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Occurrence and Associated Risk Factors of Metallo-B-Lactamase Producing Agents of Infection among Patients in Uyo, Southern Nigeria

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ABSTRACT

Background: *Carbapenemase enzymes such as metallo-β-lactamase (MβL) belong to Amber class B type of β- lactamase that cause serious clinical infections mostly feared due to the associated antibiotic resistance mechanisms resulting from their ability to hydrolyse virtually all β-lactam agents, including the carbapenems.*

Aim*: To investigate the occurrence as well as determine the associated risk factors of metallo- β -lactamase producing bacterial agents of infection among in Uyo, Southern Nigeria.*

Methods: *A clean-catch, mid-stream urine and wound samples were collected from consented patients and cultured according to standard procedure. The isolated pathogens were subjected to antibiotic susceptibility testing using the Kirby Bauer method. All Imipenem and Ertapenem resistant isolates were used for of MβL detection, using Total Metallo-β-Lactamase confirmation Kit.*

Results: *Of the 157 Gram-negative bacteria isolates from 312 clinical samples of urine and wound, metallo-beta-lactamase (MβL) was detected in 12 (7.6%). The highest MβL producing Gram-negative bacteria isolate from urine was Escherichia coli. However, Pseudomonas aeruginosa was the major producer from wound the samples. Overall, the antibiotic sensitivity profile revealed that the isolates were mostly sensitive to imipenem 123(91.0%) and highly resistant to the commonly used antibiotics in the study area. The associated risk factors of note were the participants residence, antibiotic use and duration of wound infection and they were found to be statistically significant (P = <0.05).*

Conclusion: *The prevalence of metallo-β-lactamase producing bacterial agents in Uyo, Nigeria was found to be 7.6%. In addition, while E. coli and P. aeruginosa were the major MβL producers, the participant's place of residence, antibiotics use and wound duration were found to be associated with their occurrence.*

Keywords

Metallo-β-lactamase, Risk factors, Carbapenemase, Southern Nigeria.

Introduction

Metallo-β-lactamase (MβL) is a zinc dependent enzyme belonging to Ambler class B that can hydrolyse all beta lactam antibiotics including carbapenem [1] and its production is the most common mechanism of carbapenem resistance [2]. Carbapenemases are enzyme that can efficiently hydrolyse most β-lactams, including carbapenems. Ertopenem, Doripenem, imipenem, Meropenem and Faropenem are example of carbapenems but Imipenem and Meropenem are the most commonly used carbapenems perse [3].

In recent years, the Metallo-β-lactamases (MβLs) have emerged as one of the most feared resistance mechanisms because of

their ability to hydrolyse virtually all β-lactam agents, including the carbapenems, and because their genes are carried on highly mobile elements [4], So far, six major clinically important groups of MβLs have been identified: *IMP, VIM, SIM, SPM, GIM* and *AIM* [5], And the emergence and spread of these MβLs poses not only a public health threat but also a cause of increased mortality associated with in-hospital [6].

The risk factors for colonization or infection with MβLs include prior antimicrobial use, severity of illness and admissions or stay in the intensive care unit (ICU). However, prior use of antimicrobial agents, more specifically the carbapenems, third and fourth generation cephalosporins and fluoroquinolones, increases the risk of infection or colonization. Furthermore, the host factors in combination with length of stay in the hospital particularly in ICU and indwelling devices like mechanical ventilation, primary venous catheterization, and urinary catheterization commonly predisposes the development of carbapenem-resistant isolates [7,8]. As such, this study aims to determine the occurrence and risk factors for the acquisition of Metallo-β-lactamases among patients in a tertiary hospital care facility, the University of Uyo Teaching Hospital, Akwa Ibom State, Nigeria.

Materials and Methods

Study Design

This was a descriptive cross-sectional hospital-based study.

