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PREVALENCE OF BACTERIAL COLONIZATION AT INTENSIVE CARE UNIT ADMISSIONS WITH SPECIAL REFERENCE TO ESBL AND MBL PRODUCING GRAM NEGATIVE BACTERIA

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ABSTRACT

Aims: *The incidence of infections in critically ill patients is much higher than general ward patients despite the immense advancement in therapeutic technologies. Gram Negative Bacteria (GNBs) can survive in a hospital environment because of its resistance to a variety of antimicrobials. Production of enzymes like Extended Spectrum β Lactamases (ESBLs) and Metallo β Lactamases (MBLs) could be the important factors responsible for this resistance. This study was thus undertaken to determine the spectrum of GNBs in patients admitted to an ICU and their antibiotic resistance pattern with reference to ESBL and MBL production.* ***Methods:*** *A total of 100 non-repetitive clinical isolates of Gram Negative Bacteria isolated over a period of one year were processed for their identification and antibiotic susceptibility. They were then screened for ESBLs and MBLs production.* ***Results:*** *Acinetobacter spps (30.69%) and Pseudomonas spps. (30.69%) were the most predominant GNBs isolated in the ICU patients; followed by E.coli (19.80%) and Citrobacter (10.89%). Among the 100 isolates, 45% E.coli, 50% Klebsiella spps & 63.63% Citrobacter spps were ESBL producers while 64.51% Acinetobacter spps &70.96% Pseudomonas spps were MBL producers. The major ESBL producers were E.coli, Klebsiella spps &Citrobacter spps whereas Acinetobacter spps and Pseudomonas spps were the predominant MBL producers.* ***Conclusion:*** *The Multi Drug Resistant GNBs are the most common cause of morbidity and mortality in ICUs. ESBLs and MBLs detection in these bacteria can help in selection of specific antibiotic and it can also help in strategies like antibiotic restriction, combination therapy etc.*

KEY WORDS

ESBL, ICU, MBL, MDRGNBs

INTRODUCTION

Enterobactericeae, initially considered as normal commensals in human intestine, have recently emerged as medically important pathogen. Incidence of *Enterobactericeae* infection is significantly high in patients suffering from urinary tract infection, blood stream infection, surgical sites infection, diarrhoea, abdominal infection, pneumonia, and meningitis. *Enterobactericeae* infections usually develop in previously colonized patients and can spread through the hands of health care workers and the environment. When the patients are admitted to ICU for critical care for a long duration, they may get colonized with resistant strains of Gram Negative Bacteria (GNBs). This can be a threat to the patient who is already in the low immune status.

Enterobactericeae are hardy organisms and can survive for long period on fomites. They can survive in a hospital

environment because of their resistance to a variety of antimicrobials. Gram negative bacteria represent the most common nosocomial isolates, primarily *Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp,* and *Acinetobacter spp*.

The β-lactam antibiotics are the most commonly prescribed antibiotics in ICUs worldwide, which are favoured because of their efficacy, broad spectra and low toxicity. [1] The β-lactamases are the most significant group of enzymes involved in conferring resistance to β-lactam antibiotics in some of the Gram-negative bacteria. Today there are many β-lactam antibiotics and β-lactamase enzymes, including Extended Spectrum β-lactamases (ESBLs) and Metallo β lactamases (MBLs) which are responsible for resistance to β-lactam antibiotics. These organisms pose a greater risk in ICU patients due to their immunocompromised status and prolonged stay in these hospital patients.

The Indian literature lacks evidence about the incidence and pattern of colonisations of multidrug resistant bacteria (especially ESBL and MBL producing bacteria) amongst critically ill patients.

Hence this study was thus undertaken to determine the spectrum of Gram Negative bacteria in patients admitted to an ICU and their antibiotic resistance pattern with reference to ESBL and MBL production.

