



DETECTION OF METALLO BETA-LACTAMASES AMONG GRAM NEGATIVE BACTERIAL ISOLATES FROM MURTALA MUHAMMAD SPECIALIST HOSPITAL, KANO AND ALMADINA HOSPITAL KADUNA, NIGERIA

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ABSTRACT

Over the last few years, the increase in the number of multi-resistant (MR) enterobacteria has become a major clinical problem. This study detects the occurrence and prevalence of Metallo beta-lactamase production among some clinical bacterial isolates in Murtala Muhammad Specialist Hospital, Kano and Al-Madina Specialist Hospital Kaduna, Nigeria. A total of 200 clinical isolates comprising of *E. coli* (83), *Klebsiella pneumoniae* (52), *Pseudomonas aeruginosa* (28) and *Proteus mirabilis* (37) were screened phenotypically for carbapenemase and specifically for Metallo beta-lactamase using Modified Hodges Test and EDTA Disc Synergy Test respectively. The result showed that 67(33.5%) of the isolates were found to produce carbapenemase. High production occurred in 24(35.8%) and low production occurred in 43(64.2%) of the isolates. Highest prevalence of carbapenemase was found in *Pseudomonas aeruginosa* (38.55%) followed by *E. coli* (34.8%), *Proteus mirabilis*. (29.1%) and least prevalence in *Klebsiella pneumoniae* (25.0%). The prevalence of MBLs in the study was 24.5% with highest prevalence in *E. coli* (31.32%) followed by *Proteus mirabilis*. (21.6%), *Pseudomonas aeruginosa* (21.2%) and least among *Klebsiella pneumoniae*. (14.3%). Most of carbapenemase producers produce MBL type. Urine samples were found to be with the highest prevalence of 38.3% when compared with ear swab (12.0%). Prevalence of 67.9% and 76.9% were recorded for Murtala Muhammad specialist hospital Kano and Al-madina hospital Kaduna respectively. This showed that carbapenemase-mediated resistance occurred in the selected hospitals and uncontrolled spread may lead to treatment failure and frustration.

Keywords: Metallobeta-lactamase, Carbapenemase, Enterobacteriaceae, prevalence, Hospital

INTRODUCTION

Beta-lactam antibiotics are widely used antibiotics in the treatment and management of infectious bacteria in Nigerian hospitals (Yusuf and Arzai, 2011). However, the production of single spectrum beta-lactamases, one of the resistance mechanisms encountered in *Enterobacteriaceae*, mainly *E. coli* and *K. pneumoniae*, has been associated with several treatment failures when one or more of the antibiotics are used.

Carbapenemase enzymes belonging to Ambler molecular classes A to D have been detected in various clinical isolates in Nigeria (Yusuf *et al.*, 2011). Metallo-beta-lactamases enzymes, whose genes are plasmid and integron located, hydrolyze virtually all Beta-Lactams except aztreonam (Walsh, 2008). Many of the carbapenemase producers are frequently resistant to fluoroquinolones and aminoglycosides (Heffernan *et al.*, 2006). One strategy developed by bacteria to resist action of beta-lactam antibiotics is the expression of Metallo-Beta-lactamases requiring Zinc (Zn²⁺) for activity (Heffernan *et al.*, 2006). Metallo-beta-lactamases is an enzyme that confers antibiotic resistance to Bacteria by catalyzing the hydrolysis of Beta-lactam antibiotics

(Yong *et al.*, 2009). Different enzymes of MBLs have been identified which are the IMP type Carbapenemases, VIM (Verona Integron-encoded Metallo-Beta-lactamase) and the NDM-1 (New Delhi MBLs).

Among *Enterobacteriaceae*, resistance to carbapenemase is still rare and the awareness of its occurrence and effects is low especially in Nigerian hospitals.

Metallo-Beta-Lactamases mediated resistance is a cause for concern in the therapy of clinically ill patient. The relatively new form of resistance is spreading unchallengeable as there is a current lack of potent and selective inhibitors of MBLs. Spread of MBLs deserves a study in real time of how resistance can become global. Their rapid dissemination is worrisome and necessitates not just surveillance study but also studying occurrence and prevalence in a hospital setting to formulate a policy of empirical therapy. The research therefore aim at detecting the occurrence and prevalence of MBLs among *Enterobacteriaceae* from Murtala Muhammad specialist hospital Kano and Almadina hospital Kaduna using phenotypic method.