Study area

The study was conducted at University of Uyo Teaching Hospital, Uyo, Southern Nigeria. The samples were processed at the Medical Microbiology laboratory and the Molecular laboratory of the University of Uyo Teaching Hospital and Niger Delta University, Bayelsa State respectively.

Sample Size

The sample size of patients studied was determined based on the prevalence rate of 24.5% obtained from a study in North West Nigeria by Yusuf, et al. [9]. The estimated sample size used for this study together with 10% attrition rate was 312.

Sample collection, Culture and Identification of Bacterial Isolates

A total of 312 wound and urine samples were aseptically collected following standard protocol [10], from consenting patients in various wards and clinics including General Out-patients Department, Obstetrics and Gynaecology Department, Orthopaedic, Medical and surgical wards, as well as Burns and Plastic Surgery unit of the hospital.

All urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED) and Blood agar (BA) plates while the wound swab samples were inoculated on blood agar (BA) and MacConkey agar (MA) plates. All plates were incubated aerobically for 18-24 hours. All isolates were first presumptively identified using Gram-stain followed by biochemical method using Microbact 24E (MB24E) (Oxoid, UK) system for Gram-negative bacteria.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method on Müller-Hinton agar plate was used to perform antibiotic susceptibility tests on all isolates as recommended by Clinical Laboratory Standard Institute [10]. The standard antibiotics (Oxoid, UK) used for susceptibility testing include, Amoxicillin/clavulanate (30µg), Ceftazidime (30 µg), Ertapenem (10 µg), Cefepime (30 µg), Imipenem (10 µg), Ceftriaxone, (30 µg), Gentamicin (30 µg) and Ciprofloxacin (5 µg). And the zones of inhibition were interpreted according to Clinical Laboratory Standard Institute (CLSI) [11].

Phenotypic Detection of MβL-producing Gram-negative bacilli

Metallo-β-Lactamase (MβL) detection was carried out on isolates resistant to Carbapenems (Imipenem, Ertapenem), using Total Metallo-β-Lactamase confirmation Kit 98016 from ROSCO Diagnostica (ROSCO Diagnostica A/S, Taastrupgaardsvej, Denmark) according to the manufacturer's instructions. Two imipenem discs were placed on agar plates containing lawn of test organism and incubated. After incubation, the diameter of inhibition zones was measured and recorded. A 'no zone' around a disc corresponded to a 9 mm inhibition zone. The zones of inhibition around imipenem 10 µg to that of imipenem+DPA and imipenem+EDTA were compared. If all zones were within 3 mm of each other, the organism was not expressing MβL activity and as such is MβL negative.

For Enterobacteriaceae: The zones around Imipenem and Imipenem+DPA were compared, if a difference in zone diameter of ≥5 mm was observed, the isolate is expressing MβL activity and considered to be positive.

For *Pseudomonas* species/*Acinetobacter* species: The zones of diameter around IM+DP–IMI10 were compared, if the difference in zone diameter was observed to be ≥8 mm, the isolate is MβL positive.

Ethical Approval

The ethical approval to carry out this study was gotten from the Ethical Review Board of the University of Uyo Teaching Hospital, Akwa Ibom State, Nigeria under approval reference number UUTH/AD/S/96/VOL.XXI/229. A written consent was provided by the respondents before their inclusion in the study.

Statistical Analysis

Data analysis was performed using SPSS, 22.0 (Chicago, IL, USA). A Chi-square test for the comparative analysis of categorical variables was used to determine the independent risk factors. To identify risk factors that were independently associated with an MβL organism, a multivariate analysis was conducted using a logistic regression model. In addition, all the tests for statistical significance were 2-sided, with $P < 0.05$ denoted as statistical significance

