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Emergence of multi-drug resistant Non-fermenting gram-negative bacteria in causing Hospital-acquired infections in ICU setting of a tertiary care hospital.

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Abstract
Aims:*This study evaluated the emergence of multidrug resistant non-fermenting bacteria causing nosocomial infections in intensive care units.*
Methods and Results:*A prospective study was carried out in patients who were admitted to ICU and underwent ventilation, catheterization, had a central-line insertion, or developed wound infection and then started showing features of invasive sepsis 48 hours after their admission, were included. Endotracheal aspirate/Broncho-alveolar lavage, urine, central-line catheters and pus samples were collected for culture and antibiotic sensitivity. 126 out of 224 samples showed growth on culture media. VAP accounted for 42% of all nosocomial infections. Acinetobacter baumannii from respiratory and blood samples were susceptible only to Polymyxin B and doxycycline to some extent. Pseudomonas aeruginosa from respiratory and urine samples was sensitive to Polymyxin B.*
Conclusion*:Emerging multi-drug resistance in Acinetobacter baumannii is becoming a difficult problem to tackle with.*
Significance and Impact of Study:*With the advent of new drugs, a concurrent rise in multi-drug resistant organisms has been seen with high virulent properties that is severely affecting the mortality rates. Non-fermenters, especially Acinetobacter baumanniiand Pseudomonas aeruginosa are the two most frequently isolated organisms among the bacterial pathogens which have developed resistance to most of the anti-bacterial agents.*
Keywords: *Acinetobacter baumannii; multi-drug resistant; nosocomial infections; Polymyxin B; Pseudomonas aeruginosa*

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I. Introduction

Hospital Acquired Infections (HAI) or nosocomial infections are defined as those infections that are not present or incubating at the time of admission and manifest 48 hours after admission to the hospital.[1] HAIs are the leading cause of morbidity and mortality among hospitalized patients.

Healthcare associated infections in Intensive Care Units (ICU) in India range from 9.6% to 17.7% [2,3] The risk of HAI in ICU is 5 to 10 times greater than those acquired in general medical and surgical wards.A probable explanation for this may be the frequent requirement of invasive medical devices such as urinary catheters, central venous and arterial catheters and endotracheal tubes by critically ill patients, thus compromising normal skin and mucosal barriers.[4]

Among the nosocomial infections, ventilator-associated pneumonia (VAP), catheter-associated urinary tract infection (CAUTI) and central line-associated bloodstream infections (CRBSI) are seen in approximately 30% of ICU patients. The pathogens responsible for a substantial proportion of HAIs include vancomycin-resistant *enterococci* (VRE),methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* that produce extended-spectrum beta-lactamases, carbapenem-resistant *Acinetobacter baumannii and Pseudomonas aeruginosa*and *Enterobacter spp.* Other pathogens, such as, *Escherichia coli, Candida albicans, Clostridium difficile,* etc also play vital role in the occurrence of nosocomial infections. [5,6,7]

Antibiotic resistance among bacterial pathogens isolated in ICU infections has become a major problem worldwide. Treatment becomes more challenging in case of gram-negative organisms causing life-threatening infections in ICU, such as pneumonia, bloodstream infections, surgical site infections and meningitis; mainly due to the increasing multidrug resistance rates and limited novel antimicrobial agents. [8]

It is evident that these nosocomial infections in ICUs play a vital role in the increased duration of hospital stay, cost of treatment, higher morbidity and mortality rates. Hence, this study was done to determine the prevalence and nature of nosocomial infections in the ICU of a government tertiary care hospital in West Bengal, India, evaluate the bacteriological profile of the isolates and analyse the drug sensitivity pattern of the isolated pathogen.

II. Methods

Study Design and Setting:

A prospective analytical study was carried out in the Department of Microbiology in collaboration with the Department of Anaesthesiology and Critical Care Medicine & Department of Surgery in a tertiary care government medical college in West Bengal, India.

