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Research Article

Metallo-Beta-Lactamase Producing Gram-Negative Bacteria among Patients Visiting Shahid Gangalal National Heart Centre

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Abstract

Background: The rapid spread of acquired Metallo-Beta-Lactamases (MBL) among major Gram-negative pathogens is an emerging threat and a matter of concern worldwide as it results into fewer therapeutic options for the treatment. Therefore, this study was undertaken to determine the prevalence of MBL producing Gram-negative bacteria isolated from different clinical samples.

Methods: A total of 490 samples were analyzed, at the Microbiology Department of Shahid Gangalal National Heart Centre (SGNHC), Bansbari, Kathmandu from December 2013 to June 2014, for routine culture and antibiotic susceptibility testing. MBL detection was done by Imipenem-EDTA Combined Disc Test.

Results: Out of 490 samples analyzed, 107 showed positive growth. Forty-two percent of the Gram-negative isolates were Multi Drug Resistant (MDR). Among 107 Gram-negative isolates, 66 ceftazidime resistant isolates were screened for MBL production of which 9 (13.6%) were found to be MBL positive. All MBL positive isolates were *Pseudomonasaeruginosa*. None other Gram-negative bacteria were found to produce MBL. Prevalence of MBL producing *P. aeruginosa* was 20% and all the isolates were MDR. All the MBL producing *P. aeruginosa* were isolated from hospitalized patients.

Conclusions: This study showed MBL production in a considerable number of *P. aeruginosa* isolates with MDR phenotypes. There is a need to track the detection of MBL producers and judicious use of carbapenems is necessary to prevent the further spread of these organisms.

Keywords: MBL; MDR; *P. aeruginosa*; EDTA; Imipenem

Abbreviations

AST: Antibiotic Susceptibility Test; ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Standard Institute; EDTA: Ethylene-Diamine-Tetraacetic Acid; ESBL: Extended-Spectrum-Beta-Lactamase; ICU: Intensive Care Unit; MBL: Metallo-Beta-Lactamase (Metallo-β-lactamase); MBLs: Metallo-Beta-Lactamases; MDR: Multi-Drug Resistance; SPSS: Statistical Package for Social Science; TUTH: Tribhuvan University Teaching Hospital; ZOI: Zone of Inhibition

Introduction

Metallo-beta-lactamases belong to Amber class B type of Beta-lactamase and act on a broad spectrum of substrates including penicillins, cephalosporins, and carbapenems [1]. Over the past few years, MBL producing Gram-negative bacteria have emerged as a most widespread and clinically significant carbapenem resistance mechanism [2]. One of the last lines of treatment against high level drug resistant infections is carbapenem, a MBL class of antibiotics, which was developed to resist the beta-lactamase mediated resistance posed by infection causing microbes. However the emergence of New Delhi MBL conferring resistance to almost all beta-lactam antibiotics, including carbapenem, has brought the clinical utility of carbapenem under threat. The production of MBL has recently emerged as one of the most worrisome resistance mechanisms of *P. aeruginosa* and *Acinetobacter* species owing to their capacity to hydrolyze all beta-lactam including Carbapenems [3].

Clinical infections with organisms harboring carbapenemases pose serious therapeutic challenges, with increasing reports of poor patient outcomes and death. So, early detection of resistance strains is crucial which helps in timely implementation of strict infection control practices as well as formulation of clinical guidelines regarding the potential risks for therapeutic failure [4]. This study was conducted with an aim to determine the prevalence of MBL producing Gram-negative bacteriain clinical settings with reference to a hospital in Nepal.

Methods

The present study was conducted at Shahid Gangalal National Heart Centre (SGNHC), Bansbari, Kathmandu from December 2013 to June 2014. Various clinical samples like Urine, Sputum, ET Secretion, Suction Tip, Pus, Wound Swab specimens from both out-patients and in-patients, were included in the study as sent for routine

Table 1: Gram-negative bacteria from clinical isolates in various culture positive samples (n=107).

	E. coli	*P. aeruginosa*	*C. diversus*	*C. freundi*	*E. cloacea*	*K. pneumoniae*	Total
Urine	23	11	19	0	1	3	57
	21.5%	10.3%	17.8%	0%	0.9%	2.8%	53.3%
Sputum	0	2	0	0	0	0	2
	0%	1.9%	0%	0%	0%	0%	1.9%
ET Secretion	5	28	1	0	0	6	40
	4.7%	26.2%	0.9%	.0%	.0%	5.6%	37.4%
Suction Tip	1	0	0	1	0	0	2
	0.9%	0%	0%	.9%	.0%	.0%	1.9%
Pus	0	2	0	1	0	0	3
	0%	1.9%	0%	0.9%	.0%	.0%	2.8%
Wound Swab	0	2	0	0	0	1	3
	0%	1.9%	0%	0%	0%	0.9%	2.8%
Total	29	45	20	2	1	10	107
	27.1%	42.1%	18.7%	1.9%	.9%	9.3%	100.0%

culture to microbiology laboratory and their antibiotic susceptibility testing were performed. Identification of the organisms was carried out following the manual of American Society of Microbiology and Antimicrobial Susceptibility Test (AST) were carried out using Kirby-Bauer disc diffusion method and the result interpreted in compliance with the CLSI (2013) guideline [5].

