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Prevalence of metallo-beta-lactamase in clinical isolates of *Pseudomonas aeruginosa* and *Proteus mirabilis* in Benin City, Nigeria

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Abstract

Carbapenems are the prime choice of treatment for severe cases of infections caused by Multi-Drug-Resistant *Pseudomonas aeruginosa* and *Proteus mirabilis*. Nevertheless, Metallo-Beta-Lactamase (MBL) production by these organisms has led to carbapenem resistance which is a global threat. This study aimed to determine the prevalence and antimicrobial susceptibility profile of MBL in clinical isolates of *Pseudomonas aeruginosa* and P*roteus mirabilis* in Benin City, Nigeria. 354 non monotonous clinical isolates of Pseudomonas *aeruginosa* (282) and *Proteus mirabilis* (72) used were obtained from various clinical samples from tertiary hospitals in Benin City. Identification of these isolates were done using standard microbiological techniques. Antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method. MBL production was detected using Imipenem Ethylene-Diamine-Tetra-Acetic Acid combined disc test method. Of the total 354 clinical isolates tested, 115 (32.48%) were MBL and the prevalence of this resistant isolates was significantly higher in *Pseudomonas aeruginosa* (46.8%) compared to *Proteus mirabilis* (P=0.0001). Among the metallo-beta-lactamase producing isolates of *Pseudomonas aeruginosa* higher prevalence were reported from Pleural fluid and cerebrospinal fluid samples with 6 (100%) and 3 (100%) respectively. This difference was statistically significant (P=0.0001). Susceptibility testing showed that isolates that produced beta-lactamase demonstrated poorly against cephalosporin, amoxicillin-clavulanate, gentamicin and floroquinones than non-beta- lactamase producers. A prevalence of 46.8% was reported for MBL producing *Pseudomonas aeruginosa* and 12.5% was reported for MBL producing *Proteus mirabilis*. Isolates that produced the MBL enzyme were more resistant to antibacterial agents. Measures to control and curb the spread of MBL producing clinical isolates are highly advocated

Keywords: Prevalence; Multi-Drug Resistance; Carbapenems; Metallo-Beta-Lactamase; *Pseudomonas Aeruginosa; Proteus Mirabilis*

1. Introduction

The most effective antibacterial drugs used to treat infections brought on by multidrug-resistant gram-negative bacilli (*Pseudomonas aeruginosa* and *Proteus mirabilis*) are carbapenems which include Imipenem and Meropenem. [1]. However, the emergence of divergent beta-lactamases in a variety of Gram-negative bacterias (including *Pseudomonas aeruginosa* and *Proteus mirabilis*) is to be blame for the increased global reports of acquired resistance to carbapenems. [2]

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Pseudomonas aeruginosa and *Proteus mirabilis*, are significant nosocomial infection that has built-in drug resistance. The genetically transferrable metallo-beta-lactamases which can metabolize all beta-lactams except monobactams are the most adaptable beta-lactamases that have been discovered [3]. Early diagnosis of MBL- producing organisms is crucial because it enables rapid administration of the proper antibiotics to successfully manage illness. The dependence of MBL activity on zinc or cadmium has been well shown. [4)]To find MBL-producing species, a number of screening techniques have been developed that make use of a metal chelating agents like ethylenediaminetetraacetic acid (EDTA) which can inhibit MBL activity. [5]. The combined IPM-EDTA disk test (CDT) is the most practical phenotypic method for metallo-beta-lactamase detection and operates by comparing the zones of inhibition obtained with IPM disks with IPM disks with and without EDTA [6].

2. Materials and methods

2.1. Bacterial Isolates

A total of a 354 consecutive non repetitive clinical isolates of *Pseudomonas aeruginosa* and *Proteus mirabilis* were recovered from clinical specimens, namely urine, wound swab, ear swab, catheter tip, Intravascular abscess, throat swab, sputum, tracheal swab, Ascitic fluid, endocervical swab, cerebrospinal fluid, and pleural fluid. These specimens were collected from various Hospital in Benin City, Edo State. Sociodemographic data accompanying these specimens, such as age and gender of patients and wards the isolates were recovered from, were obtained from the laboratory records. These isolates were identified as *Pseudomonas aeruginosa* and *Proteus mirabilis* by the medical microbiology diagnostic laboratory using routine biochemical confirmatory tests.