Study Population:

The study only included patients who were intubated and on mechanical ventilation, patients with urinary catheters, with central venous catheters or with wound infections and started developing clinical features of invasive sepsis (systemic inflammatory response syndrome, SIRS) 48 hours after admission in the ICU. Their samples were collected for bacteriological culture and antibiotic susceptibility.Patients admitted to the ICU with sepsis were not included unless they developed new signs of SIRS more than 48 hours after admission. Patients having infections prior to admission in ICU and those admitted in ICU without infections were also excluded from the study.

Sample processing:

Blood cultures were processed using the BACT/ALERT 3D system (BioMerieux, Marcy L'Etoile, France) and incubated at 36°C for 7 days. When the culture bottle flagged positive, gram stain and subculture was performed. The respiratory samples (endotracheal aspirate, bronchoalveolar lavage), pus, urine and central venous catheters were plated on blood agar and MacConkey agar. All plates were incubated overnight at 37˚C.A colony count of $>10^5$ Colony forming units(CFU)/ mL was considered significant for endotracheal aspirate and urine samples. For BAL fluid, a colony count of 10^4 bacterial colonies per ml was considered significant.[9,10]For central venous catheter, significant growth was defined as ≥15 colonies by roll plate Method and ≥100 CFU/ml by flush method.[11] Antimicrobial susceptibility of the isolates were tested on Mueller-Hinton agar by modified Kirby Bauer disk diffusion method as per the recommendations of Clinical and Laboratory Standards Institute (CLSI)-2020. Vancomycin E-test was used to determine its susceptibility in MRSA isolates.

III. Results

In this study, a total of 224 samples from patients admitted in ICU were processed in the laboratory, out of which only, 126 (56.25%) of the samples were culture positive. 86% of respiratory samples, 38.01% of urine samples,100% of pus samples and 40% of blood samples showed culture positivity. [Table 1]

Type of sample	**Culture positive**	**Culture positive**	**Culture negative**	**Culture negative**
	No.	**%**	**No.**	**%**
Respiratory samples(n=64)	55	86	9	14
Urine (n=54)	21	38.01	33	61.99
Pus (n=12)	12	100	0	0
Blood (n=94)	38	40	56	60

TABLE 1: Correlation between samples and culture positivity.

Pneumonia accounted for 42% of all the nosocomial infections, followed by blood stream infections (33.5%) and urinary tract infections. (17.7%). *Acinetobacter baumannii* was found to be the most common bacteria causing blood stream infections (43%) and pneumonia (58%). Urinary Tract Infection and wound infections among ICU patients were mostly caused by *Escherichia coli* (54.5% and 48% respectively). [Table 2]

	Pneumonia N=53	**Urinary Tract Infection N=22**	**Wound infection N=9**	**Blood Stream Infection N=42**
Acinetobacter baumanii	58% N=31	-	-	43% N=18
Escherichia coli	5% N=3	54.5% N=12	48% N=4	7% N=3
Klebsiella pneumoniae	11% N=6	18.3% N=4	18.7% N=2	23% N=10
Staphylococcus aureus	3.8% N=2	-	-	10 N=4
Enterococcus spp.	3.8% N=2	-	-	7% N=3
Staphylococcus epidermidis	-	-	-	10% N=4
Pseudomonas aeruginosa	14% N=7	13.6% N=3	-	-
Proteus mirabilis	-	13.6% N=3	-	-
Enterobacter spp.	3.8% N=2	-	33.3% N=3	-

Table 2: List of organisms isolated causing nosocomial infections in ICU.

Acinetobacter baumannii isolates from both blood and respiratory samples showed a good sensitivity to Polymyxin B (88.9% and 80.6% respectively) and was moderately sensitive to Doxycycline (50% and 41.9% respectively). Meropenem had no sensitivity against the isolates from blood, and around 10% susceptibility toisolates from respiratory samples.*Enterobacteriaceae* isolates from blood, respiratory samples and pus showed high level resistance to Meropenem, whereas 75% of the isolates from urine were sensitive to Meropenem. Sensitivity was practically absent to Ciprofloxacin.*Pseudomonas aeruginosa* isolated from respiratory samples and urine showed sensitivity mostly to Polymyxin B (71.4% and 66.7% respectively) and Aztreonam (42.8% and 66.7% respectively), while being resistant to most other antibiotics. [Table 3]

