were made from the sputum specimens and stained by Gram's Method. Sputum specimens were processed within two hours after collection and were cultured onto chocolate, blood and MacConkey agar plates. The cultured plates were thereafter incubated for 24 - 48 hours at 37ºC.

Identification of microorganisms

Emergent colonies from culture plates were identified using standard microbiological techniques. Isolates that were Gram negative rods were identified using the Microbact 24E identification system (Oxoid, U.K). The microbact Gram negative system is a standardized micro-substrate system designed to simulate conventional biochemical substrates used for the identification of Enterobacteriaceae and common miscellaneous Gram negative bacilli. A suspension of the test organism was made in sterile distilled water and 100 µl was dispensed into each well containing the substrates. The substrates include Lysine, ornithine, hydrogen sulphide, glucose, mannitol, xylose, ONPG, indole, urease, voges-proskwer, citrate, tryptophan deaminase, gelatin, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin and arginine. This was then incubated for 18-24 hours at 37ºC. An octal code was thereafter gotten after each group of three reactions generated a single digit; the sum of positive indices in each group of three formed the code number. This was thereafter entered into the computer package and the identity of the test organism was noted.

Antimicrobial Susceptibility Test:

Antimicrobial susceptibility tests were performed for each isolate following the Clinical Laboratory Standards Institute (CLSI) (2013) guidelines (8). Briefly, test organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standard. Once matched, a sterile cotton-wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on the side of the test tube. The entire surface of Mueller-Hinton agar plate was seeded by swabbing in three directions with the swab. The antibiotics included Ciprofloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg), Sulfamethoxazole-trimethoprim (25µg), Cefotaxime (30µg), Cefuroxime (30µg), Cefixime (5 µg), Ceftriaxone-sulbactam (30/15 µg), Gentamicin (10 µg). Bacterial isolates which showed resistance to ≥ 3 classes of antibacterial agents were deemed multi-drug resistant (MDR).

Detection of Metallo-β-lactamase:

Metallo-β-lactamase was detected using a modification of the method described by Lee et al. (9) Bacterial isolates were seeded on Mueller-Hinton's agar using sensitivity tests above. Imipenem (10µg) and meropenem (10µg) discs were placed on either side of a 1,900µg EDTA disc, 10 mm apart from the EDTA disc (edge–to–edge) on the seeded plate. The plate was incubated overnight at 37^0C. A synergistic zone of inhibition between the EDTA disc and one or both

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discs were taken as indicative of metallo-β-lactamase production. .

STATISTICAL ANALYSIS

The data obtained were analyzed with Chi (X^2) square test or Fischer's exact test where appropriate using INSTAT® software. A p value of < 0.05 was deemed statistically significant.

RESULTS

A total of 185 Gram negative bacterial isolates were recovered from the sputum specimens of patients with signs and symptoms of LRTI during the study period. These included *Enterobacteriaceae* (160), *Acinetobacter* spp (7), *Alkaligenes* spp (4) and *P.aeruginosa* (14). A total of 9 (4.7%) out of the 185 isolates were MBL-producers.

The prevalence of MBL-production was significantly higher (p=0.0191) among isolates of *Hafnia alvei* compared with other isolates (Table 1).

In relation to source of patients, there was no significant difference (p=0.5177) in the prevalence of MBL-producing-bacteria recovered from in-patients and out-patients, although inpatients were 2 times more likely to be infected with MBL-producing bacteria (Table 2).

One hundred percent of MBL-producing organisms were MDR while 63.8% of non-MBL-producing organisms were MDR. MBL-producing organisms were most likely to be MDR (p= 0.0285). The distribution of multidrug resistant (MDR) bacterial isolates in relation to metallo-beta-lactamase (MBL) production is shown on Table 3.

Table 1: Distribution of metallo-β-lactamase enzymes among bacterial isolates recovered from patients with LRTI.

Organism	No of isolates recovered/tested (% of total)	No of MBL-positive isolates
Citrobacter spp	4	1 (25.0)
E. coli	14	1 (7.1)
Enterobacter spp	43	2 (4.7)
Klebsiella spp	82	1 (1.2)
Hafnia alvei	2	1 (50.0)
Proteus mirabilis	2	0*
P. vulgaris	2	0*
Providencia spp	6	0*
Shigella spp	1	0*
Serratia spp	4	1 (25.0)
Acinetobacter spp	7	1 (14.3)
Alkaligenes spp	4	1 (25.0)
P. aeruginosa	14	0*
Total	185	9 (4.7)

MBL vs Isolates; p = 0.0191, Number in bracket = value in percentage, MBL – Metallo-β-lactamase, *= not included in statistical analysis.