Identification of the isolates

All clinical isolates were first identified by conventional methods in a routine microbiology laboratory as recommended by the manual of American Society of Microbiology. A positive culture was defined as identification of the organism on Gram-stain followed by growth of the organism in the suitable culture medium.

Identification with staining reactions

Gram-staining was performed for the presumptive identification of the bacteria according to standard technique.

Identification with biochemical test

Typical colonies of bacterial isolates were inoculated on Nutrient broth and incubated at 370C for 4 hours. After incubation, fresh culture of test organism was inoculated into different biochemical media. Test organism was also cultured on Nutrient agar to perform other tests. All the Gram-negative bacteria from clinical isolates were characterized and identified by standard methodology as described in manual of American Society of Microbiology (using a combination of colonial morphology, Gram- stain characteristics, IMViC test, motility test, oxidative-fermentation test, catalase, citrate, oxidase tests and biochemical reactions).

Antimicrobial susceptibility tests

Antibiotic susceptibility test of all isolates was performed by Kirby Bauer disc diffusion method recommended by Clinical Laboratory Standard Institute (CLSI 2013) guidelines using the Mueller-Hinton Agar and recommended antibiotics. In this study, those isolates which acquired non-susceptibility to at least one agent in three or more antimicrobial categories were regarded as MDR [6]. Control

strains of *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were tested primarily.

Detection of MBL-producers, MBL-screening [7]

The isolates were subjected for MBL detection when the Zone of Inhibition (ZOI) for ceftazidime (30μg) was <18mm. The turbidity of inoculum AST was compared with 0.5 Mc-Farland tube then those screened isolates were subjected for MBL detection.

MBL confirmation test [8]

Two imipenem discs were placed on agar plates containing lawn of test organism. 10 μl of 0.5 M EDTA solution was applied to one of the imipenem disc, placed 25 mm apart (center to center) and the plate was incubated at 370C. After 18-24 hours of incubation, an increase of ≥7 mm in the zone diameter of imipenem-EDTA disc as compared to imipenem disc alone was considered to be positive test for the presence of MBL.

Data management and analysis

The data from the laboratory finding were entered and analyzed by SPSS version 16.0. Frequency and percentages were calculated and Chi-square test was done whenever applicable with $P < 0.05$ regarded as significant.

Results

Out of the total 490 different clinical samples, 107 (21.83%) samples showed positive growth of Gram-negative bacteria isolates, of which *P. aeruginosa* 45 (9.18%), *E. coli* 29 (5.92%), *C. diversus* 20 (4.08%), *K. pneumoniae* 10 (2.04%), *C. freundi* 2 (0.41%) and *E. cloacea* 1 (0.20%) were isolated.

Gram-negative bacteria from clinical isolates in various culture positive samples

Urine samples showed maximum number of culture positivity of Gram-negative bacteria from clinical isolates, (53.3%), followed by ET Secretion (37.4%), Pus (2.8%), Wound Swab (2.8%), Sputum (1.9%) and Suction Tip (1.9%) (Table 1).

Table 2: Antibiotic susceptibility pattern of *P. aeruginosa* (n=45).

	Sensitive	Intermediate	Resistant
Amikacin	29	0	16
	64.4%	0%	35.6%
Ciprofloxacin	5	3	37
	11.1%	6.7%	82.2%
Ceftazidime	3	0	42
	6.7%	0%	93.3%
Gentamicin	9	0	36
	20.0%	0%	80.0%
Imipenem	35	0	10
	77.8%	0%	22.2%
Meropenem	27	1	17
	60.0%	2.2%	37.8%
Piperacillin	27	1	17
	60.0%	2.2%	37.8%
Piperacillin/Tazobactam	39	0	6
	86.7%	0%	13.3%

Table 3: MDR isolates and MBL production among Gram-negative bacteria from clinical isolates.

