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Original Article

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Detection of Metallo-Beta-Lactamase (MBL) producing *pseudomonas aeruginosa* and *acinetobacter Spp.* from a Tertiary Care Hospital

Aradita C[1], Archana Rao K[*2], Lakshminarayana SA[3]

[1] *Post-graduation first year, Department of Dermatology, JSS Medical college, India*

[2] *Assistant professor, Department of microbiology, Rajarajeswari medical college and hospital. Bengaluru, India*

[3] *Professor and Head, Department of microbiology, Rajarajeswari medical college and hospital. Bengaluru, India*

***Corresponding authors:** Dr Archana Rao K, **Address**: No 108, First Floor, MIG, KHB Colony, 2nd Stage,6th Cross, Basaveshwaranagar, Bangalore-560079, **Email**: archanaswaroop79@gmail.com, **Tel**: +918105933216

Abstract

***Background & Aims*:** Non-Fermenting Gram-Negative Bacilli [NFGNB] which are considered as environmental contaminants have emerged as multidrug-resistant bacteria and are of serious concern to the treating physician. The aim of this study was to determine the proportion of metallo-beta-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in clinical samples received in the Microbiology Laboratory and to study the antibiotic sensitivity pattern of metallo-beta-lactamase (MBL) producing strains.

Materials & Methods: Various samples (pus, sputum, urine, blood and body fluids, etc.) were processed according to standard protocols. *P.aeruginosa and Acinetobacter spp* were isolated and identified with the help of various culture media, staining methods and biochemical reactions. Antibiotic susceptibility test was done by using Kirby-bauer disc diffusion method. MBL producers were identified using CLSI guidelines.

***Results*:** Out of 600 positive culture isolates from various samples, 65 (10.8%) were non-fermenting gram-negative bacilli, 40 (61.53%) were *Pseudomonas aeruginosa*, and 25 (38.47%) were *Acinetobacter* species. The overall incidence of MBL positive isolates in our study was 12.3% (8 of 65). All the isolates of *Pseudomonas aeruginosa* and *Acinetobacter*species were 100% sensitive to Colistin and Tigecycline.

***Conclusion*:** The study helps in understanding the antibiotic resistance pattern of isolates causing nosocomial infections, helping clinicians in making appropriate antibiotic choices as an empirical therapy and the policy-makers to bring out the measures in controlling the superbugs.

***Keywords*:** *Acinetobacter* Species, Multidrug Resistant, Metallo-Beta-Lactamase, Non-Fermenting Gram-Negative Bacilli, *Pseudomonas Aeruginosa*, Superbugs

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Introduction

Non-Fermenting Gram-Negative Bacilli (NFGNB) are a group of taxonomically diverse organisms that make their explicit mark for their ubiquitous nature. Being a part of soil, water, air, and surfaces, and they are considered as environmental contaminants (1,2). Also, they are present abundantly in the hospital environment and are recovered routinely from devices such as

catheters, ventilators, endotracheal tubes and intra-vascular devices (3,4). Among NFGNB, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are dreaded pathogens in the hospital settings (5). It has been reported that non-fermenters approximately account for 15% of all the gram-negative bacilli isolated in the microbiology laboratory. Of these, commonly isolated organisms are *Pseudomonas aeruginosa* [*P. aeruginosa*] followed by *Acinetobacter baumannii* [*A. baumannii*] *(*6,7). The spectrum of infection caused by these pathogens ranges from urinary tract infections, burn infections, to ventilator-associated pneumonia, severe sepsis (5).

The major threat is mainly because of their capability to possess intrinsic and extrinsic antibiotic resistance to a wide variety of antimicrobial agents. Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* and *Acinetobacter spp* has emerged which have the capacity to hydrolyze virtually all beta-lactam agents including the carbapenems. This poses a serious therapeutic problem in hospitals because carbapenems are often antibiotics of last resort for the treatment of serious infections. The World Health Organization has recognized antimicrobial resistance (AMR) as "a global health security threat that requires action across government sector and society as a whole." (8). The Centers for Disease Control and Prevention (CDC) has estimated the excess direct healthcare costs associated with AMR to be as high as $20 billion, and additional costs to society for lost productivity as high as $35 billion a year in the United States alone (9). Detection of *P. aeruginosa* and *A.baumannii* is crucial for optimal treatment of patients and control the spread of resistant bacteria in the hospital scenario (1, 6). The purpose of this study was to evaluate the metallo-beta-lactamase (MBL) production among multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter spp*., isolated from clinical specimens. We also determined the proportion of metallo-beta-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in clinical samples received in the Microbiology Laboratory of RRMCH, Bengaluru and studied the antibiotic sensitivity pattern of MBL producing strains.