Results

In this study, of the 312 samples of urine and wound, 157 (50.3%) yielded growth of Gram-negative bacterial isolates while 123 (39.4%) and 32 (10.3%) yielded Gram-positive isolates, and no significant growth respectively. Out of the 157 positive samples of Gram-negative bacteria which consisted predominantly of *Escherichia coli* and *Pseudomonas aeruginosa*, 95 (60.5%) isolates were from urine while 62 (39.5%) were from wound (Table 1). Overall, the Gram-negative isolates were mostly sensitive to Ertapenem and Imipenem (Table 2). Furthermore, these carbapenem-resistant isolates shown to consist of 24 (15.3%) each of both Imipenem and Ertapenem; 13 (54.2%) were from urine (Figure 1) and 11 (45.8%) were from wound (Figure 2) samples. In addition, out of these 24 isolates, 12 (50%) were MβL-producing and which consisted of 7 (58.3%) isolates from wound and 5 (41.7%) isolates from urine (Figures 1 and 2). The assessed risk factors that have statistically significant association with the prevalence of Mβl were; the participants residence in urban area, the participants prior antibiotic use and the duration of wound infection ($p \leq 0.05$) (Tables 3, 4 and 5 respectively).

Table 1: Antimicrobial resistance of Isolated Gram-negative bacteria according to sample types.

Organisms	Total	Urine	Wound
Acinetobacter baumanii	3	1	2
Escherichia coli	32	25	7
Enterobacter aerogenes	12	8	4
Enterobacter agglomerans	3	3	0
Klebsiella aerogenes	1	0	1
Klebsiella ornitholytica	2	2	0
Klebsiella oxytoca	12	8	4
Klebsiella pneumoniae	27	19	8
Morganella morganii	7	7	0
Proteus mirabilis	19	14	5
Providencia stuartii	3	3	0
Pseudomonas aeruginosa	32	3	29
Pseudomonas putida	3	1	2
Stenotrophomonas maltophilia	1	1	0
Total	**157**	**95 (60.5)**	**62 (39.5)**

Table 2: Antimicrobial resistance pattern of Gram-negative bacteria isolates.

Organism Isolated	Antibiotics							
	AMC (30µg)	CAZ (30 µg)	ETP (10 µg)	CEP (30 µg)	IMP (10 µg)	CRO (30µg)	CN (30µg)	CIP (5µg)
Acinetobacter baumanii	2	0	0	1	0	1	1	2
Escherichia coli	18	9	3	9	3	15	23	18
Enterobacter aerogenes	8	3	1	2	1	7	7	6
Enterobacter agglomerans	2	2	2	2	2	2	3	2
Stenotrophomonas maltophilia	1	1	0	1	0	1	1	1
Klebsiella aerogenes	1	0	0	0	0	0	0	0
Klebsiella ornitholytica	1	1	0	0	0	0	1	0
Klebsiella oxytoca	4	2	2	4	2	7	7	9
Klebsiella pneumonia	15	6	3	6	3	14	19	12
Morganella morganii	3	4	1	1	1	2	4	3
Proteus mirabilis	14	4	4	5	4	7	12	14
Providencia stuartii	3	2	1	1	1	1	2	1
Pseudomonas aeruginosa	24	11	6	11	6	18	21	20
Pseudomonas putida	3	1	1	2	1	2	2	2
Total	**99**	**46**	**24**	**45**	**24**	**77**	**103**	**90**

Keys: AMC-Amoxicillin/clavulanate, CAZ-Ceftazidime, ETP-Ertapenem, CEP-Cefepime, IMP-Imipenem, CRO-Ceftriaxone, CN-Gentamicin and CIP-Ciprofloxacin.