MATERIALS AND METHODS

Sample Collection

a) Clinical Isolates

A total of 200 clinical isolates of members of the family Enterobacteriaceae were collected from pathology department of Murtala Muhammad Specialist Hospital Kano and Almadina Hospital Kaduna. Isolates were collected along with information on the sample type, hospital number, age and sex of the patients.

b) Control Strain

E. coli ATCC 25922 was used as control and was collected from Aminu Kano Teaching Hospital

Screening of pathogen for Carbapenemase Production

Modified Hodges Test was adopted as described by Walsh *et al.* (2011) and CLSI (2009).

A 0.5 McFarland suspension of the control *E. coli* ATCC 25922 was inoculated on a well dried Mueller Hinton Agar (MHA) (Oxoid, UK) using a sterile wire loop. Meropenem (MEM 10µg Oxoid, UK) disc was placed using a sterile forceps on the surface at the middle of the MHA plate. In a straight line, the test organism was streaked from the edge of the MEM disc to the edge of the culture plate. The plates were incubated for 24hrs at 37°C. After 24hrs of incubation, the plates were examined for flattening and indentation at the intersect of the organism and *E. coli* ATCC 25922 within the zone of inhibition of the MEM susceptibility disc as described by Clinical laboratory standard institute (2009).

Inhibitory Based Test for MBLs

EDTA-disc synergy test or combined disc test was used as described by Walsh *et al.*, 2011 and CLSI (2009). A carbapenemase producing isolate was streaked on the surface of the MHA (Oxoid UK) using

a sterile wire loop. Two meropenem (MEM) (10µg Oxoid UK) discs, one containing EDTA and the other without EDTA were placed on the surface of the MHA using a sterile forceps at a distance of about 30mm apart. The plates were incubated for 24hrs at 37°C. Zones of inhibition were recorded after 24hrs of incubation. Organisms that showed difference in diameter of zone of inhibition by ≥4mm on the MEM containing EDTA and MEM alone were confirmed for the production of MBLs.

RESULTS

Out of the total 200 isolates screened, 67(33.5%) were found to produce carbapenemase. Highest prevalence of carbapenemase was found among *Pseudomonas aeruginosa* (38.55%) followed by *E. coli* (34.8%), *Proteus mirabilis* (29.1%) and least prevalence in *Klebsiella pneumoniae*. (25.0%) (Table1). Of the carbapenemase producers, 24(35.8%) were found to produce strong carbapenemase with indented zones and 43(64.2%) produces a weak type of the enzymes with flattened zones. (Table 2). The prevalence of MBLs in the study was 24.5% (Table 3), highest prevalence was recorded among *E. coli* (31.32%) followed by *P. mirabilis* (21.6%), *P. aeruginosa* (21.2%) and least among *K. pneumoniae* (14.3%) (Table 3). Based on clinical samples, urine was found with high prevalence of MBLs (38.3%), followed by wound swab (30.9%), semen and sputum (25.0%) (Table 5).

While distribution of MBLs based on sex recorded 26.9% and 23.3% among male and female respectively, the distribution of MBLs according to Hospital, recorded a prevalence of MBLs was 67.9% and 76.9% in Murtala Muhammed specialist hospital Kano and Al-madina hospital Kaduna respectively (Table 6).

Table 1: Occurrence of Carbapenemase among the Clinical bacterial Isolates

Clinical Isolates	Total Number of Isolates screened	Number of Isolates producing carbapenemase	% Prevalence
<i>E. coli</i>	83	29	34.9
<i>P. aeruginosa</i>	52	20	38.5
<i>K. pneumoniae</i>	28	7	25.0
<i>P. mirabilis</i>	37	11	29.7
Total	200	67	33.5

Table 2: Strength of carbapenemase produced by the isolates based on indentation and flattening of zone of inhibition

