

EMERGENCE OF METALLO- β -LACTAMASE PRODUCING GRAM-NEGATIVE BACTERIA IN A HOSPITAL WITH NO HISTORY OF CARBAPENEM USAGE IN NORTHWEST NIGERIA

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ABSTRACT

Carbapenems are among the antibiotics of last resort against infections caused by antibiotic resistant bacteria. However, resistance to this important class of antibiotic is on the increase due to expression of metallo-beta-lactamases (MBLs). This study investigated the occurrence of MBL-producing bacteria in a healthcare facility in Sokoto, Nigeria. Swabs were collected from the rectum ($n = 29$) and bed linens ($n = 27$) of patients within the surgical wards of the hospital between March and August, 2018 and processed using Centers for Disease Control and Prevention (CDC) broth enrichment method for isolation of carbapenem resistant Gram-negative bacteria (CR-GNB). In addition, 110 bacteria isolated from clinical specimens submitted to microbiology laboratory of the hospital were collected and tested for susceptibility to antibiotics using the disc diffusion method. The carbapenem resistant isolates were further evaluated for MBL-production using the combined disc method. Overall, 31(28.2%) of the Gram-negative bacterial isolates were carbapenem resistant. The most predominant isolated bacterium in this study was *E. coli* (18; 36%). The isolates were highly resistant to cephalosporins (74%) and fluoroquinolones (52.7%) while remaining moderately susceptible to gentamicin (38.8%) and amoxicillin-clavulanate (45.7 %). Majority of the CR-GNB were extensively drug resistant (19; 38%) and pan drug resistant (10; 20%). The MBL-production test revealed that 19 (38.0%) of the CR-GNB were MBL-positive. The study revealed a high prevalence of MBL-producing CR-GNB in a hospital with no history of its use. This report documents for the first time the independent emergence of such resistance in our hospital. Implementation of adequate antibiotic stewardship program is therefore imperative so as to contain this emerging threat.

Keywords: Carbapenem Resistant Bacteria, Metallo- β -lactamase, Carbapenemase, MDR.

INTRODUCTION

Carbapenem is a broad-spectrum beta-lactam (β -lactam) antibiotic, developed as one of the last resort antibiotics for treatment of serious and life-threatening infections caused by multitude of multidrug resistant (MDR) Gram-negative bacterial infections, including extended spectrum- β -lactamase (ESBL)-producing Gram-negative bacteria (El-gamal *et al.*, 2017; Hsu *et al.*, 2017). The carbapenems were introduced in the late 1980s in response to emergence of ESBL-producing Gram-negative bacteria (Iovleva and Doi, 2017). This was soon overwhelmed by the resilience of bacteria. The plasmid borne genes which encoded Gram-negative β -lactamase mutated to evolve into more efficient mutants, leading to emergence of first carbapenem resistant Gram-negative bacteria (CR-GNB) in Japan in the early 1990s (Iovleva and Doi, 2017).

Since then, bacteria resistant to carbapenem has emerged and spread in the healthcare settings worldwide (Dortet *et al.*, 2014; Duin and Doi, 2017; Logan and Weinstein, 2017). This constitutes immediate threat to public health with attendant morbidity and mortality and thus requiring urgent and aggressive action (CDC, 2013). In the United States, CR-GNB causes approximately 9300 infections per year and up to half of all CR-GNB bloodstream infections result in death (CDC, 2013). A higher likelihood of death (more than 40% deaths) has been observed in patients with carbapenem-resistant infections compared with patients infected with carbapenem susceptible organisms (Falagas *et al.*, 2014; Xu *et al.*, 2017). Moreover, longer duration of hospital stays and consequently increased healthcare cost is associated with CR-GNB infections (Magiorakos *et al.*, 2017).

