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Occurrence of *bla* genes encoding carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from Intensive Care Unit in a tertiary care hospital

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Abstract:

CONTEXT: ICU shows increasing incidence of infection associated with the use of invasive procedures for the diagnostic purpose as well as the indiscriminate use of antibiotics. *Pseudomonas aeruginosa* and *Acinetobacter species* are "very successful" pathogen and the emergence of the Metallo-β-Lactamases (MBL) is becoming a therapeutic challenge.

AIMS: To isolate the *Nonfermenting* Gram negative bacilli from the ICU samples. To identify the metallo betalactamase producers and to detect the *bla* gene presence among the *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

SETTINGS AND DESIGN: The Nonfermenting Gram negative bacilli isolates from the ICU samples were taken over for 5 years (2009-2014) in a tertiary care hospital.

METHODS AND MATERIALS: The isolates of *Pseudomonas species* and *Acinetobacter species* were confirmed by API analyser and processed according to standard procedures. Detection of the MBL producers were done by E strip method and subjected for *bla* gene detection by PCR method.

RESULTS: In our study a total of 195 isolates of *NFGNB* were obtained from various ICU. Of these MBL producers, 26 % were *Pseudomonas aeruginosa* and 25 % were *Acinetobacter baumannii*. The subtypes of bla_{VIM} MBL producing *P.aeruginosa* were 26%.The predominant gene coding for MBL activity in *A.baumannii* were found to be bla_{OXA} gene 11.9%. The gene accession numbers were KF975367, KF975372.

CONCLUSIONS: We have to control the development and dissemination of these superbugs among the ICU's.

Key words:

Acinetobacter baumanni, *bla* genes, ICU, Metallo-β-lactamases, *Pseudomonas aeruginosa*

Introduction

Isolation of nonfermenters from the clinical specimens obtained from Intensive Care Unit (ICU) shows that increasing incidence of infection associated with the use of invasive procedures, indiscriminate use of antibiotics, inadequate sterilization, and immune compromised condition due to lifestyle disease have also contributed.[1]

Among the nonfermenters, *Pseudomonas aeruginosa* is inherently resistant and *Acinetobacter* species capable of surviving in various environmental conditions are adapted at acquiring resistance.[2,3] The digestive tracts of patients within ICUs often serve as reservoirs for multidrug-resistant (MDR) isolates.[4,5]

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P. aeruginosa resistance is a global disease burden[6] and it is a therapeutic challenge.[7] The *Acinetobacter* baumanii complex is emerging multidrug resistant nosocomial and community acquired pathogen.The incidence of infection by these species among the patients receiving the mechanical ventilation are quite increasing.[8] These organisms are "very successful" pathogen which possesses both acquired and intrinsic mechanisms of resistance to various classes of antibiotics.[9-11]

Infections in the ICU patients were commonly associated with ventilator-associated pneumonia, urinary tract infection, and bacteremia caused by MDR organism Gram-negative bacilli with increasing morbidity and mortality.[12,13] The emergence of the metallo-β-lactamases (MBL) is becoming a therapeutic challenge.[12] Antimicrobial resistance pattern has emerged as an important determinant of the outcome for patients in the ICUs.[14]

In our study, drug-resistant isolates in the ICUs were detected and the gene encoding carbapenem resistance in *P. aeruginosa* and *Acinetobacter baumannii* was identified. The resulting sequences were compared with those available in GenBank.

Methods

All the suspected colonies of the *NFGNB* were identified by Gram staining, colony characteristics, oxidase test, motility, and standard biochemical reactions, and further confirmation of the species was carried out by API analyzer. The study was carried out in a tertiary care hospital (2009–2014). All the organisms identified were tested for the susceptibility according to the standard Clinical and Laboratory Standards Institute guidelines.[15] The sensitivity pattern of first- and second-line drugs was tested.

For *Pseudomonas* species, the following 15 drugs were used: amikacin (Ak-30 μg), aztreonam (Az-30 μg), colistin (Cl-10 μg), ciprofloxacin (Cip-5 μg), ceftazidime (Caz-5 μg), cefepime (Cpm-5 μg), carbenicillin (Cb-100 μg), gentamicin (G-10 μg), imipenem (Imp-10 μg), meropenem (Mr-10 μg), netilmicin (Net-30 μg), ofloxacin (Of-5 μg), piperacillin-tazobactam (Pit-100 μg/10 μg), polymyxin B (Pb-300 units), tobramycin (Tb-10 μg).