Clinical Isolates	carbapenemase Positive	Strong AmpC	Weak AmpC
<i>E. coli</i>	29	10(34.5)	19(65.5)
<i>P. aeruginosa</i>	20	6(30.0)	14(70.0)
<i>K. pneumoniae</i>	07	3(42.9)	4(57.1)
<i>P. mirabilis</i>	11	5(45.5)	6(54.5)
Total	67	24(35.8)	43(64.2)

Figures in parenthesis are percentages

Table 3: Occurrence of MBLs among the Isolates using EDTA-disc synergy test

S/N	Clinical isolates	Total No. of Isolates screened	No. of Isolates producing MBLs	% prevalence
1	<i>E. coli</i>	83	26	31.3
2	<i>P. aeruginosa</i>	52	11	21.2
3	<i>K. pneumoniae</i>	28	4	14.3
4	<i>P. mirabilis</i>	37	8	21.6
Total		67	49	24.5

Table 4: Occurrence of MBLs among Carbapenemase producing Bacteria

S/N	Clinical isolates	No. of isolates producing carbapenemase	No. of isolates producing MBLs	% prevalence
1	<i>E. coli</i>	29	26	89.7
2	<i>P. aeruginosa</i>	20	11	55.0
3	<i>K. pneumoniae</i>	7	4	57.1
4	<i>P. mirabilis</i>	11	8	72.7
Total		67	49	73.1

Table 5: Distribution of MBLs producing isolates based on Sex of patients

Sex	No of isolates Screened	Positive MBLs	% prevalence
Male	63	17	26.9
Female	137	32	23.3
Total	200	49	49.2

Table 6: Prevalence of MBLs among clinical samples

S/N	Clinical samples	No. of Isolates screened	No. of Isolates producing MBLs	% prevalence
1	Urine	47	18	38.3
2	Stool	30	5	16.7
3	High vaginal swab	43	9	20.9
4	Ear Swab	25	3	12.0
5	Wound Swab	10	3	30.0
6	Semen	20	5	25.0
7	Sputum	16	4	25.0
8	Blood culture	9	2	22.2
Total		200	49	24.5

Table 7: Distribution of MBLs production based on Hospitals

S/N	Clinical Isolates	Murtala Muhammad Specialist Hospital			Almadina Hospital		
		No.	No. producing carbapenemase (%)	No. Producing MBLs (%)	No.	No. producing carbapenemase (%)	No. producing MBLs (%)
1	<i>E. coli</i>	21	7(33.3)	6(85.7)	52	22(42.3)	20(90.9)
2	<i>P. aeruginosa</i>	32	8(25.0)	4(50.0)	25	12(58.3)	7(58.3)
3	<i>K. pneumoniae</i>	18	4(22.2)	2(50.0)	11	3(66.7)	2(66.7)
4	<i>P. mirabilis</i>	27	9(33.3)	7(77.8)	14	2(50.0)	1(50.0)
Total		98	28(28.6)	19(67.9)	102	39(38.2)	30(76.9)

DISCUSSION

The first report of carbapenemase detection among clinical bacterial pathogens in Kano was in 2010 (Yusuf and Arzai, 2011). The prevalence of 14.0% was reported. Few months later another research published by Yusuf *et al.*, (2011) reported a prevalence of 13.32% in Kano Nigeria. In comparison with current study, the prevalence is high. This shows that the carbapenemase resistance is on rapid increase in Kano. This may be as a result of

unawareness of their occurrence, effects but only the consequences is being felt as a number of treatment failures have been reported when antibiotics such as cephalosporins, penicillins, quinolones are used. A higher prevalences were reported elsewhere for example Bush, (1998) also reported 59% in New York. This high difference in prevalence could be due as a result of unavailability and cost of the antibiotics here where only few can afford to buy it.

The high prevalence among *E. coli* and *P. aeruginosa* is of clinical importance as these species are among the most frequent Gram-negative bacteria involved in hospital-acquired infections and nosocomial outbreaks worldwide (Coque *et al.*, 2002). In addition, *P. aeruginosa*, which is a bacterium commonly implicated in nosocomial infections with highest prevalence of (38.5%) is currently one of the most antibiotic resistant organisms known. It exhibits resistance by the use of translocases (efflux pump), and decreased porin transport. They have metabolic versatility/biochemical omnipotence i.e. ability to breakdown a wide spectrum of substrates (including disinfectants, dyes, antibiotics, antiseptics, salts) and ability to grow without organic growth factors.

