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Original Research Article

Detection of New Delhi Metallo-B-Lactamase Production from *Klebsiella pneumoniae* Isolated from Clinical Samples

Ocheme Julius Okojokwu[1*], Innocent Ajegba Onaji[2], Parfa Dashe[1], Entonu Elijah Entonu[1], Amos Obaje Ogaji[1], Francis Ukah Ofuowoicho[1], Inemesit Dankat Bigwan[1], Rukaiyat Adamu[1], Mitu Cecilia Gideon[1]

[1]Department of Microbiology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria

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Abstract: The rapid spread of New Delhi metallo-β-lactamase (NDM) production in *Klebsiella pneumoniae* proved to be a major challenge for the treatment and control of infectious diseases. The aim of the study was to detect New Delhi metallo-β-lactamase production in *Klebsiella pneumoniae* isolated from clinical samples. A total of 103 clinical samples were collected and analysed for the presence of *Klebsiella pneumoniae* was isolated from 26 (25.2%) of the clinical samples. Antibiotic susceptibility testing was carried out for all the isolates. Out of the 26 isolates screened, only 3 and 5 were resistant to meropenem and imipenem respectively. These isolates were screened for New Delhi metallo-β-lactamase production using the combined-disk test. The 8 isolates were New Delhi metallo-β-lactamase producer. New Delhi metallo-β-lactamase producing *Klebsiella pneumoniae* in Plateau State Specialist Hospital Jos, Plateau State, Nigeria as seen in this study occurs at the rate of 30.8%. Having established the presence of New Delhi metallo-β-lactamase among patients in Plateau State Specialist Hospital, Jos, efforts should be geared towards limiting their spread.

Keywords: Antimicrobial resistance, Modified Hodge's test, Carbapenem, Carbapenemase, *Klebsiella pneumonia*, New Delhi metallo-β-lactamase.

1. INTRODUCTION

There is a worldwide clinical concern due to increasing resistance to carbapenems which is antibiotic of last resort against many multidrug resistance bacteria. This resistance is due to production of carbapenem hydrolysing enzymes by Enterobactericeae such as *Klebsiella pneumoniae*, *Escherichia coli* (Hemalatha *et al*., 2005).). Carbapenems are class of β-lactams, broad spectrum antibiotic that inhibit cell wall synthesis and used against Gram negative organisms. Combinations of carbapenems with other antimicrobial agents are good therapy for the treatment of severe hospital acquired infection. They exhibit bactericidal effect against a targeted organism by binding to the penicillin binding protein (PBP) therefore preventing the linking of peptidoglycan strands and further synthesis of bacterial cell wall (Hussaini *et al*., 2017). According to Ambler classification method, carbapenemases can be divided into classes A, B and D. Class A and D carbapenemases are serine β-lactamases and class B carbapenemases are metello-β-lactamases (MBL) such as New Delhi metallo-β-lactamase (Ambler,1980). Metallo-β-lactamase (MBL) is the most diverse class of carbapenemases that represent an important clinical threat and present hydrolytic activity against β-lactam antibiotics (except for monobactams); they are inhibit able by divalent cation chelators such as ethylene di-amine tetra acetic acid (EDTA) and sodium mercaptoacetate (MAS), and escape the action of all β-lactamase inhibitors for clinical use, such as clavulanic acid and sulbactam. β-lactam has been the drug of choice for treatment of serious bacterial infection (Koraei *et al*., 2018). New Delhi metallo-β-lactamase is a newly described metallo-β-lactamase, which was first identified in 2008 in a single isolate of *Klebsiella pneumoniae* and *Escherichia coli* from a patient who was repatriated to Sweden after treatment in a hospital in New Delhi, India. Like other acquired metallo-β-lactamase, New Delhi metallo-β-lactamase -1(NDM-1) hydrolyses all β-lactam antibiotics except aztreonam, which is usually inactivated by co-produced extended spectrum or AmpC beta-lactamase (Struelens *et al*., 2010).