HAI	Causative organism	PIT	CIP	MRP	PB	3rd GEN CEP	DOX	COT	AZM	GEN	NIT
Pneumonia	*Acinetobacter baumanii*	0%	0%	10%	80.6 %	10%	41.9 %	12.7 %	25.8 %	25.8 %	-
	Escherichia coli	33.3 %	33.3 %	33.3 %	100%	33.3 %	33.3 %	33.3 %	33.3 %	33.3 %	-
	Klebsiella pneumoniae	16.7 %	16.7 %	16.7 %	83.3 %	16.7 %	33.3 %	16.7 %	33.3 %	33.3 %	-
	Pseudomonas aeruginosa	0%	0%	28.5 %	71.4 %	0%	0%	-	42.8 %	28.5 %	-
	Enterobacter spp.	50%	50%	50%	100%	50%	50%	50%	50%	50%	-
Urinary Tract Infection	*Escherichia coli*	33.3 %	0%	75%	75%	33.3 %	-	-	50%	75%	33.3 %
	Klebsiella pneumoniae	25%	0%	75%	75%	25%	-	-	50%	75%	25%
	Pseudomonas aeruginosa	33.3 %	0%	33.3 %	66.7 %	33.3 %	-	-	66.7 %	66.7 %	66.7 %
	Proteus mirabilis	33.3 %	0%	66.7 %	0%	33.3 %	-	-	66.7 %	66.7 %	33.3 %
Wound infection	*Escherichia coli*	25%	0%	25%	75%	0%	25%	25%	25%	25%	-
	Klebsiella pneumoniae	0%	0%	50%	100%	0%	50%	0%	0%	0%	-
	Enterobacter spp.	33.3 %	0%	33.3 %	66.7 %	0%	33.3 %	33.3 %	33.3 %	33.3 %	-

Blood Stream Infection	*Acinetobacter baumanii*	0%	0%	0%	88.9 %	11.1 %	50%	34%	27.7 %	22.3 %	-
	Escherichia coli	33.3 %	33.3 %	33.3 %	100%	33.3 %	66.7 %	33.3 %	66.7 %	33.3 %	-
	Klebsiella pneumoniae	40%	10%	10%	100%	40%	30%	10%	20%	40%	-

Table 4: Antibiotic sensitivity rates of different gram-negative isolates.

[PIT: Piperacillin-Tazobactam, CIP: Ciprofloxacin, MRP: Meropenem, PB: Polymyxin B, CEP: Cephalosporin; Ceftazidime was used for *Acinetobacter baumannii*and *Pseudomonas aeruginosa*; Ceftriaxone was used for Enterobacteriaceae. DOX: Doxycycline, COT: Cotrimoxazole, AZM: Azithromycin NIT: Nitrofurantoin]

All gram-positive isolates from respiratory samples were 100% sensitive to Vancomycin and Linezolid, however, two Linezolid resistant *Staphylococcus epidermidis* (LRSE) isolates were found in blood samples. All *Staphylococcus aureus* isolates were Cefoxitin resistant strains (Methicillin resistant *Staphylococcus aureus*). [Table 4]

		VA	LZ	CX	CXM	CD	RO	GEN	CIP
Pneumonia	*Staphylococcus aureus*	100%	100%	0%	0%	0%	0%	100%	50%
	Enterococcus spp.	100%	100%	-	-	0%	0%	100%	0%
Blood Stream Infection	*Staphylococcus aureus*	100%	100%	0%	0%	50%	50%	25%	0%
	Enterococcus spp.	100%	100%	-	-	-	66.7%	66.7%	0%
	Staphylococcus epidermidis	100%	50%	-	25%	50%	50%	25%	0%

Table 5: Antibiotic sensitivity rates of different gram-positive isolates.

[VA: Vancomycin, LZ: Linezolid, CX: Cefoxitin, CXM: Cefuroxime, CD: Clindamycin, RO: Roxithromycin, GEN: Gentamicin, CIP: Ciprofloxacin.]