Materials & Methods

This is a cross-sectional was conducted at the Department of Microbiology, Rajarajeswari medical college. Clinical samples received by the Microbiology Laboratory, Rajarajeswari Medical College and Hospital (a tertiary care hospital), showing the growth of Acinetobacter and Pseudomonas aeruginosa was included in the study. Various samples (pus, urine, sputum, vaginal swabs, ear swabs, pleural fluid, and blood) were included in the study. All the samples were processed following the standard procedure. Clinical data (age, gender, ward, clinical history, antibiotic use) will be recorded using a standardized proforma (10).

Processing of specimens:

Microscopy: Direct smear with gram stain was observed for the presence of inflammatory cells and the type and morphology of the microbial flora.

Culture: Specimens will be inoculated on chocolate agar and MacConkey agar. The plates are inoculated aerobically at 370c and examined for growth at 24 and 48 hours.

Identification: Gram-negative bacterial isolates were identified by their colony characteristics and subjected to various biochemical reactions. The isolates were identified based on Gram stain, catalase test, oxidase test, nitrate test, Triple sugar iron test, urease test, indole test, citrate test and also various sugar fermentation and amino acid utilization tests.

Antimicrobial Susceptibility Testing: Isolates were screened initially using Kirby-Bauer method on Mueller Hinton agar as per CLSI guidelines.48 All MBL producing isolates are confirmed by using combined disc test (CDT) with antibiotic discs containing Imipenem (10 µg) either alone or in combination with EDTA (750 µg). An isolate was considered to be an MBL producer if the zone of inhibition around the Imipenem/EDTA disc is > 7 mm than the zone around the Imipenem disc alone. Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by Clinical Laboratory Standard Institute (CLSI).

Quality control: ATCC Pseudomonas aeruginosa 27853 (11,12).

Results

In our study, out of 600 culture positive isolates from various samples, 65 (10.8%) were non-fermenting gram-negative bacilli, 40 (61.53%) were *Pseudomonas aeruginosa* and 25 (38.47%) were *Acinetobacter* species.

Table 1. Age-wise distribution

Age (yrs.)	Pseudomonas aeruginosa n=40 (%)	Acinetobacter species n=25 (%)
1 to 10	4(10%)	5(20%)
11 to 20	3(7.5%)	2(8%)
21 to 30	5(12.5%)	5(20%)
31 to 40	4(10%)	3(12%)
41 to 50	5(12.5%)	0
51 to 60	9(22.5%)	3(12%)
61 to 70	10(25%)	7(28%)

In our study, the age of the patients ranges from 1 to 70 years. The mean age of the patient was 60.5. Majority of *Pseudomonas aeruginosa* (25%) and *Acinetobacter spp.* (28%) were isolated from the age group of 61-70 years. Out of 40 isolates of *Pseudomonas aeruginosa*, the majority of the patients were males 24 (60%), followed by females 16 (40%). In case of 25 isolates of *Acinetobacter* species, the majority of the patients were males 19 (76%) and the number of females were 6(24%).

Table 2. Sample wise distribution of isolates

	Pseudomonas aeruginosa n=40 (%)	Acinetobacter species n=25 (%)
Pus	9 (22.5%)	4 (16%)
Sputum	7 (17.5%)	14 (56%)
Urine	5 (12.5%)	3 (12%)
Ear discharge	17 (42.5%)	2 (8%)
Tissue	1 (2.5%)	0
Throat swab	1 (2.5%)	0
Blood	0	2 (8%)

Among the 40 isolates of *Pseudomonas aeruginosa,* majority are from ear discharge (otitis media, otitis externa, etc.) followed by pus (22.5%) which includes wound infections, burn infections, etc. Whereas among the 25 isolates of *Acinetobacter* species, 14 (56%) were from sputum samples, followed by 4 (16%) from pus samples.