To date, antibiotic resistance by bacteria is one of the most important global health problem introduced by World Health Organization (WHO) and most annual mortality due to hospital acquired infection occurs

***Corresponding Author:** Ocheme Julius Okojokwu

because of this challenge. Carbapenem resistant Enterobacteriaceae is one of the three main resistance threats (Koraei *et al*., 2018). Major global public health problem that lead to increasing health care cost, extra length of hospital stays and treatment failure is the antibiotic resistance. There are many reports about increasing of antimicrobial resistance in Gram negative pathogen. Example; *Klebsiella pneumoniae* (Lovayava *et al*., 2014).

This study will help investigate and provide relevant insights on the occurrence and prevalence of New Delhi metallo-β-lactamase production in *Klebsiella pneumoniae* and their resistance to antimicrobial drugs among patients in Plateau State Specialist Hospital, Jos. It will also enlighten the general public on the importance of proper administration and intake of antimicrobial agents.

2. MATERIALS AND METHODS

This hospital-based descriptive cross-sectional study was conducted in the Department of Microbiology, Faculty of Natural Sciences, and University of Jos after samples were collected from Plateau State Specialist Hospital Jos, Plateau State from November 2019 to April 2020. One hundred and three (103) samples were collected and analysed.

Study population

The study was conducted on the samples of inpatients and outpatients of 5-60 years (both male and female) from Plateau State Specialist Hospital Jos, Plateau State.

Ethical considerations

Prior to conduct of the study, ethical clearance with reference number PSSH/ADM/ETH.CO/2019/005 was obtained from the ethical committee of Plateau State Specialist Hospital, Jos, Plateau State, Nigeria. Thereafter, informed consent was sought and obtained from the patients before samples were collected from consenting patients.

Specimen collection

The isolates were obtained from the following samples; urine (n=75 sample), stool (n=20), and sputum (n=8) of patients.

Bacterial Identification and Characterization

Specimens were inoculated on MacConkey agar. They were then incubated for 24 hours aerobically at 36 – 37°C, colonial appearance and characteristics of isolates on MacConkey agar were noted, and they were then subjected to Gram staining methods according to the standard methods. All suspected isolates of *Klebsiella pneumoniae* were confirmed by the following biochemical tests according to the manufacturer instruction; Indole Test, Oxidase Test, Citrate Utilization Test, Catalase Test, Methyl Red Test, Triple Sugar Iron Agar, Hydrogen Sulfide (H_2s) Production Test, Urease Test.

Antimicrobial Susceptibility Testing

Susceptibility testing was performed using disk diffusion method on Muller-Hinton agar plate with the followings antibiotic discs; imipenem (10µg), meropenem (10µg), gentamicin (10µg), ciprofloxacin (10µg), norfloxacin (10µg), amoxil (20µg), streptomycin (30µg), rifampicin (20µg), erythromycin (30µg), chloramphenicol (30µg), ampiclox (20µg), and levofloxacin (20µg). Isolated colonies were inoculated on Muller-Hinton agar plates with the help of a sterile wire loop. Antibiotic discs were placed aseptically to the surface of each plate by appropriate arrangement with the help of a sterile forceps and incubated at 37^0C for 24 hours. Then results were interpreted following their zone of inhibition; sensitive, intermediate and resistant according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014).

All isolates that showed resistant pattern (isolate with an inhibition zone of <25mm in diameter) against one or more carbapenem were included for phenotypic detection of metallo-β-lactamase using combined-disk test.

Phenotypic evaluation of Metallo-β-lactamase producers

Phenotypic detection of metallo-β-lactamase was performed by combined-disk test (CDT) according to Franklin *et al*. (2006). Initially, a bacterial suspensions equivalent to 0.5 McFarland standard inoculum were prepared using peptone water then inoculated by streaking the cultured isolates on Mueller-Hinton agar by means of a sterilized wire loop. Subsequently, two imipenem disks one of which was impregnated with 10 µL of 0.1 M (292 µg) anhydrous ethylene diamine tetraacetic acid (EDTA) were placed on medium 25 mm apart. An increase in the diameter of the inhibition zone by ≥ 7mm with imipenem-EDTA disk compared to that of the imipenem disk alone, after 18-24 hours incubation at 37°C was interpreted as metallo-β-lactamase positive.