MATERIALS AND METHODS

After taking approval from the Institutional Ethics Committee, the present study was carried out for a period of one year in department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai. A total of 100 strains of GNBs were collected from Urine, Pus, Sputum, Endotracheal tips, Catheter tips, Blood and other body fluids like Suction tip, ET tube, Foley's tip, Pleural fluid, Tracheaostomy tube etc. All the samples were inoculated onto nutrient agar, blood agar and incubated at 37ºC overnight. The GNBs were identified using standard biochemical tests. The antibiotic sensitivity was performed by Kirby Bauer disc diffusion technique on Muller Hinton Agar. [2] *Acinetobacter spps* (30.69%) and *Pseudomonas spps* (30.69%) were the most predominant GNBs followed by *E.coli* (19.80%) and *Citrobacter* (10.89%). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. [3]

Detection of ESBL- The colonies of Gram negative bacilli were picked up from MacConkey's agar, inoculated in Peptone water and 0.5 McFarland's turbidity standard suspension was prepared. Lawn culture was made on Muller Hinton Agar (MHA) plate with this inoculum. Discs of Cefepime and Amoxicillin+ Clavulanic acid (20 mcg/10 mcg) were placed aseptically at a distance of 20mm on the surface of MHA. An increase of 5mm in zone of inhibition of the combination discs in comparison to the Cefepime disc alone confirmed to be ESBL producer. [3]

Detection of MBL- This was performed by Imipenem EDTA combined disc test. Imipenem disc was placed on a plate inoculated with the test organism, at a distance of 20-25mm and 10µl of 0.5 M EDTA solution was added to one disc. A zone diameter difference of ≥ 7 mm between Imipenem discs & Imipenem plus EDTA discs interpreted as Metallo-β-Lactamase positive. [4]

(Refer photograph-1 showing ESBL and MBL production at the end).

RESULTS AND DISCUSSION

The present study which was carried out for a period of 1 year revealed presence of various pathogens from the patients admitted in the ICU

Table 1 shows the spectrum of all GNBs isolated from ICU patients.

TABLE.1 Spectrum of Gram negative bacteria isolated from various ICU patients.

Total no. of organisms isolated	Gram negative bacteria isolated from various ICU patients			Total GNBs isolated
	MICU(n=60)	PICU(n=8)	SICU(n=33)	n=101
E.coli	13(21.66%)	2(25%)	5(15.15%)	20(19.80%)
Klebsiella species	6(10%)	1(12.5%)	1(3.12)	8(7.92%)
Acinetobacter species	15(25%)	2(25%)	14(42.42%)	31(30.69%)
Citrobacter species	8(13.33%)	-	3(9.09%)	11(10.89%)
Pseudomonas species	18(30%)	3(5%)	10(30.30%)	31(30.69%)

Table 1 shows that amongst all GNBs isolated from ICU patients, *Acinetobacter* spps. (30.69%) and *Pseudomonas* spps. (30.69%) were the most predominant GNBs followed by *E. coli* (19.80%) and *Citrobacter* (10.89%). While in study by Kamalraj *et al*., *Pseudomonas* (83.5%) was the most predominant GNBs followed by *Acinetobacter* spps (15.5%) & *Shewanella* spp. (0.9%). [5] In 2010, Azal Azim *et al*. showed that *P. aeruginosa* (57%) & *Acinetobacter baumanii* (39%) were the most common colonizers followed by Enterobacteriaceae. [6] In a study done by Rajput & Naik *et al*. (2015), it was observed that *E.coli* (33.3%) was the most predominant organism isolated, followed by *Pseudomonas* spp(23.8%), *Acinetobacter* spp (20.6%), *Klebsiella* spp (19%), *Providencia* spp & *Burkholderia cepecia* (1.5%). [7]

It also shows that *Psuedomonas* spp (30%) was the most predominant GNB isolated from MICU followed by *Acinetobacter* spp (25%) & *E.coli* 21.66%). *E.coli* (25%) & *Acinetobacter* (25%) were the most frequently isolated GNBs from the PICU patients. *Acinetobacter* spps. (42.42%) was the most predominant GNB isolated from SICU followed by *Pseudomonas* (30.30%) & *E.coli* (15.15%).

Acinetobacter & *Pseudomonas* strains can lead to serious nosocomial infections in ICU. They can adapt to different environmental conditions and acquire resistance to antibiotics. This contributes to their pathogenicity which can cause septicaemia, pneumonia and UTI in the hospitalized patients. These bacteria can survive in a hospital environment & persist for extended periods of time on surface which makes them a frequent cause for health care associated infections & lead to multiple outbreaks. *Acinetobacter* is the most frequent organism causing nosocomial bloodstream infections and risk factors predisposing to bacteraemia are pneumonia, trauma, surgery, presence of catheters or intravenous lines, dialysis and burns. *Acinetobacter* blood stream infections may be associated with high morbidity and mortality.