Table 3. Distribution of isolates in different clinical departments

Department	Pseudomonas aeruginosa=40(%)	Acinetobacter species n=25 (%)
Medicine	5 (12.5%)	4 (16%)
Surgery	7 (17.5%)	3 (12%)
ENT	16 (40%)	1 (4%)
Pediatrics	3 (7.5%)	5 (20%)
OBG	1 (2.5%)	1 (4%)

Department	Pseudomonas aeruginosa=40(%)	Acinetobacter species n=25 (%)
Ortho	1 (2.5%)	0
Urology	1 (2.5%)	0
NICU	0	2 (8%)
MICU	4 (10%)	8 (32%)
TBCD	2 (5%)	1 (4%)

Majority of *Pseudomonas aeruginosa* were isolated from ENT department i.e., 16 isolates (40%), followed by 7 from surgery ward (17.5%). In case of *Acinetobacter* species majority of isolates were from MICU 8 (32%), followed by Pediatrics ward 5 (20%).

Table 4. Antibiotic sensitivity pattern of isolates

	Pseudomonas aeruginosa n=40 (%)		Acinetobacter species n=25(%)	
	SENSITIVE	RESISTANT	SENSITIVE	RESISTANT
Piperacillin	22(55%)	18(45%)	3(12%)	22(88%)
Piperacillin+Tazobactam	28(70%)	12(30%)	18(72%)	7(28%)
Ceftazidime	20(50%)	20(50%)	15(60%)	10(40%)
Cefepime	25(62.5%)	15(37.5%)	16(64%)	9(36%)
Gentamycin	25(62.5%)	15(37.5%)	15(60%)	10(40%)
Tobramycin	28(70%)	12(30%)	20(80%)	5(20%)
Amikacin	30(75%)	10(25%)	20(80%)	5(20%)
Netilmycin	32(80%)	8(20%)	20(80%)	5(20%)
Ciprofloxacin	15(37.5%)	25(62.5%)	7(28%)	18(72%)
Ofloxacin	20(50%)	20(50%)	10(40%)	15(60%)
Imipenem	30(75%)	10(25%)	18(72%)	7(28%)
Meropenem	30(75%)	10(25%)	18(72%)	7(28%)
Aztreonam	35(87.5%)	5(12.5%)	20(80%)	5(20%)
Cotrimoxazole	10(25%)	30(75%)	5(20%)	20(80%)
Colistin	40(100%)	0	25(100%)	0
Tigecycline	40(100%)	0	25(100%)	0

Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* by disc diffusion method showed 87.5% sensitivity to Aztreonam followed by 80% to Netilmycin,75% to Imipenem, Meropenem, Amikacin and 70% to Tobramycin, Piperacillin-Tazobactam. Least sensitivity was towards cotrimoxazole 25% followed by 37.5% to ciprofloxacin. Among *Acinetobacter* species, 80% were sensitive to Aztreonam, Netilmycin, Amikacin, Tobramycin, 72% to Imipenem, Meropenem and Piperacillin-Tazobactam. Least sensitivity was to Piperacillin which was 12%, followed by 20% sensitivity to Cotrimoxazole and 25% to Ciprofloxacin.

Table 5. Tests for MBL detection

	Pseudomonas aeruginosa n=40 (%)	Acinetobacter species n=25 (%)
Carbapenem resistance	10 (25%)	7 (28%)
MBL screening by CDT	3 (7.5%)	5(20%)

10 (25%) isolates of *Pseudomonas aeruginosa* showed Carbapenem resistance (resistance to Imipenem or Meropenem or both) by disc diffusion method. Among these 10 isolates, MBL screening was positive in 3(7.5%) by CDT.

Whereas, 7 (28%) isolates of *Acinetobacter* species showed resistance towards Carbapenem by disc diffusion method. Among these 7 isolates, MBL screening was positive in 5(20%) by CDT. The overall incidence of MBL positive isolates in our study was 12.3% (8/65).

All the MBL producing isolates of *Pseudomonas aeruginosa* and *Acinetobacter* species were multidrug resistant with sensitivity pattern different from that of the MBL non-producers**.** All the isolates of *Pseudomonas aeruginosa* and *Acinetobacter* species were 100% sensitive to Colistin and Tigecycline.