3. STATISTICAL ANALYSIS

The data were stored and analysed using the Microsoft Excel 2010 and Statistical Package for the Social Sciences (SPSS) respectively. The variables were reported as frequencies and percentages. The results were expressed as descriptive statistics and presented in tables.

4. RESULTS

A total of 103 clinical samples were diagnosed for *Klebsiella pneumoniae* according to Clinical Laboratory Standard Institutes (CLSI) guidelines. Seventy-five [75(72.8%)] were urine samples, 20 (19.4%) were stool samples, and 8(7.8%) were sputum samples. In terms of growth, 43 (57.3%) of urine,

16(80%) of stool and 0(0%) of sputum respectively showed suspected growth of *Klebsiella pneumoniae*, while the total growth observed on the samples was 59 (57.3%). Out of the 59 samples that had colonies, 26 (44.1%) were identified as *Klebsiella pneumoniae*. Two [2(7.7%)] were resistant to meropenem, 4(15.4%) to imipenem, 12(46.2%) to ciproflaxacin, 9(34.6%) to norfloxacin, 11(42.3%) to gentamycin, 15(57.7%) to amoxil, 14(53.8%) streptomycin, 12(46.2%) to rifampicin, 9(34.6%) to erythromycin, 12(46.2%) to chloramphenicol, 13(50.0%) to ampiclox and 8(30.8%) to levofloxacin as shown on Table 1. Table 2 indicates that *Klebsiella pneumoniae* were isolated from urine (41.9%) and stool (50.0%) giving a total of 26 isolates. The 26 *Klebsiella pneumoniae* isolates were subjected to antimicrobial susceptibility test. Two (7.7%) of the isolates were resistant to meropenem while 4 (15.4%) were resistant to imipenem. One each showed intermediate response to meropenem (3.8%) and imipenem (3.8%) (Table 3).

Three (11.5%) meropenem- and 5 (19.2%) of imipenem-resistant isolates (with zone of inhibition <25mm) were metallo-β-lactamase positive. Therefore, the prevalence of New Delhi metallo-β-lactamase in this study was 30.8% as indicated in Table 4. All the 8 isolates that were subjected to the combined disk test were found to express New Delhi Metallo-β-Lactamase (Table 5).

Table-1: Pattern of Growth in Individual Specimen

Sample	No. of Sample (%)	Significant Growth (%)	No. of Growth (%)
Urine	75	43 (57.3%)	32 (42.7%)
Sputum	8	0 (0.0%)	8 (100%)
Stool	20	16 (80.0)	4 (20.0%)
Total	103	59 (57.3%)	44 (42.7%)

Table-2: Identification of *Klebsiella pneumoniae*

Sample	Type of Isolate	Number of Samples (%)
Urine (n=43)	*Klebsiella pneumoniae*	18 (41.9)
	Salmonella Typhi	8 (18.6)
	Salmonella Paratyphi A	3 (7.0)
	Escherichia coli	13 (30.2)
	Proteus mirabilis	1 (2.3)
	Total	43 (57.3)
Stool (n=16)	*Klebsiella pneumoniae*	8 (50.0)
	Salmonella Typhi	2 (12.5)
	Escherichia coli	4 (25.0)
	Shigella species	2 (12.5)
	Total	16 (80.0)

Table-3: Antimicrobial Sensitivity Test

Antibiotics		*Klebsiella pneumoniae* (n=26)				
	Susceptible		Intermediate		Resistant	
	N	%	N	%	N	%
Meropenem	23	88.5	1	3.8	2	7.7
Imipenem	21	88.0	1	3.8	4	15.4
Ciprofloxacin	4	15.4	10	38.5	12	46.2
Norfloxacin	12	46.2	5	19.2	9	34.6
Gentamicin	15	57.7	0	0.0	11	42.3
Amoxil	11	42.3	0	0.0	15	57.7
Streptomycin	12	46.2	0	0.0	14	53.8
Rifampicin	14	53.8	0	0.0	12	46.2
Erythromycin	17	65.4	0	0.0	9	34.6
Chloramphinocol	11	42.3	3	11.5	12	46.2
Ampiclox	13	50.0	0	0.0	13	50.0
Levofloxacin	11	42.3	7	26.9	8	30.8