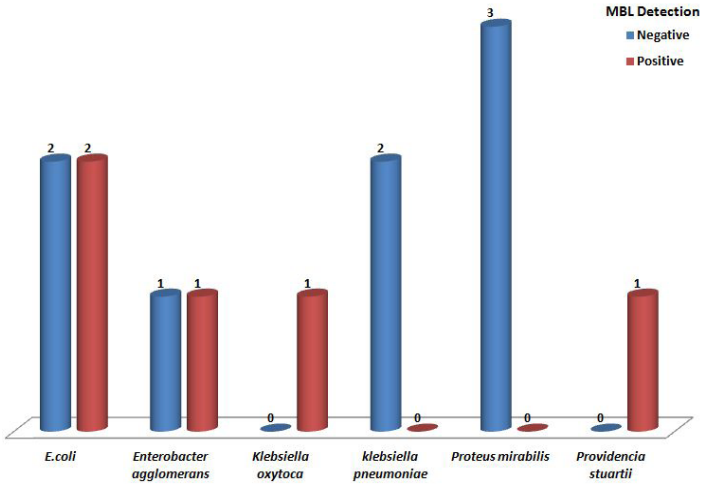

Figure 1: Prevalence of Mβl in Urine.

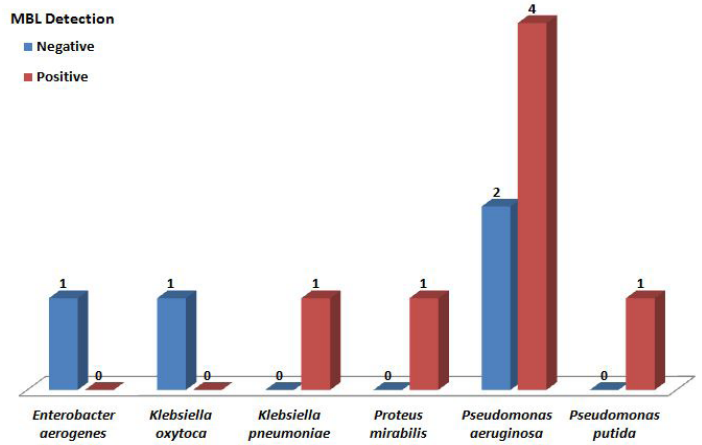

Figure 2: Prevalence of Mβl in Wound.

Table 3: Risk Factors based on Participants Sociodemographic characteristics and their Relationship with Mβl Prevalence.

Variable	Category	Mβl negative (n=145)	Mβl positive (n=12)	p-value
Age group	15-24	18	0	0.59
	25-34	31	5 (41.7)	
	35-44	44	4 (33.3)	
	45-54	29	1 (8.3)	
	>54	23	2 (16.7)	
Gender	Male	69	4 (33.3)	0.74
	Female	76	8 (66.7)	
Place of Residence	Urban	89	8 (66.7)	0.001
	Rural	56	4 (33.3)	
Occupation	Business/Trade	17	0	0.21
	Civil Servant	56	3 (25)	
	Student	65	9 (75)	
	Unemployed	13	0	
	Retired	6	1	

Table 4: Association between Mβl-production and Clinical History of UTI among Participants.

History	Mβl negative (n=145)	Mβl positive (n=12)	p-value
Does participant have a recent (3 months) history of UTI?			
Yes	47	2 (16.7)	0.77
No	108	10 (83.3)	
Is participant on a urethral catheter?			
Yes	22	2 (16.7)	0.31
No	123	10 (83.3)	
Did participant recently (within the previous 2 weeks) remove a urethral catheter?			
Yes	10	0	0.65
No	135	12 (100)	
Previous Antibiotic Use			
Yes	40	11 (91.7)	0.001
No	105	1 (8.3)	

Table 5: Association between Mβl-production and infected wound characteristics among participants.