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Table 2: Frequency of metallo-β-lactamase enzyme detection in relation to source of clinical isolates (Gram negative rods)

Clinical isolates	No Tested	No positive (%)	OR	95% CI	p
Inpatient	54	4 (7.4)	2.016	0.520, 7.818	0.5177
Outpatient	131	5 (3.8)			

Table 3: Prevalence of multi-drug resistant bacteria in relation to metallo-β-lactamase production

	No of isolates	Multi-drug resistant (%)
MBL	9	9 (100)
Non-MBL	176	112 (63.6)
Total	185	121 (65.4)

Isolates vs multi-drug resistance, p= 0.0285

DISCUSSION

For this study, the prevalence of MBL-producing bacteria was 4.9%. This value is lower than a previous study in northern Nigeria, where 25% of bacterial isolates from sputum were MBL-producing (10). However, the sample size in that study was comparatively lower than our study and the sampling technique for bacterial isolates was not stated in the study. Similarly, a higher prevalence was observed in a three month study in Benin where 27.4% of isolates from sputum was MBL positive (11). The etiologic agents of LRTI and their antibiogram have been shown to vary with time, geographical location and season (12). This may explain our findings. Also, *Hafnia alvei* was more likely to be MBL-producing in comparison to any other bacterial isolate recovered during the study period. Although plasmid- borne AmpC type β-lactamase genes originate from diverse Gram-negative bacilli such as *Hafnia alvei*, *Morganella morganii*, and *Aeromonas* spp (13), we haven't found data on the detection of MBL in *H. alvei*. However horizontal transfer of plasmids has been known to occur in pathogenic strains of *Enterobacteriaceae* and MBL strains are largely plasmid-borne (4,6,7). More studies exploring carriage of plasmid bearing MBL genes in *H. alvei* isolates would give clarity. To the best of our knowledge, this organism has not been

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previously reported as causing LRTI in our region (10, 11, 14). Case report of the organism being implicated in chronic obstructive pulmonary disease (COPD) have however been reported in Ankara, Turkey (15). Our study for the first time reports MBL-producing *H. alvei* as being implicated in LRTI in Benin, Nigeria.

In relation to source of patient, inpatients were two times more likely to have LRTI due to MBL-producing bacteria, though the finding was not statistically significant. As had been previously reported, the β-lactams are administered as antibiotic coverage for patients undergoing surgery as well as are available over-the-counter in our region (5, 11). It has been hypothesized that practices like this create selective pressure, ensuring the survival of β-lactamase producing isolates (11). The detection of MBL-producing isolates among symptomatic outpatients in this study is therefore worrisome for patient management and the possibility of spread of infection in the community with these superbugs.

All MBL-producing strains were MDR. This finding was not too surprising as plasmid harboring MBL genes also carries resistance determinants to several other antibiotics; VIM-1 positive isolates showed a statistically significant relationship with *qnrS1* gene (quinolone resistance gene) in a previous study (16). Similarly, most NDM-1 isolates have been demonstrated to carry 16S methylase genes which confer resistance to all aminoglycosides (17). A recent study in the Edo state, Nigeria which applied whole genome sequence on carbapenemase (serine and MBL) producing isolates showed that the isolates harbored large number of antibacterial resistance genes which included *qnr*, SHV, TEM, *aac(6')lb-cr*, *aac(3)-lla* and so on, borne on several plasmids (18). While the association between MBL-producing strains and resistance to the carbapenems and other antimicrobial classes may explain 100% MDR observed, it is noteworthy that 63.7% of non-MBL-producing bacterial isolates were MDR. This finding further highlights the existence of MDR bacterial isolates with other mechanisms of resistance. In recent times, ESBL- and AmpC-producing bacteria have been isolated from sputum samples of symptomatic patients and other clinical specimens in Benin (5, 11). These enzymes specifically inactivate the third generation cephalosporins, cephamycins and have been associated with resistance to aminoglycosides and sulfamethoxazole-trimethoprim, many of which were used in this study (5, 11, 19). The carbapenems are expensive and are drugs of last resort in our setting. This study therefore advocates caution and laboratory guidance before use of these drugs as resistant superbugs causing LRTI were recovered form symptomatic patients residing in hospital and community settings.

CONCLUSION

The prevalence of MBL-producing bacteria causing LRTI in this study was 4.7%, Metallo-β-lactamase producing bacteria showed 100% multi-drug resistance when compared to non-MBL-producing organisms. The study harps on prudence in the use of antibiotics in order to minimize the emergence and spread of resistant strains in our setting.

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