2.2. Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was determined using Kirby – Bauer disc diffusion method [7] on nutrient agar. Test organism (*Pseudomonas aeruginosa* and *Proteus mirabilis*) were emulsified in sterile water and the turbidity matched with 0.5 McFarland standards. After 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 the nutrient agar plate was seeded by swabbing in three directions with the swab. The isolates were tested to the following panel of antibiotics Amoxicilin-Clavulanate (30µg), Ofloxacin (5µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Gentamicin (10µg).

2.3. Detection of Metallo- Beta-lactamase production.

Combined disk test was used for the detection of metallo-beta-lactamase producing clinical isolates. Briefly, an EDTA solution with 0.5M strength was prepared by dissolving 2g of Disodium EDTA in 100ml of distilled water. The pH was adjusted to 8.0 and the solution was sterilized by autoclaving. A culture of pure isolate was inoculated into normal saline and was adjusted to a turbidity of 0.5 McFarland standard and was spread on the surface of a Muller Hinton Agar plate. Two 10µg imipenem disks was placed on either side of the seeded plate 10mm away. With a clean auto pipette 10µl of 0.5M EDTA was poured onto one of them After overnight incubation at 37ºC, the increased inhibition zone of _>7mm with the imipenem-EDTA disk was compared to the imipenem disk alone and was considered as MBL positive. [8]

3. Results and Discussion

Of all the 354 clinical isolates of *Pseudomonas aeruginosa* and *Proteus mirabilis*, 141(39.8) were Metallo-beta-lactamase producers. The prevalence of metallo-beta-lactamase was significantly higher (P=0.001) in *Pseudomonas aeruginosa* (36.8%) compared to *Proteus mirabilis* (12.5%) (Table 1). Among the metallo-beta-lactamase producing isolates of *Pseudomonas aeruginosa* higher prevalence were reported from Pleural fluid and cerebrospinal fluid sample with 6 (100%) and 3 (100%) respectively (Table 2).

Table 1 Prevalence of Metallo-beta-lactamase in clinical isolate of *Pseudomonas aeruginosa* vs *Proteu smirabilis*

Organism	No Tested	No positive	% positive	P value
Pseudomonas aeruginosa	282	132	46.8	0.0001
Proteus mirabilis	72	9	12.5	
Total	354	141	39.83	

Beta Lactamase positive isolates showed low activity against Cephalosporins, Amoxicilin-Clavulanate, gentamicin and the fluoroquinolones in relation with Non-Beta-Lactamase producers. Imipenem was the most active antibacterial agent against Beta-lactamase and non-beta-lactamase producing bacterial isolates. (Table 3 and 4)

Table 2 Prevalence of MBL in Clinical Isolates of *Pseudomonas aeruginosa* and *Proteus mirabilis* based on Specimen

Organism	Specimen	No Tested	No Positive	% positive	P value
Pseudomonas aeruginosa	Wound swab	78	36	46.1	0.0001
	Urine	90	51	56.7	
	Ear Swab	45	12	26.7	
	Catheter tip	27	2	44.5	
	IVS Abscess	3	0	0	
	Throat Swab	3	0	0	
	ECS	18	12	66.7	
	Ascitic Fluid	3	0	0	
	Tracheal Swab	18	12	66.7	
	Cerebrospinal Fluid	3	3	100	
	Pleural Fluid	6	6	100	
	Sputum	0	0	0	
	Total	282	122	43.3	

ECS= Endo Cervical Swab, IVS Abscess=Intravascular Abscess.

Table 3 Susceptibility Profile of *Pseudomonas aeruginosa* and *Proteus mirabilis* isolate producing Beta-Lactamase

Organism	Number tested	AUG (30µg)	CAZ (30µg)	CXM (30µg)	CN (10µg)	OFX (5µg)	CRO (30µg)	CIP (5µg)	IMP (10µg)
Pseudomonas aeruginosa	282	19 (6.7%	95 (33.7%)	5 (1.8%)	133 (47.1%)	190 (67.4%)	76 (26.9%)	152 (53.0%)	150 (53.2%)
Proteus mirabilis	0(0%)	0(0%)	68(86.1%)	0(0%)	18(25%)	24(33.3%)	6(8.3%)	30(41.7%)	12(16.67%)

AUG= Amoxicillin-Clavulanate, CAZ=Ceftazidime, CXM=Cefuroxime, CN=Gentamicin, OFX=Ofloxacin, CRO=Ceftriaxone, CIP=Ciprofloxacin, IMP= Imipenem

Table 4 Susceptibility Profile of Non-Beta-Lactamase Producing *Pseudomonas aeruginosa* and *Proteus mirabilis* Isolates.