Discussion

NFGNB, earlier which were only considered to be environmental contaminants, have now emerged as important nosocomial pathogens *(*13). In our study, out of 600 culture positive isolates from various samples, 65 (10.8%) were non-fermenting gram-negative bacilli which is in parallel to studies done by *Rit et al (*14) *and Benachinmardi et al (*15). The most common NFGNB isolated in our study was *Pseudomonas aeruginosa* 61.53% and 38.47% were *Acinetobacter* species which is similar to the results obtained by Malini et al (13) WHO reported *P. aeruginosa* as the most common isolate accounting for 104/189 (53.8%) isolates, followed by A. baumannii (43/189, 22.2%). Similarly, the study done by Rit et al (14) also found *P. aeruginosa* to be the predominant isolate (101/201, 50.24%), followed by A. baumannii (50/201, 24.8%). Among various etiological agents causing healthcare-associated infections, NFGNB poses a major threat, because of their ubiquitous nature surviving on humidifiers, ventilators other medical equipment, also their ability of acquiring antibiotic resistance especially in immunocompromised hosts. Due to irrational and indiscriminate use of antibiotics, there is an increased multidrug resistant pattern among these isolates. Carbapenems are the currently recommended therapeutic option for infections due to multi-drug-resistant pathogens such as *Pseudomonas aeruginosa and Acinetobacter species.* Spread of MBL mediated resistance among these pathogens and transfer to other gram-negative bacteria will significantly restrict treatment options (16, 17). In our study, 26.15% of the patients were under the age group of 61-70 (Table 1). NFGNB are known to cause infection in extremes of age which was also seen in our study similar to the study of Gardner et al. (18) and Sachdev et al (19) which could be due to subnormal immune system. In the present study, majority of the *Pseudomonas aeruginosa* isolates were from ENT ward (40%) and *Acinetobacter* species from MICU (32%). Our study is in concordance with the study of Neethu Gupta et al (20). According to the results of antibiotic sensitivity pattern performed by us, it shows that *Pseudomonas aeruginosa* were 87.5% sensitive to Aztreonam, followed by 80% to Netilmycin, 75% sensitivity to Amikacin, Imipenem and Meropenem which is comparable to the study by Sujatha Karjigi et al (21). *Acinetobacter* show 80% sensitivity to Aminoglycosides, Carbapenems and Aztreonam which is similar to the study by Z Chang-Tai et al (22). The overall proportion of MBL positive isolates in our study was 12.3% (8/65). We noted that 7.5% of *Pseudomonas aeruginosa* and 20% of *Acinetobacter* species were MBL positive. Agarwal et al (23). reported 8.05% of *Pseudomonas aeruginosa* and Rit K (24) et al. reported 22% of *Acinetobacter* species, which were MBL positive. The predominant source of MBL positive strains in the current study was sputum, which is similar to the findings of Nautiyal S et al (25). The antibiotic sensitivity pattern of MBL producers in our study showed significant difference with respect to Cephalosporins, Aminoglycosides, Fluoroquinolones and Carbapenems, as compared to the MBL non-producers. MBL genes are carried on mobile genetic elements, that also code for resistance genes to Aminoglycosides and Fluoroquinolones (26). This explains for the concurrent high resistance rates to these two classes of antibiotics along with MBL production. Colistin and Tigecylcine showed 100% sensitivity

among MBL producing *Pseudomonas aeruginosa and Acinetobacter* species. Other authors have also recorded similar findings. The second most active drug against MBL isolates was Aztreonam, with sensitivity rate of 87.5% and 80% of *Pseudomonas aeruginosa* and *Acinetobacter* species respectively. Theoretically, MBLs can hydrolyze all beta-lactam antibiotics except Aztreonam.

Conclusions

In conclusion, this study documents the proportion of MBL producers among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter* species at 12.3% in our hospital. Emergence of MBL-producing *Pseudomonas aeruginosa* and *Acinetobacter* species in this hospital is alarming due to the looming possibility of hitting therapeutic dead ends in the absence of novel therapeutic MBL inhibitors. The early detection of MBL-producing isolates would be important for the reduction of mortality rates of infected patients and also to avoid the intra-hospital dissemination of such strains. Isolation of multidrug-resistant *P.aeruginosa* and *Acinetobacter Spp* in the present study is an alarm and indicates emerging antibiotic resistance in this group of bacteria in our region. Proper screening, early detection of non-fermenters especially in nosocomial settings and systematic assessment of their antibiotic susceptibility profiles, evaluation of reports for multidrug resistance and encouraging the clinicians for judicious use of antibiotics, to follow protocols of antibiotic policy that is choosing right drug at right dose plays a major role for effective management of the infections caused these superbugs.

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Nil.

Conflict of interests

The authors declare that they have no conflicts of interest.

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No funding was required for the study.

Data Availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

Ethical Statement

To protect the confidentiality and privacy of the patients, the samples were anonymized and coded using a unique identifier. The clinical data of the patients, such as age, gender, ward, clinical history, and antibiotic use, were recorded using a standardized proforma and stored securely in a password-protected database. The study adhered to the ethical principles of beneficence, non-maleficence, respect for autonomy, and justice, as outlined by the Declaration of Helsinki.

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