TABLE.2 Spectrum of GNBs isolated from various Specimens.

Samples / Orangisms	Blood (11)	Pus (13)	Urine (20)	Sputum (2)	Miscellaneous (Suction tip, ET tube, Foley tip, Bed sore, Pleural fluid, Trachaealstomy tube) (40)
E.coli	1(9.09%)	5(38.46%)	5(25%)	1(50%)	8(20%)
Klebsiella	1(9.09%)	-	-	1(50%)	6(15%)
Acinetobacter spps	7(63.63%)	7(53.84%)	2(10%)	-	15(37.5%)
Citrobacter spps	-	-	5(25%)	-	6(15%)
Pseudomonas spps	2(18.18%)	2(15.38%)	9(45%)	-	18(45%)

Table 2 shows the spectrum of GNBs isolated from various specimens. *Acinetobacter* spps. (63.63%) was the most predominant pathogen isolated from Blood sample of ICU patients having bacteraemia. In this study 53.84% of Pus & Wound swabs grew *Acinetobacter* spps. and (38.46%) samples grew *E.coli.* In study of Pillai *et al*. (2015), *Klebsiella* spps. (44.44%) were the most common pathogen followed by *Acinetobacter baumannii* (22.22%), *P. aeruginosa* (16.66%), *E.coli* (11.11%) &*Proteus mirabilis* (5.55%). [8]

Suction tip, ET tube, Foley tip, Bed sore, Pleural fluid, Tracheostomy tube of the critically ill patients showed that growth of variety of GNBs like *Acinetobacter* spp (37.5%), *Pseudomonas* spp (45%), *E.coli* (20%), *Klebsiella* spp (15%) & *Citrobacter* spp (15%). In a similar study by Shymaa *et al.* (2016), the most common organism isolated from Pleural fluid were *P. aeruginosa* (15.6%) followed by *Klebsiella* spp (3%) & *E.coli* (7.5%)). [9] While in study of Chaudhary AK et al. (2016), the most common organism isolated from ET secretion were *P. aeruginosa* (26.2%) followed by *E.coli* (4.7%), *K. pneumoniae* (5.6%) & *C. diversus* (0.9%) and also it was observed that *C. freundii* (9%) was the most predominant organism isolated from Suction tip followed by *E.coli* (0.9%). [10]

TABLE 3 Antibiotic Resistance Pattern of GNBs.

Organisms / Antibiotics	*E.coli*	*Klebsiella spp.*	*Acinetobacter spps*	*Citrobacter spps*	*Pseudomonas Spps*
Augmentin (AMC)	100%	100%	100%	100%	100%
Tobramycin (TOB)	35%	50%	41.93%	36.36%	25.80%
Cefazolin (CZ)	65%	87.5%	80.64%	100%	83.87%
Gentamicin (GEN)	30%	37.5%	67.74%	63.63%	22.58%
Cefuroxime (CXM)	60%	100%	87.09%	81.81%	83.87%
Amikacin (AK)	20%	50%	77.41%	45.45%	22.58%
Cefotaxime (CTX)	80%	100%	100%	100%	100%
Ciprofloxacin (CIP)	80%	75%	93.54%	100%	90.32%
Cefoperazone (CPZ)	80%	75%	93.54%	90.90%	48.38%
Ofloxacin (OF)	75%	62.5%	74.19%	81.81%	35.48%
Ceftazidime (CAZ)	85%	75%	83.87%	90.90%	51.61%
Tetracycline (TE)	55%	37.5%	64.51%	63.63%	61.29%
Imipenem (IPM)	75%	50%	70.96%	81.81%	80.64%
Meropenem (MRP)	60%	50%	83.87%	81.81%	74.19%
Polymixin B (PB)	50%	50%	19.35%	54.54%	48.38%