For *Acinetobacter species*, amikacin (Ak-30 μg), cefepime (Cpm-5 μg), ceftazidime (Caz-5 μg), ciprofloxacin (Cf-5 μg), cefotaxime (Ce-5 μg), colistin (E strip), cotrimoxazole (Cot-5 μg), gentamicin (G-10 μg), imipenem (IMP-10 μg), meropenem (Mr-10 μg), piperacillin-tazobactam (Pt-100 μg/10 μg), and polymyxin B (E strip) were used, and in case of urine samples, nitrofurantoin (Nit-300 μg) disks were used.

The study was confined to the MBL-producing *P. aeruginosa* and *A. baumannii* species. The antibiotic discs used in our study were purchased from HiMedia. The E strip was purchased from HiMedia, Biomerieux, and Radianz biotechnologies. Screening for MBL production was done in imipenem-resistant isolates by the E strip method using the ceftazidime and ceftazidime + ethylenediaminetetraacetic acid.[16]

The MBL-producing resistant strains of *P. aeruginosa* were screened for the *bla* genes – $bla_{VIM,\ KPC,\ NDM,\ IMP}$[1,17-21] [Table 1]. For *A. baumannii*, $bla_{VIM,\ IMP,\ OXA,\ NDM}$ genes [Table 2] were carried out.[1,18,21-23]

Polymerase chain reaction amplification

The reaction conditions were as follows: predenaturation

Table 1: The primers used for *bla* gene detection in *Pseudomonas aeruginosa*

Gene	Primers		Product size
$bla_{VIM\ A}$	Forward primer	5'TCT ACA TGA CCG CGT CTG TC-3'	748 bp
$bla_{VIM\ B}$	Reverse primer	5'TGT GCT TTG ACA ACG TTC GC-3'	
bla_{IMP}	Forward primer	5' CCA GAT TTA AAA ATA GAG AAG CTT G-3'	587bp
bla_{IMP}	Reverse primer	5' TGG CCA AGC TTC TAC ATT TGC GTC -3'	
bla_{NDM}	Forward primer	5' GGT TTT GGC GAT CTG GTT TTC 3'	522 bp
bla_{NDM}	Reverse primer	5' CGG AAT GGC TCA TCA CGA TC 3'	
bla_{KPC}	Forward primer	5' GCT ACA CCT AGC TCC ACC TTC-3'	989bp
bla_{KPC}	Reverse primer	5' ACA GTG GTT GGT AAT CCA TGC-3'	

Table 2: The primers used for *bla* gene detection in *Acinetobacter baumannii*

Gene	Primers		Product size
bla_{VIM}	Forward primer	-5'-GTGCTTTGACAACGTTCGCT-3'	442bp
bla_{VIM}	Reverse primer	-5'-TCCACGCACTTTCATGACGA-3'	
bla_{IMP}	Forward primer	- 5' -TTTTGCAGCATTGCTACCGC-3'	220 bp
bla_{IMP}	Reverse primer	- 5' -CACGCTCCACAAACCAAGTG-3'	
bla_{OXA}	Forward primer	-5'-AGTATTGGGGCTTGTGCT-3'	398bp
bla_{OXA}	Reverse primer	-5'-AACTTCCGTGCCTATTTG-3'	
bla_{NDM}	Forward primer	5' GGT GCA TGC CCG GTG AAA TC 3'	660 bp
bla_{NDM}	Reverse primer	5' ATG CTG GCC TTG GGG AAC G 3'	
		Internal control	400 bp

at 94°C for 2 min, followed by 30 amplifications cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 sec, with final extension step of 72°C for 5 min. The cycling parameters for the $bla_{IMP,\ VIM,\ NDM,\ OXA}$ genes were as follows: initial denaturation: 94°C for 3 min, denaturation: 94°C for 1 min, annealing: 58°C for 1 min 35 cycles, extension: 72°C for 1 min, final extension: 72°C for 5 min. After screening for the MBL, the positive PCR products were sequenced. Sequencing the amplified products, the BLAST results were analyzed.