This resistance has been attributed to a number or combination of diverse mechanisms. These include the production of carbapenemase enzymes, modification of outer membrane permeability and up-regulation of efflux system (Potter *et al.*, 2016). Most notably among these mechanisms is the production of carbapenemases, which are group of broad spectrum β -lactamase enzymes with hydrolytic activities against all cephalosporins, carbapenems and penicillins (Potter *et al.*, 2016). The enzymes are functionally divided into those possessing serine group at their active site (Ambler classes A and D) and those possessing divalent metal ion(s) at their active site (Ambler Class B or the metallo- β -lactamase) (Iovleva and Doi, 2017; Walsh, 2005). Metallo-beta-lactamases (MBLs) efficiently hydrolyse carbapenems and other β -lactams (except monobactams) and are not inhibited by the clinically available β -lactamase inhibitors (Potter *et al.*, 2016). These enzymes are more commonly detected in *Klebsiella* spp. and *Escherichia coli* (Hsu *et al.*, 2017). The rapid global spread of this bacteria and accompanied virulence is facilitated by resident of most carbapenemase genes on ambulatory genetic elements (Potter *et al.*, 2016). This is responsible for clonal transfer among bacteria of the same species and horizontal acquisition among bacteria of different species.

In tandem with global trend, the prevalence of CR-GNB in Nigeria is increasing (Aibinu *et al.*, 2007; Ibrahim *et al.*, 2017). The resistance of clinical isolates to carbapenems has increased by about ten-folds within a decade from 4.1% in 2007 to 40.3 % in 2017 (Aibinu *et al.*, 2007; Ibrahim *et al.*, 2017). Molecular detection methods have also shown high circulation of carbapenemase genes in many Nigerian hospitals (Ogbolu and Webber, 2014; Mohammed *et al.*, 2015).

Though, emergence of carbapenem resistance has been mostly linked to prior carbapenem exposure, its occurrence among patients with no known medical history of its usage has however been documented in several countries including Nigeria (Mohammed *et al.*, 2015). This is similar to the emergence of colistin resistant bacteria among colistin naive patients (Olaitan *et al.*, 2016).

Because of the threat constituted by these organisms, prompt detection of patients with a CR-GNB infection is necessary not only for patient treatment decision and guiding infection control but also for epidemiological surveillance efforts aimed at limiting its spread (Magiorakos *et al.*, 2017). This study therefore aimed to determine the occurrence of MBL-producing bacteria isolates in a healthcare facility in Sokoto, Nigeria.

MATERIALS AND METHODS

Ethical Consideration

Ethical approval to conduct this research was obtained from the hospital ethics and research committee of the hospital with the reference number, SHS/SUB 133/VOL.1. After adequate briefing of patients or their relatives on the objective of the study, informed consent was obtained from all individuals enrolled in the study.

Study Design and Bacterial Isolates

This prospective observational study was conducted in a tertiary healthcare facility in Sokoto, Nigeria. Non-duplicate clinical isolates of Gram-negative bacteria isolated from routine clinical specimens brought to the microbiology laboratory of the hospital, were collected between March and August, 2018. Validated questionnaires were also administered to collect information on socio-demographic and clinical characteristics of patients from the patients' medical record.

Sample Collections

Rectal ($n = 29$) and bed linens ($n = 27$) swabs of both male and female patients of all ages on admission within the surgical wards of the hospital during the study period were collected. The swabs were processed within two hours after collection using the standard microbiological techniques such as culturing, isolation and identification. In addition, 110 bacteria isolated from clinical specimens submitted to microbiology laboratory of the hospital were collected and tested for susceptibility to antibiotics using the disc diffusion method.

Screening, Isolation and Identification of Carbapenem-Resistant Bacteria

The screening and isolation of CR-GNB was

done in accordance with the United States Centers for Disease Control and Prevention (CDC) isolation protocol for CRB as previously described (CDC, 2011). Briefly, swabs were inoculated into 5 ml of sterile trypticase soy broth (TSB) in which one 10 μ g meropenem disc has been previously placed aseptically and then incubated in ambient air overnight at 37 °C. The overnight culture was then sub-cultured onto MacConkey agar plate and incubated again. All the bacterial isolates were identified using standard laboratory techniques (Cowan and Steel, 1993).

The CDC broth enrichment method employed in this study utilizes materials that are readily available even in resource constrained settings, which makes it easy to implement and most suitable for laboratories where molecular detection techniques and other highly sensitive phenotypic methods may be unavailable (Aguirre-Quiñonero and Martinez-Martinez, 2017; Richter and Marchaim, 2017).