Table-4: Prevalence of New Delhi Metallo-β-Lactamase

Carbapenems	Resistant	
	N	%
Meropenem	3	11.5
Imipenem	5	19.2
Total	8	30.8

Table-5: Combined Disc Test Result

Sample	IMI (10µg)	IMI + EDTA	Difference	NDM
U1	15mm	22mm	≥7mm	Enzyme present
U14	20mm	27mm	≥7mm	Enzyme present
U16	15mm	24mm	≥9mm	Enzyme present
U42	17mm	27mm	≥10mm	Enzyme present
U56	18mm	25mm	≥7mm	Enzyme present
U62	15mm	25mm	≥10mm	Enzyme present
U72	20mm	28mm	≥8mm	Enzyme present
S5	15mm	22mm	≥7mm	Enzyme present

IMI = Imipenem, IMI + EDTA = Imipenem + EDTA.

Interpretation = An increase in the diameter of the inhibition zone by ≥7mm with imipenem-EDTA disk compared to that of the imipenem disk alone, after 18-24 hours incubation at 37°C was interpreted as metallo-β-lactamase positive.

5. DISCUSSION

In this study, the prevalence of carbapenem resistance among clinical isolates of *Klebsiella pneumoniae* in Plateau State Specialist Hospital is 30.8%. This is quite high for a drug which is a last resort for the treatment of resistant strain of Klebsiella pneumuniae. In contrast, other studies report a lower prevalence of carbapenem-resistant Enterobacteriaceae when compared with this study. For instance, in a study carried out in Enugu, Nigeria the prevalence of carbapenem-resistant Enterobacteriaceae was 2.5% (Ejikeugwu *et al*., 2012). A similar study performed in China, determined the prevalence of carbapenem resistance to be 1.2% (Xia *et al*., 2012).

Also, the result obtained in this study was slightly higher than that obtained from a study carried out in Kano (Yusuf *et al*., 2014) where the prevalence of carbapenemase production was determined to be 10.2%, Studies carried out in Morocco (2.8%) and Taiwan (8.6%), respectively (Wartiti *et al*., 2012, Lai *et al*., 2014) when compared with that of this study. However, our result was lower than that obtained in Tanzania where the prevalence of carbapenemase production was 35.24% (Mushi *et al*., 2014).

In this research, based on antibiotic sensitivity testing (AST) findings, most of the *Klebsiella pneumoniae* isolates showed high sensitivity(>85%) to carbapenem drugs tested which was in concordant to previous studies in Iran (Shahcheraghi *et al*., 2013; Firoozeh *et al*., 2017). Susceptibility rates for meropenem and imipenem were 88.5% and 80.8% respectively. The low occurrence rate of carbapenem resistant *Klebsiella pneumoniae* and low level of resistance demonstrated by the isolates to meropenem and Imipenem in this study could be due to the fact that Meropenem, Imipenem and other carbapenems usage is still low in Nigeria; carbapenems are expensive as such they are not subjected to abuse, they are not readily available and are reserved for life threatening Gram negative infections and are administered intravenously and they are not always prescribed.

6. CONCLUSION

Having established the presence of New Delhi metallo-β-lactamase among patients in Plateau State Specialist Hospital, Jos, efforts should be geared towards limiting their spread. Preventive efforts such as infection control procedures, hand hygiene among healthcare workers, antibiotic stewardship as well as careful use of carbapenems and third generation cephalosporins should be aggressively pursued within the health care setting. This is because the treatment options for infections caused by carbapenemase‑producing bacteria are limited.

Accurate detection of the carbapenemase‑producing Enterobacteriaceae is also very important. Where molecular tests are not available, phenotypic test such as the combined disc test used in this study may be employed. They are easy to perform, interpret and introduce into the workflow of the clinical laboratory.

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