Results

The nonfermenters isolated from ICU were found to be notorious as there were possibilities of drug-resistant strains being horizontally spread among the patients. In our study, a total of 195 isolates of *NFGNB* were obtained from various ICUs. Among them, 61 (31.2%) were *Pseudomonas spp* and 134 (68.8) were *Acinetobacter spp*. Among the 84 isolates of *NFGNB*, 32 (38%) were *P. aeruginosa* and 61.9% *A. baumannii* were isolated from surgical ICU. Distribution of *NFGNB* – *P. aeruginosa* and *A. baumannii* in different ICUs is shown in Table 3. Among 61 *P. aeruginosa* from ICU patients, 19 (31.1%) were from males and 42 (68.8%) were from females. Distribution of *P. aeuginosa* from ICU among different sexes is shown in Chart 1. Among 134 *A. baumannii* from ICU patients, 89 (66.4%) were from males and 45 (33.5%) were from females [Chart 2].

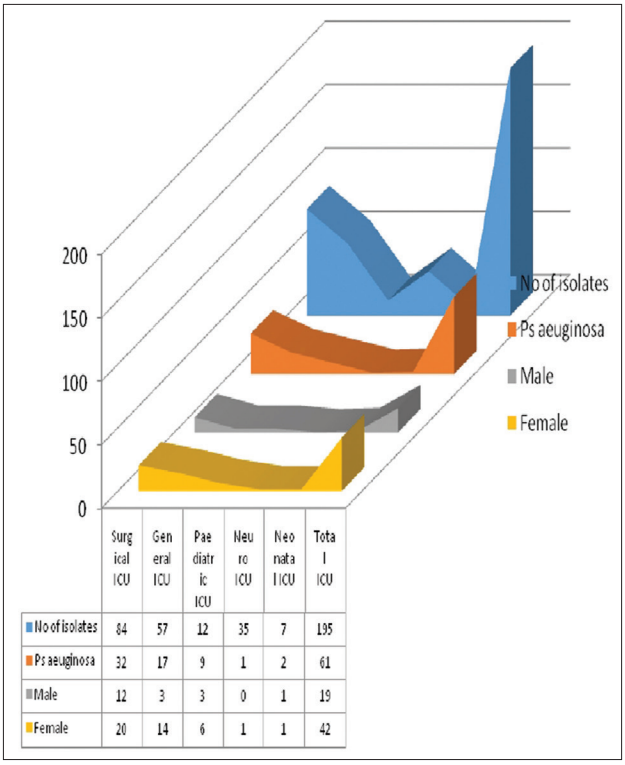

Chart 1: Distribution of *Pseudomonas aeruginosa* from Intensive Care Unit among different sexes

Among 195 *NFGNB* isolates from ICU, 89 (45.6%) were drug resistant. Out of these, 26 (13.33%) were *P. aeruginosa* and 63 (32.3%) were *A. baumannii*. Overall, the MDR isolates from ICU were 33.33%. The MBL producers from ICU were 49 (25.12%) [Table 4]. Of these MBL producers, 16 (26.22%) were *P. aeruginosa* and 33 (24.62%) were *A. baumannii* [Table 5 and Chart 3].

The maximum numbers of MBL producers were in surgical ICU followed by general ICU. Among ICUs, 6.1% of isolates were from pediatric ICU and one isolate of *P. aeruginosa* was MBL producer [Table 6]. Among the MBL producers in ICU, *P. aeruginosa* was obtained from

Table 3: Distribution of *P.aeruginosa* and *A.baumannii* in different ICUs

Source	No of isolates	P.aeuginosa (%)	A.bauamannii (%)
Surgical ICU	84	32 (38)	52 (61.9)
General ICU	57	17 (29.8)	40 (70.1)
Paediatric ICU	12	9 (75)	3 (25)
Neuro ICU	35	1 (2.8)	34 (97.2)
Neonatal ICU	7	2 (28.5)	5 (71.4)
Total ICU	195	61	134

Table 4: Drug resistance in ICU isolates

Isolates from ICU (*n*)	Drug resistant isolates	Multidrug resistant isolates	Metallo beta lactamase producers
P.aeruginosa (61)	26	23	16
A.baumannii (134)	63	42	33
Total (195)	89	65	49

Table 5: Showing MBL positive by Estrip method

Method (E strip–MIC E strip)	*P. aeruginosa* (*n*=26)	*A. baumanii* (*n*=63)
CDT - Imp, Imp + EDTA	16	33