Organism	Number Tested	AUG (30µg)	CAZ (30µg)	CXM (30µg)	CN (10µg)	OFX (5µg)	CRO (30µg)	CIP (5 µg)	IMP (10µg)
Pseudomonas Aeruginosa	282	52(34.7 %)	91(32.2 %)	26(9.2) %	130(46 %)	143(50.7 %)	1(32.2%)	143(50.7 %)	141(50 %)
Proteus Mirabilis	72	25(34.7 %)	35(46.8 %)	35(46.8 %)	40(55.5 %)	20(27.8%)	20(27.8 %)	25(34.7%)	46(63.8 %)

AUG= Amoxicillin-Clavulanate, CAZ=Ceftazidime, CXM=Cefuroxime, CN=Gentamicin, OFX=Ofloxacin, CRO=Ceftriaxone, CIP=Ciprofloxacin, IMP=Imipenem

4. Discussion

The current study aimed at detecting the prevalence of Metallo-Beta-Lactamase in clinical isolates of *Pseudomonas aeruginosa* and *Proteus mirabilis* which have recently emerged as one of the most worrisome resistance mechanisms because of their capacity to hydrolyze all beta lactam antibiotics including penicillin, cephalosporins and imipenem except for aztreonam. Hospital acquired infections producing metallo-beta-lactamase positive isolate of *pseudomonas aeruginosa* and *Proteus mirabilis* is important to identify because they pose not only therapeutic problem but also a serious global concern for Centre for Disease Control [9]

In this study a total of 354 bacterial isolates from different clinical samples were studied for their susceptibility profile and resistance to antibiotics before they were screened for metallo-beta-lactamase. Here, 115(39.83%) of the isolates were imipenem resistant i.e., MBL producers. This was higher than the 16.52% observed by Ogefere *et al*., (2013) (10). The difference could be attributed to the types of isolates used. Multi drug resistant Gram-negative bacilli was used by Ogefere *et al.,* (2013) [10]. while only *Pseudomonas* aeruginosa and *Proteus mirabilis* and a larger sample size was used in this study and the isolates were obtained from patients who were treated with narrow spectrum antibiotics. All the isolates were screened for the presence of metallo-beta-lactamase using Imipenem-EDTA combined disc method. Among the imipenem-resistant isolates the prevalence of MBL producers was observed to be (39.83%) in this study. This is also higher than the 28.7% previously reported among gram negative bacillis. [10]. Ogefere *et al*., (2013) [10]. in Nigeria reported (14.29%) *Pseudomonas* aeruginosa isolates that were MBL producers Figures by Ogefere *et al.,* (2013) were lower than the figures in this present study. In this present study highest numbers of MBL producers were noted from Cerebrospinal fluid and pleural fluid specimen (100%) followed by tracheal swab, urine, wound swab, catheter tip and ear swab with (66.7%), (56.7%), (46.1%), (44.5%) and (26.7%) respectively and non were detected from other specimen. Ibadin *et al*., (2017) [11] in Nigeria reported a lower number of MBL producers in all the specimens as against the higher number of MBL Producers in the present study that were isolated from cerebrospinal fluid and pleural fluid. This reveals that such bacterial isolates might have been acquired by the patients from the hospital environment. In the present study P*seudomonas aeruginosa* 132(46.8%) were susceptible to imipenem, *Proteus mirabilis* 9(12.5%) were susceptible to imipenem and other anti- bacterial agents were not effective against the bacterial isolates as shown in table 3 & 4

5. Conclusion

A prevalence of 39.8% was observed for Metallo-Beta-Lactamase in clinical isolate of *Pseudomonas aeruginosa* and *Proteus mirabilis* for this study. Isolates that produced the metallo-beta-lactamase enzyme were more resistant to antibacterial agents. Therefore, measures to control and curb the spread of MBL producing clinical isolates are advocated.

Compliance with ethical standards

Acknowledgement

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Disclosure of Conflicts of interest

All authors declared that there was no conflict of interest

Statement of ethical approval

The present work contain studies performed on humans subjects and ethical approval was approved by the Edo state Ministry of Health through their letter referenced Ha.723/181.

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