Isolation of Carbapenem Resistant Bacteria and Antimicrobial Susceptibility Testing

All the isolates collected from the laboratory during the study period were phenotypically screened for carbapenem resistance using the disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). Two standard carbapenem (imipenem (10 μ g) and meropenem (10 μ g)) discs sourced from Oxoid, UK, were used. The isolates with reduced susceptibility (total or intermediate) to carbapenem were regarded as carbapenem resistant. The carbapenem resistant bacterial isolates were subjected to antimicrobial susceptibility testing using the modified Kirby Bauer disc diffusion method on Mueller-Hinton agar plates (Oxoid Limited, Basingstoke, UK) according to the CLSI guideline. The antimicrobial agents used in this study (Oxoid Limited, Basingstoke, UK) were levofloxacin (5 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), ceftriaxone (30 μ g), amoxicillin-clavulanate (20/10 μ g), cefotaxime (30 μ g). The inhibition zone diameter was measured and interpreted according to CLSI interpretative criteria.

Detection of MBL Production

The CR-GNB isolates were subjected to confirmatory test for MBL-production by the

combined discs method as previously described (Aguirre-Quiñonero *et al.*, 2017). This was performed by using two imipenem discs (10 μ g), one containing 10 μ l of 0.1 M anhydrous ethylene diamine tetra-acetic acid (EDTA). The discs were placed 25 mm apart on Mueller-Hinton agar plates inoculated with the standardized inoculum of the test organisms. An increased inhibition zone diameter of 7 mm with imipenem-EDTA disc compared to imipenem disc alone was considered as MBL-positive.

Statistical Analysis

The data were analysed using GraphPad Prism[®] 7.0 software (GraphPad Software Inc., USA). Demographic characteristics (gender and age) and clinical (sample sources and source of bacterial isolates) variables were analysed by univariate analysis. Associations among variables were determined using Pearson's chi-square. In all cases, significance was assessed at $p < 0.05$.

RESULTS

Patient Characteristics

The study involved a total of 110 bacteria isolated from 47 female and 63 male patients with mean age of 28.09 ± 2.12 (Range: 9 months-79 years). Majority of the bacteria (58; 52.7%) were isolated from urine samples. Others were obtained from stool samples (27; 24.6%) and wound swab (22; 20%) (Table 1).

Occurrence of Carbapenem Resistant Bacteria in the Hospital

Overall, 31(28.2%) of the Gram-negative bacterial isolates were carbapenem resistant. Of these, 14 (29.8%) and 17 (27.0%) were isolated from female and male patients, respectively (Table 2). The CR-GNB isolates were predominantly isolated from patients aged 18-50 years (Table 2). A statistically significant association was observed between the gender ($p < 0.001$) and age ($p < 0.048$) of the patients. About 50% and 25.92% of bacteria isolated from wound and stool samples were carbapenem resistant. The result of chi-square test showed no significant association between sample sources and the isolation of carbapenem resistant bacteria ($p > 0.05$).

Furthermore, a total of 19 (33.93%) CR-GNB

were isolated from 56 samples collected from the bed linens and rectum of patients involved in this study. The samples from the rectum and bed linens yielded 10 (34.5%) and 9 (33.3%) CR-GNB, respectively.

The distribution of the CR-GNB isolated from

the hospital is presented in figure 1. *E. coli* (18; 36%) was the most prevalent CR-GNB isolated from the hospital. This was followed in decreasing order of prevalence by *Klebsiella* spp. (11; 22%), *Salmonella* spp. (6; 12%) and *Pseudomonas aeruginosa* (6; 12%).

Table 1: Characteristics of Patients Involved in the Study

Characteristics	Frequency	Percentage
Gender		
Female	47	42.7
Male	63	57.3
Age categories		
0-17 years	38	29.5
18-50 years	55	42.6
51 years and above	17	13.2
Isolates sources		
High Vaginal swab	2	1.8
Sputum	1	0.9
Stool Sample	27	24.6
Urine Sample	58	52.7
Wound swab	22	20.0
Total	110	100

Table 2: Occurrence of Carbapenem Resistant Bacteria in the Hospital.