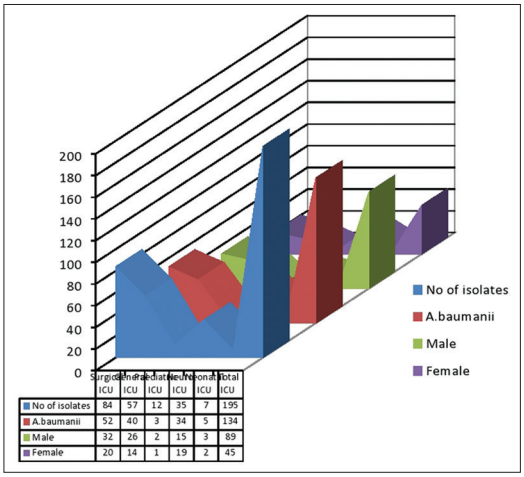

Chart 2: Distribution of *Acinetobacter baumannii* from Intensive Care Unit among different sexes

11 males and 5 females and *A. baumannii* was isolated from 13 males and 20 females.

MDR and MBL producers were more from general ICU and surgical ICU. Among the 63 drug-resistant *A. baumannii*, 42 (66.6%) were multidrug resistant and 33 (52.3%) were MBL producer.

Clinical sources of the MBL-producing *P. aeruginosa* are shown in Table 7. The subtypes of bla_{VIM} MBL-producing *P. aeruginosa* were 26% and strains of *P. aeruginosa* from ICU were negative for other $bla_{KPC,\ NDM,\ IMP}$ genes. Distribution of all three subtypes of MBL-producing *P. aeruginosa* was as follows: 13.1% bla_{VIM-4}, 9.8% bla_{VIM-5}, and 3.2% bla_{VIM-38} strains [Chart 4].

Table 6: Distribution in Paediatric ICU

Organisms	No of isolates	No of MDR isolates	No of MBL isolates
P.aeruginosa (*n*=61)	9	2	1
A.baumannii (*n*=134)	3	1	0
Total (*n*=195)	12 (6.1%)	3 (1.5%)	1 (0.5%)

Table 7: Clinical source of *P.aeruginosa* with bla_{VIM} gene subtypes

Sample source	bla_{VIM38}	bla_{VIM5}	bla_{VIM4}
Tracheal aspirate	-	1	3
Wound swab	-	2	2
CVP tip	2	1	-
Pleural fluid	-	1	-
Pus	-	1	1
Blood	-	-	2

Table 8: Clinical source of *A.baumannii* with *bla* gene subtypes

Sample source	bla_{OXA}	bla_{VIM}	bla_{IMP}
Tracheal aspirate	2	-	-
Wound swab	-	4	3
CVP tip	4	-	-
Pleural fluid	2	-	-
Pus	8	-	4
Blood	-	2	-

In MBL-positive *A. baumannii*, bla_{VIM} gene was demonstrated in 4.4% strains, bla_{OXA} gene was seen in 11.9%, and bla_{IMP} gene was seen in 5.2%. Thus, the predominant gene coding for MBL activity was found to be OXA. Distribution of genes responsible for MBL activity in *A. baumannii* and its clinical source is shown in Table 8. The resulting sequences were compared with those available in GenBank (www.ncbi.nih.gov/*BLAST*) and the gene accession numbers were KF975367, KF975372.

Discussion

There is an increase in infection caused by the MBL-producing *NFGNB* in the ICUs, along with the significant morbidity and mortality. The incidence of infection in ICUs, especially the nosocomial infections, is a rising trend with a spectrum of clinical conditions. They may be in the range from impaired immunity, lapse in the sterilization, use of various invasive devices, and procedure to indiscriminate use of antibiotics.

A study by Aliskan *et al*.[24] showed that there was a decrease in susceptibility pattern of *A. baumannii* and *P. aeruginosa* isolates from the ICU samples. In our study, maximum *P. aeruginosa* and *A. baumannii* were from tracheal aspirates, followed by wound swab which was in concordance with study of Jaggi *et al*.[25]

In a study by Orrett, 17.3% of *P. aeruginosa* were from ICU.[26] The prevalence of *Acinetobacter species* from various parts of our country was 3%,[27] 4.5%,[28] 9.6% in West Bengal.[29] In our study, the prevalence of *P. aeruginosa* (31.2%) and *A. baumannii* (68.8%) in ICU was higher when compared with above study. Among the