IV. Discussion

Nosocomial infections in intensive care units with increasing incidence of multidrug resistance organisms is a matter of serious concern today. A proper understanding of the source of infection, the pathogens

involved and administration of drugs which have proven to be susceptible to these resistant organisms can significantly help to decrease the morbidity and mortality associated with these hospital-acquired infections.This study was focussed on understanding theemerging drug resistant pathogens causing different types of HAIs in ICU patients. Out of all the infections reported,ventilator associated pneumonia had the highest incidence rate of 42% followed by central-line blood stream infections (33.5%) and urinary tract infections (17.7%).European one-day prevalence study (EPIC)[12] conducted in 1992 showed a similar distribution of ICU-acquired infections with 46.9% cases of pneumonia, 17.6% urinary tract infections and 12% bloodstream infections. The study by Richards M J et al.[13] reported a 31% occurrence of nosocomial pneumonia, 23% urinary tract infections and 14% primary bloodstream infections out of all nosocomial infections in a surgical ICU.

Acinetobacter baumannii was found to be the commonest organism causing blood stream infections (43%) and pneumonia (58%) in this study. In the study by Chopra T etal.[14]*Acinetobacter baumannii* was observed as themain organism causing bloodstream infections in health care settings.Hartzell JD etal.[15]studied on the increasing incidence of multidrug resistant strains of *Acinetobacter baumannii* causing nosocomial pneumonia.However, in another study by Kombadeet al.[16]from India in 2010, *Pseudomonas aeruginosa* (23.8%) was the commonest isolate obtained in respiratory samples, followed by *Acinetobacter baumannii*(21.2%) and *Klebsiellapneumoniae* (15.2%).

In the present study, Urinary Tract Infection was mostly caused by *Escherichia coli* (54.5%), followed by *Klebsiella pneumoniae* (18.3%), *Pseudomonas aeruginosa* and *Proteus mirabilis* both accounting 13.6%. Vyawahare CR et al.[21] in his study on the occurrence of catheter-associated urinary tract infection in critical care units found *E. coli* 39 (57%) as the most common bacterial pathogen, followed by *Klebsiella spp.* 14 (20%), and *Pseudomonas spp.* 5 (7%). Infact, Karina et al. [22] found a similar pattern in his study with *E. coli*, being the most common urinary pathogen (27%).

All gram-positive isolates from respiratory samples were sensitive to Vancomycin and Linezolid, however, two Linezolid resistant *Staphylococcus epidermidis* (LRSE) isolates were found in blood samples. A similar finding was observed in a study by Stephanie A Folan et al.[17] where 40% of *Staphylococcus epidermidis* isolates causing bloodstream infections were Linezolid resistant.

Acinetobacter baumannii from both blood and respiratory samples showed 88.9% and 80.6% sensitivity to Polymyxin B respectively and was moderately sensitive to Doxycycline. Ciprofloxacin was found to be the least susceptible drug in this study. The study by Ngamprasertchai T et al.[18] also observed similar results in pneumonia patientswhere 51.5% multidrug resistant *Acinetobacter spp.* isolates were susceptible to colistin.In the 2009 MYSTIC report,[19] 67% of *Acinetobacter* isolates were resistant to ciprofloxacin.

Pseudomonas aeruginosa isolated from respiratory samples and urine showed 71.4% and 66.7% susceptibility to Polymyxin B respectively and also moderately to Aztreonam (42.8% and 66.7% respectively). *Pseudomonas aeruginosa* causing UTI showed 96% susceptibility to colistin and only 54% to aztreonam in the study done by Lamas Ferreiro JL et al.[20]

V. Conclusion

Increasing incidence of *Acinetobacter baumannii* causing nosocomial pneumonia and blood stream infections and resistant to almost all antibiotics but Polymyxin B is a matter of grave importance.Carbapenems like meropenem and fluoroquinolones which used to have very good activity against gram negative pathogens, are almost of no use nowadays. Doxycycline still has a moderate susceptibility to these multidrug resistant pathogens. Linezolid still remains to be a very active drug against gram positive infections, though LRSE strains among central-line associated blood stream infections are frequently documented these days.

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Both listed authors have agreed to be the authors for this research paper and have contributed equally to the work's conception, design and performed the acquisition of data for the work.
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