Out of the carbapenemase producers, 24(35.8%) were found to produce strong carbapenemase with indented zones and 43(64.2%) produces a weak type of the enzymes with flattened zones. This showed that most of the enzymes produced by the isolates studied are strong type capable of rendering antibiotics useless faster.

The prevalence of MBLs in the study was 24.5%. Highest prevalence was recorded among *E. coli* (31.32%) followed by *P. mirabilis* (21.6%), *P. aeruginosa* (21.2%) and least among *K. pneumoniae* (14.3%). MBLs are a type of carbapenemase requiring Zinc⁺ for activity. This coincides with other findings in Taiwan reporting higher prevalence in *E. coli* with 36.9% (Walsh *et al.*, 2011), 28.6% in Brazil (Bryce *et al.*, 2000) and 26.4% in Spain (Kollef *et al.*, 2007). This gradual increase could be as a result of self medication with antibiotics which are rampant in these parts of the countries or as a result of poor hygiene among medical personnel that leads to their uncontrolled spread.

However, majority of carbapenemase producers in this study (73.1%) produces MBLs type. Highest of such was found in *E. coli* (89.7%) and least prevalence was recorded for *Pseudomonas aeruginosa* (55.0%). An earlier study by Desphande *et al.*, 2010 also revealed higher production of MBLs from carbapenemase producing organisms which was 91.7%. This indicated that these clinical pathogens will hydrolyze virtually all Beta-lactams except aztreonam used in the treatment of the infection they caused (Walsh, 2008). In addition, many of the carbapenemase producers are frequently resistant to fluoroquinolones and aminoglycosides (Heffernan *et al.*, 2006). This will result in outright delay in recovery and delays the admission of new patients requiring urgent attention into wards thereby causing havoc between the patients and the clinicians. Because this carbapenemase is encoded on plasmids that may jump from bacteria to bacteria easily facilitating rapid

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dissemination and spread between different bacterial species by lateral gene transfer favoured by globalization and travel represent a high risk of a worldwide pandemic among *Enterobacteriaceae* (Lee *et al.*, 2007). Moreover, the spread poses a number of problems in the hospitals such as lack of a routine standardized phenotypic test for MBL detection, consequent probable high prevalence of unrecognized asymptomatic carriers, allowing an international dissemination of such bacteria, scarcity of available effective antibiotics so far, and the possibility to disseminate in many different Gram-negative bacteria.

Based on clinical samples, urine was found with high prevalence of MBLs (38.3%), followed by wound swab (30.9%), semen and sputum (25.0%). Nordman *et al.*, 2009 and Peirano, 2010 also reported higher MBLs production in urine as 27.0% and 30.0% in France and Australia respectively. Urinary tract infection is on increase worldwide and a multitude of antibiotic is used in their treatment. The reports of urinary pathogen's resistant to wide range of antibiotics in Kano, Nigeria mediated by extended spectrum beta Lactamases, AmpC beta lactamases and carbapenemases have been reported by Yushau *et al.*, 2007; Yusuf *et al.*, 2011 and Yusuf and Arzai, 2011 respectively. This may indicate that majority of the carbapenemase producing type reported earlier may be MBLs type. According to the distribution of MBLs based on sex, 26.9% and 23.3% were recorded among male and female respectively. Statistically the result shows no significant difference between them, that is MBLs infection is not sex dependent ($P \leq 0.05$).

The distribution of MBLs according to Hospital, the prevalence of MBLs was 67.9% and 76.9% in Murtala Muhammed specialist hospital Kano and Almadina hospital Kaduna respectively. This difference could be as a result of patient related factors which are the major drivers of inappropriate use of antibiotics and the fact that such enzymes have not been reported in Kaduna, hence no awareness of their existence.

CONCLUSION

From the result obtained in this study, it is concluded that

- Carbapenemases particularly MBLs occurred among clinical isolates in the two hospitals studied.
- Majority of the carbapenemase producing isolates also produce MBLs.
- MBLs production is not sex dependent but varies between hospitals.
- Prevalence of MBLs was high among isolates from urine samples.

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