Sample Sources	No screened	Frequency of carbapenem resistant isolates	X ² -value	p-value
Bed linens	29	10 (34.48 %)	0.693	0.628
Rectal swab	27	9 (31.03 %)		
Isolates sources				
HVS	2	0 (0 %)	7.373	0.117
Sputum	1	0 (0 %)		
Stool sample	27	7 (25.92 %)		
Urine sample	58	13 (22.41 %)		
Wound swab	22	11 (50 %)		
Gender				
Female	47	14 (29.8 %)	35.294	<0.001
Male	63	17 (27.0 %)		
Age (years)				
0-17 years	38	9(23.7%)	6.090	0.048
18-50 years	55	13(23.6%)		
51 years and above	17	9(52.9%)		
Total	110	31 (28.20 %)		

Antimicrobial Susceptibility Testing

The bacterial isolates showed varying pattern of resistance to the various antibiotics as shown in figure 2. Of the 50 CR-GNB isolates, a high resistance rate to cephalosporins was observed. Specifically, 76.7% and 71.3%, of the isolates were resistant to ceftriaxone and cefotaxime, respectively. In addition, the results of the fluoroquinolone testing showed that 42.6% and 62.8% isolates were, respectively, resistant to ciprofloxacin and levofloxacin, while approximately 38.8% of the isolates were resistant to gentamicin. Interestingly, the isolates were

more susceptible to amoxiclav, exhibiting only about 45.7% resistance. The majority of the CR-GNB isolates were multidrug (13; 26%) and extensively (19; 38%) drug resistant, as defined by (Magiorakos *et al.*, 2012) (Figure 3).

Prevalence of Metallo-β-Lactamase Enzymes

The phenotypic MBL-production test revealed a high prevalence (19/50; 38.0%) of MBL- positive isolates in the hospital as shown in figure 4. MBL-production was more prevalent among *Klebsiella* spp. (7/19; 36.8%), followed by *P. aeruginosa* (3/19; 15.8%) and *Proteus* spp. (3/19; 15.8%).

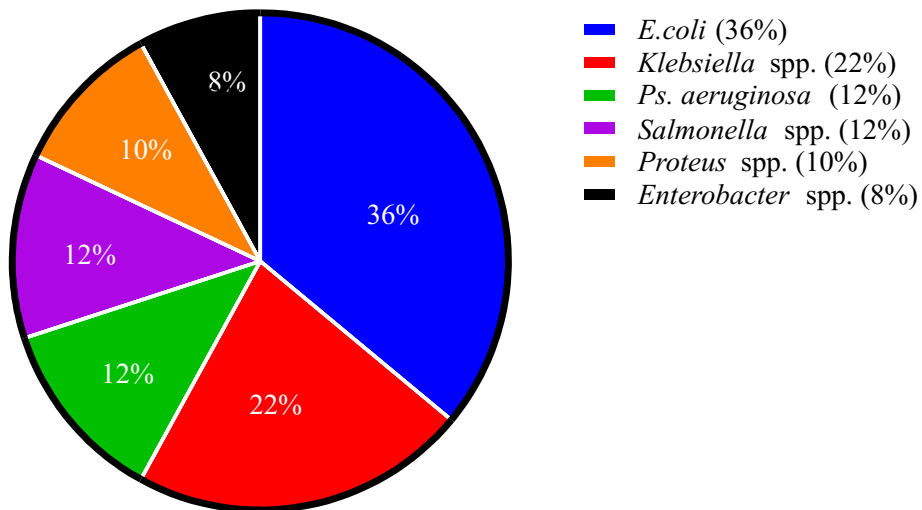


Figure 1: Frequency distribution of the isolated carbapenem resistant bacteria.