	MDR isolates			MBL Production		
	Positive	Non-MDR	Total	Positive	Negative	Total
E. coli	10	19	29	0	29	29
	34.5%	65.5%	27.1%	0%	100.0%	27.1%
P. aeruginosa	26	19	45	9	36	45
	57.8%	42.2%	42.1%	20.0%	80.0%	42.1%
C. diversus	1	19	20	0	20	20
	5.0%	95.0%	18.7%	0%	100.0%	18.7%
C. freundi	2	0	2	0	2	2
	100.0%	0%	1.9%	0%	100.0%	1.9%
E. cloacea	1	0	1	0	1	1
	100.0%	0%	0.9%	0%	100.0%	0.9%
K. pneumoniae	5	5	10	0	10	10
	50.0%	50.0%	9.3%	0%	100.0%	9.3%
Total	45	62	107	9	98	107
	42.1%	57.9%	100.0%	8.4%	91.6%	100.0%

Table 4: MBL positive *P. aeruginosa* in different clinical samples.

	Positive	Negative	Total
Urine	2	55	57
	22.2%	56.1%	53.3%
Sputum	0	2	2
	0%	2.0%	1.9%
ET Secretion	6	34	40
	66.7%	34.7%	37.4%
Suction tip	0	2	2
	0%	2.0%	1.9%
Pus	0	3	3
	0%	3.1%	2.8%
Wound swab	1	2	3
	11.1%	2.0%	2.8%
Total	9	98	107
	8.4%	91.6%	100.0%

the maximum sensitivity was observed for piperacillin/tazobactam (86.7%) followed by imipenem (77.8%), amikacin (64.4%), meropenem (60.0%) and piperacillin (60.0%) (Table 2).

Distribution of MDR isolates and MBL production among Gram-negative bacteria from clinical isolates

In this study, out of 107 Gram-negative bacteria from clinical isolates, 45 were found to be MDR and maximum MDR was observed in *P. aeruginosa* (42.1%), followed by *E. coli* (27.1%), *C. diversus* (18.7%), *K. pneumoniae* (9.3%), *C. freundi* (1.9%), *E. cloacea* (0.9%) respectively.

Out of 107 Gram-negative isolates, 9 isolates (8.4%) were MBL producers. The production of MBL was seen only in 20 % *P. aeruginosa* among 45 isolates (Table 3).

Distribution of MBL positive *P. aeruginosa* in different clinical samples

Out of 9 MBL positive isolates, higher incidence of MBL producing strains were found among samples of Endo-Tracheal-Secretion (n=6) (Table 4).

Distribution of MBL producing *P. aeruginosa* among In-patient and Out-patient
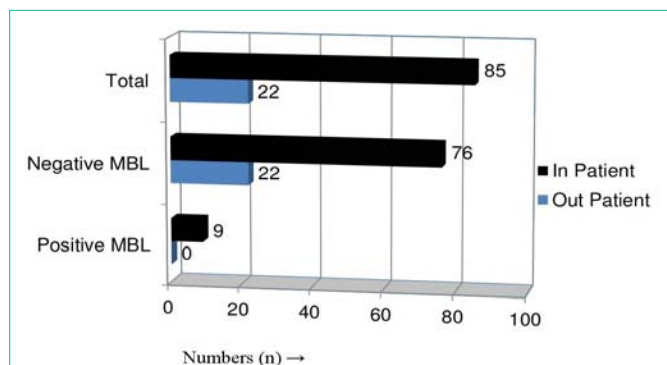
Isolates from In-patients were found to carry MBL-encoded resistance property. All MBL positive *P. aeruginosa* were isolated from in-patients only (Figure 1).

Antibiotic susceptibility pattern of MBL Positive *P. aeruginosa*

The antibiotic susceptibility pattern of MBL Positive *P. aeruginosa* revealed that the maximum sensitivity was observed for piperacillin/tazobactam 77.77% followed by amikacin 44.44%, piperacillin 33.33% and Imipenam 11.11%. Hundred percent resistances were observed for ceftazidime, gentamicin, ciprofloxacin and meropenem (Table 5).

Discussion

The rapid spread of acquired MBLs among major gram-negative pathogens is a matter of particular concern worldwide and in Nepal

Antibiotic susceptibility pattern of Gram-negative bacteria from clinical isolates

The antibiotic susceptibility pattern of Gram-negative bacteria from clinical isolates revealed that the most sensitive drug was nitrofurantoin (89.1%) among Urine samples. The most sensitive drug was piperacillin/tazobactam (82.5%), followed by imipenem (82.1%) and amikacin (71.9%) among samples other than Urine. Similarly, the most resistant drug was ampicillin (87.1%) followed by cephalexin (83.9%), ceftazidime (68.75%) and cefixime (67.7%) respectively.

Antibiotic susceptibility pattern of *P. aeruginosa*

The antibiotic susceptibility pattern of *P. aeruginosa* revealed that

Figure 1: MBL producing *P. aeruginosa* among In-patient (n=85) and Out-patient (n=22).

Table 5: Antibiotic susceptibility pattern of MBL Positive *P. aeruginosa* (n=9).