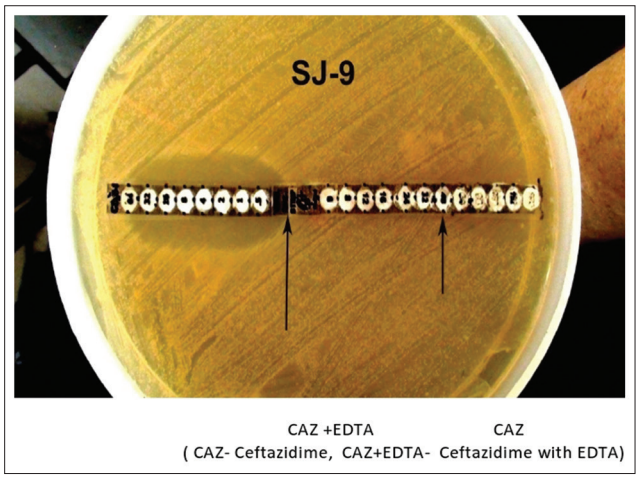

Chart 3: MIC E strip

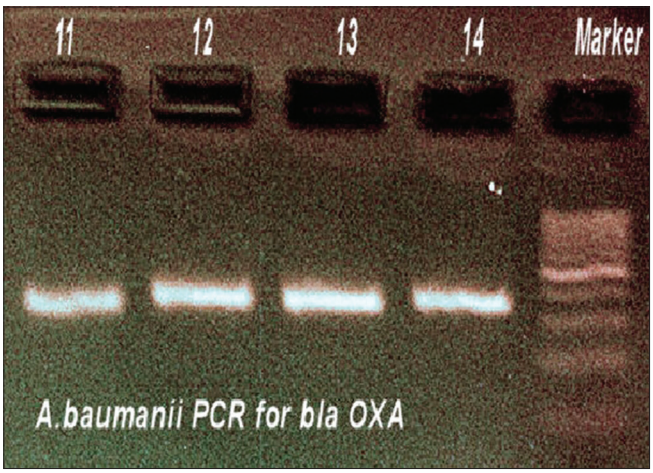

Chart 4: Polymerase chain reaction for *Acinetobacter baumannii* bla_{OXA}

A. baumannii strains isolated from ICU, 65%–70% were resistant and they were not in concordance with our study[3,30,31] which is higher when compared to our study.

The percentage of MDR *A. baumannii* isolates increased from 4% to 55% and 2%–8% in *P. aeruginosa* isolates. According to Yan *et al*., 56.7% and 58.3% of *P. aeruginosa* were found to be imipenem resistant.[3,32]

Many studies have reported <50% of resistance to imipenem and meropenem in *P. aeruginosa*. Imipenem resistance according to Livermore[33] was 77.5% and Lone *et al*.[34] was 25.6%. Tan[35] reported that 9.6% carbapenem-resistant *P. aerugionsa* and 27.2% carbapenem-resistant *P. aerugionsa* were from ICU reported by Hsu *et al*.[36] In our study, carbapenem-resistant *Acinetobacter spp.* isolated from ICU were 25% and lesser than the resistance pattern (69%) reported by Tan.[35]

A study by Hsu *et al*.[36] showed that carbapenem resistance of *Acinetobacter* was 49.6%. Lagatolla *et al*.[37] showed that 70% of carbapenem-resistant *P. aeruginosa* were MBL producers. A study by Kabbaj *et al*.[38] showed that, among 57.4% imipenem-resistant isolates of *Acinetobacter bauamnii*, 74% were MBL producers and in concordance with our study. An Indian study stated that MBL producers among the *A. baumannii* were 70.9%,[39] and another study reported that 21%[40] of *A. baumannii* were MBL producers.

Tanzinah Nasrin showed the high level of MBL producers isolated from ICU unlike our study. Studies from the Indian subcontinent have shown the bla_{IMP1} gene carried by meropenem-resistant isolates.[41] Our study confirmed the presence of *bla* gene (bla_{VIM} 26% and bla_{OXA} 12%) among the isolates of *P. aeruginosa* and *A. baumannii* from the ICU samples and comparable with the study of Gautam *et al*.,[30] 25% prevalence of NDM-1 *A. baumannii* in ICU isolates.

Conclusion

We have to control the development and dissemination of these superbugs among the ICUs. Insight into the incidence of these superbugs alarms the need of every institution to have the interventional strategies to prevent these infections. The prevalence in ICU emphasizes the need for early detection of beta-lactamases-producing organisms.

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Conflicts of interest

There are no conflicts of interest.

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