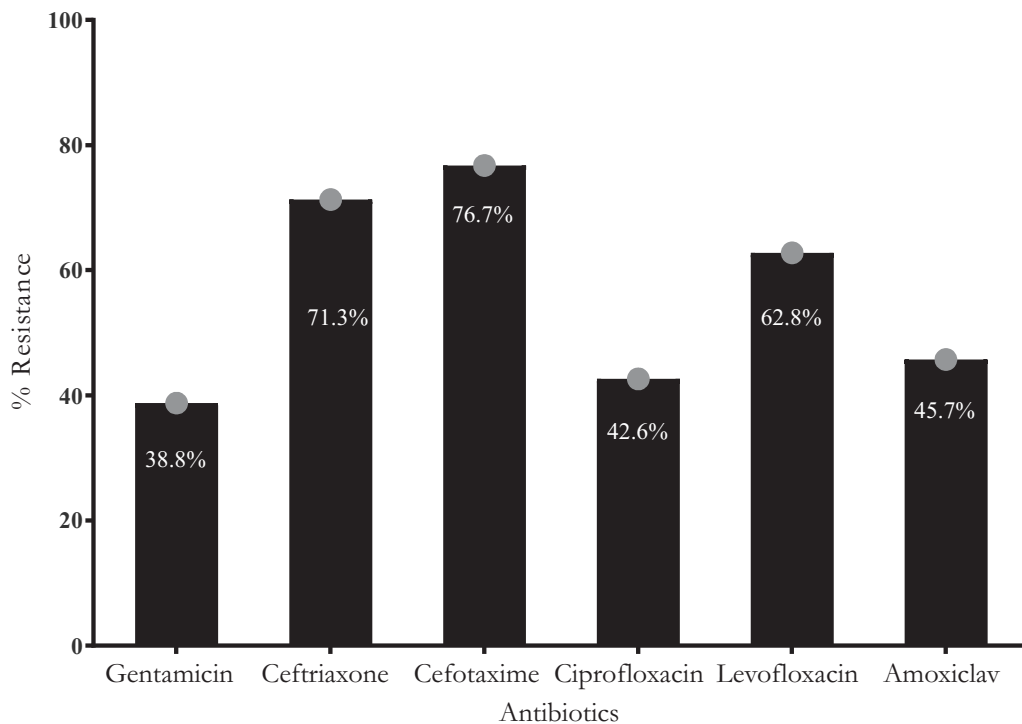


Figure 2: Antibiotic resistance profile of carbapenem resistant bacteria in the hospital.

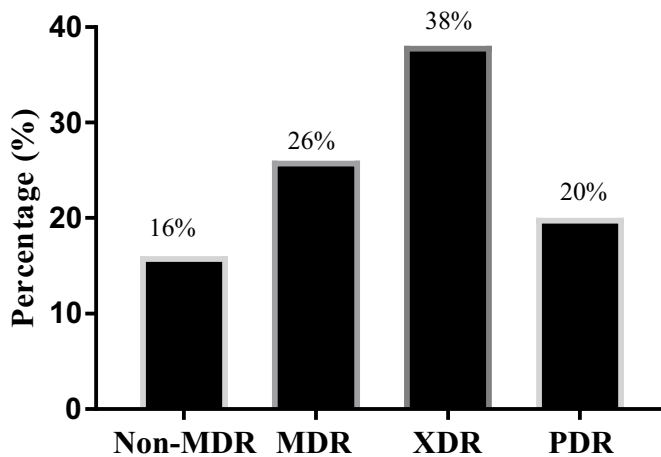


Figure 3: Distribution of MDR among carbapenem resistant isolates in the hospital.

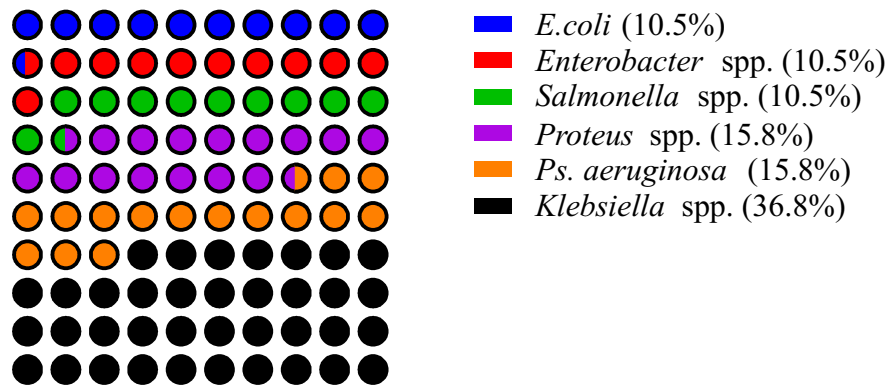


Figure 4: Prevalence of MBL-positive isolates in the hospital.