	Sensitive	Intermediate	Resistant
Amikacin	4	0	5
	44.4%	0%	55.5%
Ciprofloxacin	0	0	9
	0%	0%	100.0%
Ceftazidime	0	0	9
	0%	0%	100.0%
Gentamicin	0	0	9
	0%	0%	100.0%
Imipenem	1	0	8
	11.11%	0%	88.88%
Meropenem	0	0	9
	0%	0%	100.0%
Piperacillin	3	0	6
	33.33%	0%	66.66%
Piperacillin/Tazobactam	7	0	2
	77.77%	0%	22.22%

as well. This study showed a high incidence of MBL- producing gram-negative bacteria in different clinical samples. In Nepal first study of MBL was conducted at Department of Microbiology, Tribhuvan. University Teaching Hospital (TUTH) from June to November 2008, in which 6 (1.3%), 3 isolates each of *P. aeruginosa* and *Acinetobacter* spp. of the total 448 Gram-negative isolates were found to be MBL producer [7]. However, several studies from Nepal and India indicated higher prevalence of MBL producing gram-negative isolates [9-12], which might have resulted from overuse of carbapenems in the hospital settings.

This study showed the increasing rate of antimicrobial resistance among Gram- negative bacteria especially towards ampicillin and Cephalosporins. Among MDR isolates, the most sensitive drug was Piperacillin/Tazobactam, followed by Imipenem and Amikacin. All of MBL producers in this study were MDR and resistant to most of the antibiotic used. This is due to the fact that the MBL genes are often carried along with other resistance genes resulting in multi-drug resistance limiting treatment options [13]. Similar results were obtained in previous studies [7,14].

Although, PCR method is simple to use in detecting MBL producing isolates, it has become more difficult with the increased number and types of MBL. Combined disc test is simple to perform and highly sensitive in differentiating MBL-producing isolates. Thus, implementation of simple method using imipenem-EDTA disk for MBL detection is quick, specific, sensitive and reproducible [15]. Out of 107 Gram- negative isolates, 9 isolates (8.4%) were found to be MBL producers. The production of MBL was seen in 20 percent *P. aeruginosa* among 45 isolates.

In this study, among the 9 MBL-producers, only one isolate was sensitive to imipenem. As seen with extended-spectrum-beta-lactamase and AmpC beta-lactamases with cephalosporins, MBL carrying organisms may also appear susceptible to carbapenems using current CLSI breakpoints but they may not be effective in *in vivo* [5]. The method, utilizing ceftazidime resistance as the selective criterion for the phenotype test to detect MBL, was fruitful. If only carbapenems resistant cases were selected, one MBL carrying isolate would have been missed.

Hundred percent resistances to ceftazidime, gentamicin, ciprofloxacin and meropenem were observed among MBL producers, which was consistent with Mishra et al. [7]. In this study 77.77% of MBL producers were susceptible to piperacillin/tazobactam which was similar to the finding of Varaiya et al. [10] and 44.44% were sensitive to amikacin which was nearly similar to the study conducted by Gupta et al. [16].

Emergence of MBL producing *P. aeruginosa* in intensive care units is alarming and reflects overuse of carbapenems. There is intense selection pressure, due to high usage of broad spectrum antibiotics in ICUs. This results in eradication of competitive flora and subsequent selection of multidrug-resistant strains [11]. All MBL producing *P. aeruginosa* were isolated from In-patients and out of 9 MBL positive isolates 5 were from Endo Tracheal secretion. De et al. [17] reported prolong hospitalization > 8 days; mechanical ventilation and endotracheal intubation were common risk factors of MBL producing in ICU patients. Zavascki et al. [18] have shown that prior exposure of a Beta-lactam or fluoroquinolone, neurological disease, Urinary Tract Infection, renal failure and ICU stay were significant risk factors for MBL producing infections. This may be the reason behind MBL detection only among Inpatients in this study.

Carbapenems being the effective therapeutic agents against highly resistant pathogens such as *Pseudomonas* spp. and gram negative bacteria, spread of this resistance among these pathogens and transfer to other Gram-negative bacteria would seriously restrict therapeutic options. The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem because the only therapeutic alternative remains the potentially toxic polymyxinB and colistin. The accurate identification and reporting of MBL-producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

Conclusion

Among different Gram-negative bacteria isolated, only *P. aeruginosa* showed MBL production. None of the other Gram-negative bacteria was found to be MBL positive. This study demonstrates Gram-negative bacteria exhibiting the increased rate of

resistance to wide variety of antibiotics from different antimicrobial categories with additional burden of MBL producers within them. All MBL producers are multi-drug-resistant, leaving very few antibiotics for treatment options. This study highlights the need to do regular surveillance for the detection of MBL producers, judiciously use carbapenems to prevent their spread and use effective antibiotics after sensitivity testing for treatment.

Acknowledgements

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