Wound Characteristics	Mβl negative (n=93)	Mβl positive (n=7)	p-value
Wound Type			0.96
Gunshot wound	33	2 (28.6)	
Burns wound	12	1 (14.3)	
Abscess	15	1 (14.3)	
Surgical wound	10	1 (14.3)	
RTA wound	6	0	
Others	17	2 (28.6)	
Wound Duration			
< 1 week	11	1 (14.3)	0.02
1-2 weeks	20	0	
3-4 weeks	47	1 (14.3)	
Over a month	15	5 (71.4)	
Frequency of wound dressing			
Daily	22	1 (14.3)	0.59
Alternate days	66	5 (71.4)	
Longer plans	5	1 (14.3)	

Discussion

The organisms isolated from this study were more of Gram-negative bacilli 157 (50.3%) when compared to the Gram-positives 123 (39.4%). This agrees with studies conducted in India where Gram-negative bacilli constituted a higher number of the organisms isolated [12,13]. There were more recorded samples from females 175 (56.1%) than from male participants 135 (43.9%), and the majority of these participants were of the age group, 35-44 years 87 (29.7%). This can be attributed to the fact that the sex and age group are more likely to seek attention in the hospital, and are involved more in outdoor activities, which exposes to higher health risks. Some Nigerian studies also reported similar findings [14,15].

The prevalence rate of metallo-beta-lactamase (MβL) as revealed by this study was 7.6%. This is however, a deviation from the lower rates of 2.5% and 4.2% earlier reported by studies from Abuja in the North Central region of Nigeria and Edo State in the Southern Nigeria respectively [16,17]. There are no immediate deducible reasons for this, however, low exposure of patients to the carbapenems and improved hygienic measures may be contributory factors to low prevalence rates [15]. Although similar rates have been reported by other studies [14,18], nevertheless, higher prevalence rates ranging from 24.5% to 60% have been reported by studies from other Nigeria regions and sub-Saharan Africa [9,19,20].

Urine, which accounted for more than half (67.9%) of the sample type used, yielded higher numbers *Escherichia coli* as the most frequent uropathogen 23(10.6%), followed by *Klebsiella pneumoniae* 19(9%) and then *Proteus mirabilis* 14 (6.6%), still showed similar pattern with report from ealier studies carried out in the study center. Thereby further confirming that *Escherichia coli* is the predominant uropathogen [15].

The assessed risk factors among the participants in this study were based on the sociodemographic characteristics, clinical history of UTI and wound characteristics [21,22]. As regards the association between the sociodemographic factors and prevalence of Mβl, only the participants' place of residence was found to have statistically significant relationship with Mβl prevalence. Furthermore, the clinical history of the participants with UTI, recent history of UTI, presence of indwelling urethral catheter and recently removal of urethral catheter were all found to have no association with Mβl production. This maybe so because, being on or off urethral catheter does not increase the transmission or spread of these Mβls but prior use or indiscriminate use of antimicrobial agents (more specifically the carbapenems, 3rd and 4th generation cephalosporins and fluoroquinolones) may increase the risk of infection or colonisation with Mβl. Nevertheless, there was statistically significant relationship between Mβl-production and the participant's previous history of antibiotic use, which agrees with the fact that frequent exposure to antibiotics increases the risk of its resistance by pathogens [23-25].

Wound infection by bacterial agents plays a leading role in the development of chronicity with the consequence of delay in the healing process, which ultimately will lead to morbidity and reduced the quality of life of participants. It is of importance therefore, that wound infection was revealed to harbor the highest 7(58.3%) MβL-producing Gram-negative isolates in this study just as already documented by some studies [14]. Interestingly, the assessed risk factors of wound characteristics, showed the duration of the wound to have statistically significant relationship with the production of MβL by bacteria. This was not different from reports by studies carried out elsewhere [26,27].

Conclusion

The prevalence rate of Metallo-beta-lactamase producing bacteria agents in Uyo was 7.6% and while they were mostly resistant to the commonly used antibiotics such as Gentamicin and Amoxicillin/ Clavulanate they still showed sensitive to Entrapenem and Imipenem. Furthermore, the study revealed the risk factors of note for the occurrence of MβL producing agents to be, the participants' place of residence, use of antibiotics and duration of wound infection which were all found to have statistically significant association with the prevalence of the MβL producing Gram-negative bacteria.

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