DISCUSSION

The carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare and community associated infections. This has wide ranging implications on patient's management. The present study was conducted to investigate the occurrence of carbapenem resistant Gram negative bacterial (CR-GNB) isolates in a hospital with no history of carbapenem usage. The finding of 28.2% CR-GNB among clinical isolates in this study contrasted with the higher 36.8% carbapenem resistance observed among clinical isolates collected from four tertiary hospitals in southwest Nigeria (Enwuru *et al.*, 2011). It was however higher than 15.2% and 10.2% carbapenem resistance reported in Lagos and Maiduguri, southwest and northeast Nigeria, respectively (Oduyebo *et al.*, 2015; Mohammed *et al.*, 2015). The isolation of CR-GNB isolates from the bed linens of the hospital concur with a report that has shown extensive reservoir of carbapenem

resistant organism in the hospital environment (Gordon *et al.*, 2017). Similarly, the high faecal intestinal carriage of CR-GNB (31.03%) observed in this study is alarming as this can serve as reservoirs for cross-transmission in health care settings (Viau *et al.*, 2016).

Many factors may account for the isolation of CR-GNB among patients with no history of previous exposure to carbapenem. Cross-resistance between carbapenem and other β -lactam antibiotics may result in independent emergence of CR-GNB. Beta-lactam antibiotics (cephalosporins and penicillins) are extensively used antibiotics in our hospitals, resistance to which has been well documented (Ibrahim *et al.*, 2017; Olowo-okere *et al.*, 2018). Furthermore, the observed CR-GNB might have been imported into this region by patients returning from other countries where CR-GNB is endemic. Reports of importation of antibiotic resistant bacteria across geographical border has been documented

(Okeke and Edelman, 2001). Also, a recent systematic review has shown a high prevalence of antibiotic resistant bacteria at hajj which could be imported by pilgrimage to their respective countries on return (Leangapichart *et al.*, 2017). In addition, the localisation of most carbapenemase genes on highly ambulatory genetic element may contribute to ease of acquisition and transmission of acquired carbapenem resistance among bacterial isolates in a hospital (Potter *et al.*, 2016).

The high resistance of the isolates to other β -lactam antibiotics may be attributed to co-production of both extended spectrum betalactamase (ESBL) and carbapenemase enzymes, resulting in hydrolysis of almost all β -lactam antibiotics. In Kano, Nigeria, isolates co-producing ESBL and MBL has been reported (Ibrahim *et al.*, 2017). Despite the high resistance of the isolates to third generation cephalosporins and other β -lactam antibiotics, most of the isolates were susceptible to amoxiclav. This may also be due to the production of ESBL enzymes, as the hydrolytic activities of ESBL cannot be inhibited by β -lactamase inhibitors (Shaikh *et al.*, 2015).

Our finding of 38.0% MBL-positive isolates in the hospital is contrasting with the lower 4.2%, 8.5% and 14.4% MBL-producing Gram-negative bacteria reported in Edo, Lagos and Kano, Nigeria, respectively (Oduyebo *et al.*, 2015; Yusuf *et al.*, 2015; Jesumirhewe *et al.*, 2017) and higher than 73.1% reported in a multicentre study conducted in Kaduna and Kano, northwest, Nigeria (Yusuf *et al.*, 2013). In sub-Saharan Africa, the prevalence of carbapenemase producing bacteria ranged from 9% to 60% (Manenzhe *et al.*, 2015). The foregoing has shown that MBL-production is a major mechanism of acquired carbapenem resistant in most Nigerian hospitals.

This study involves only a single centre within the study area and so the findings cannot be generalized to the entire northwest region. Nevertheless, we believe the findings of this study are robust and exciting as it provides baseline data for the occurrence of CR-GNB in our environment.

CONCLUSION

This study revealed a high prevalence of metallo- β -lactamase-producing carbapenem-resistant Gram-negative bacteria in a hospital with no history of its use. This report documents for the first time the independent emergence of such resistance in our hospital. Urgent implementation of adequate antibiotic stewardship program and other infection control measures is therefore imperative so as to contain this emerging threat.

Conflicts of Interest: We declare that we have no relevant conflict of interest.

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