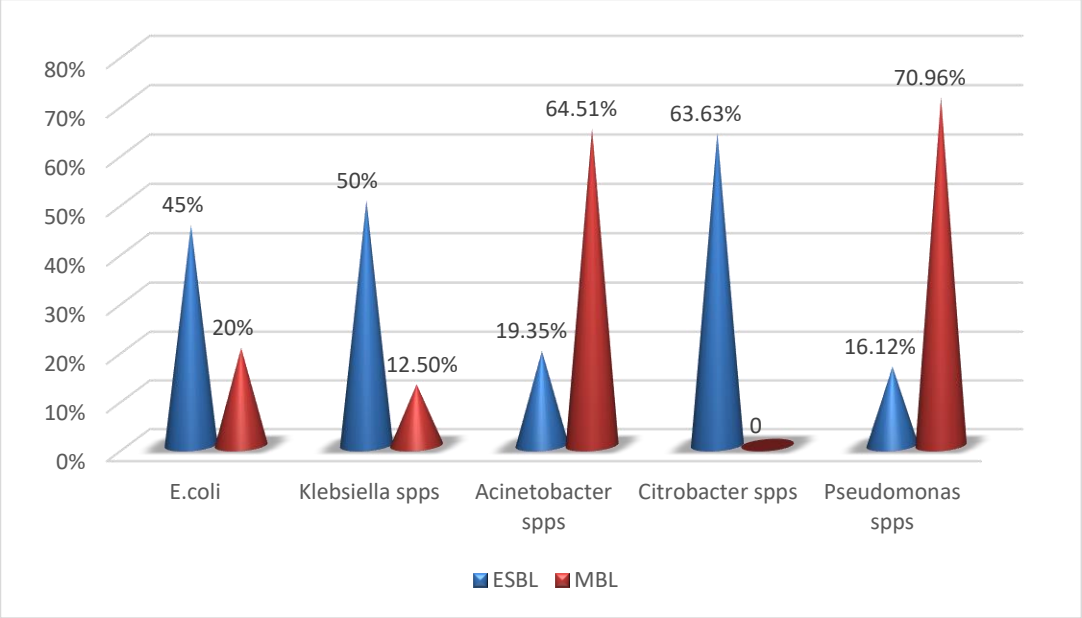
Table 3 shows Antibiotic Resistance pattern of various GNBs isolated. *E.coli* showed resistance to Augmentin (100%), Cefotaxime (80%), Ciprofloxacin (80%), Cefoperazone (80%). Maximum resistance of *Klebsiella* spps was noted against Cefuroxime (100%), Cefotaxime (100%), Cefazolin (87. %). Majority of *Acinetobacter* spps isolates were resistant to Augmentin (100%), Cefotaxime (100%), Ciprofloxacin 5 (93.54%) & Cefoperazone (93.54%). *Citrobacter* spps showed resistance to Augmentin (100%), Cefazolin (100%), Cefotaxime (100%), Ciprofloxacin (100%), Cefoperazone (90.90%) & Ceftazidime (90.90%). *Pseudomonas* spp isolates were found resistant to Augmentin (100%), Cefotaxime (100%) & Ciprofloxacin (90.32%).

Antibiotic Sensitivity test of isolated bacteria showed very high degree of resistance to all the routinely used antibiotics and majority of higher antibiotics. Hence these isolates were Multidrug resistant GNBs (MDR GNBs) in a study carried out by A Ramya *et al*. (2017), *Klebsiella pneumoniae* was more resistant to Ampicillin, Amoxyclav, Cephalosporins and Ciprofloxacin. [11] While in another study carried out by Zaveri Jitendra *et al*. (2012), Cefazolin, Cefuroxime and Imipenem were highly resistant to all isolated Gram-negative organisms. [12] In a study done by Milad Masaeli *et al*. (2012), it was reported that more than 50% of the Enterobacteriaceae isolates were resistant to Ampicillin (79.9%), Tetracycline (78.3%), Co-trimaxozol (64.8%) and Gentamcin (69.6%). [13]

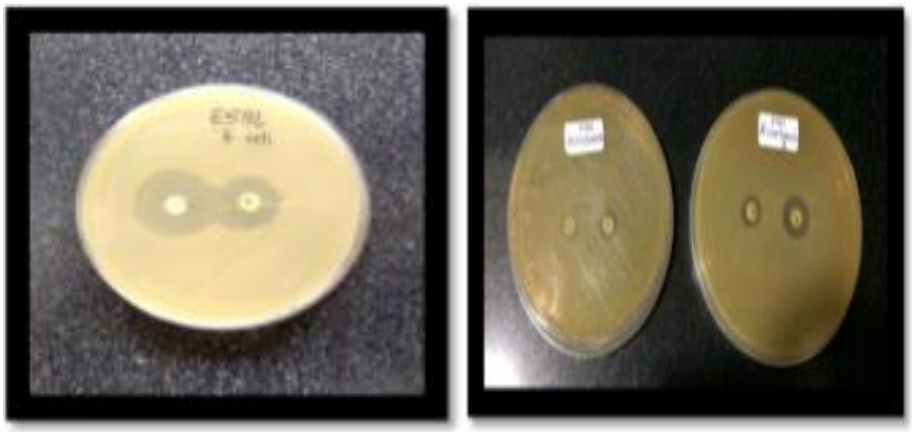
The patients of ICU who have bacterial infections are under lot of selective pressure by the antibiotics. Most of the GNB are multidrug resistant and the physicians are left with a very limited choice of antibiotics. This drug resistance in MDR GNBs could be because of various reasons. The most important reason is production of β lactamases. ESBL and MBL productions have been the major cause of development of resistance to the Cephalosporins & other β lactam antibiotics in these MDR GNBs. Hence all the MDR GNBs were subject to ESBL and MBL test.

Figure 1 shows ESBL and MBL production in the isolated GNBs.

Fig 1. Bar Diagram showing ESBL & MBL production in GNBs



Photograph-1 ESBL & MBL production.



It was observed that 45% of *E.coli* isolates were ESBL producers & 20% of the *E.coli* isolates were MBL producers. Amongst *Klebsiella* spps 50% of isolates were ESBL producers and 12.5% were MBL producers. 20% of *Acinetobacter* spps isolates were MBL producers and 19.35% were ESBL producers. Out of *Citrobacter* spps isolates 7(63.63%) were ESBL producers. However, MBL production was not shown by *Citrobacter* isolates. Amongst 31 *Pseudomonas* spps, 22(70.96%) isolates were MBL producers and 16.12% were ESBL producers. This indicates that ESBL production was the most commonly observed phenomenon in *E.coli, Klebsiella* & *Citrobacter*, whereas MBL production was more common in *Pseudomonas* and *Acinetobacter*.

In 2013 Loveena Oberoi *et al*. reported that the major ESBL producers were *E.coli* (56.25%) followed by *P. aeruginosa* (18.75%) & *K. pneumoniae* (15.62%), while the MBL production were mainly observed in *K. pneumoniae* (33.4%) & *P. aeruginosa* (26.67%). [1] Chaudhary AK et al. (2016), reported that MBL production was observed in *P. aeruginosa* (20%) among 45 isolates. [10] Zainab A *et al*. (2014) in their study, reported that the most frequent MBL producers were *P. aeruginosa* (43.5%), *A. baumanii* (43.5%) and *K. pneumoniae* (13%). [14] In another study carried out by Gupta *et al*. (2016), it was observed that ESBL producers were *Pseudomonas* spp (14.3%) and *Acinetobacter* spp (10%), while MBL producers were *Pseudomonas* spp (11.4%) & *Acinetobacter* spps (10%). [15]

The pattern of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another, as well as from one hospital to other and even among ICUs within one hospital. Resistance to antibiotics poses a serious and growing problem

because these infectious diseases are becoming more difficult to treat. Resistant bacteria do not respond to the antibiotics and continue to cause infection. Some of these resistant bacteria can be treated with more powerful antibiotics but some infections are difficult to cure even with new or experimental drugs.

CONCLUSION:

Current study highlights the high prevalence of MBL and ESBL production among the GNBs isolated from various ICUs patients. Critical action needs to be taken from the standpoint of both therapy and infection control. Clinical Microbiology Laboratories should do monitoring for detection of MBL & ESBL enzymes routinely so that the proper antimicrobial therapy can be instituted. The dissemination of the isolates may be prevented by employing suitable control measures. The above data suggest the utility of ESBL & MBL tests in GNBs which can be an important step for successful antimicrobial treatment in the future.

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