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Study of Microorganisms Isolated From Clinical Samples of ICU Patients.

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Abstract

Introduction: Health care Associated Infection (HAI) in ICUs is a global problem. In ICU patients, the invasive medical devices act as a portal of entry for microorganisms and a site for biofilm formation.

Aim & Objectives: Hence the present study was conducted to detect different microorganisms isolated from different clinical samples of ICU patients. The antimicrobial susceptibility profile and biofilm production by these microorganisms were also studied.

Material & methods: 100 microorganisms isolated from different clinical samples of ICU patients were studied. The clinical samples were cultured on blood agar, MacConkey's agar and Sabouraud's Dextrose agar. The organisms grown were characterized by conventional method. Antimicrobial susceptibility test was done by Kirby Bauer disc diffusion method. Different types of β-lactamases e.g. Extended Spectrum β-lactamases, AmpC β-lactamases and Metallo β-lactamases produced by Gram negative bacilli were detected. Methicillin resistance, inducible Clindamyin resistance for Staphylococci and High Level Amioglycoside Resistance (HLAR) for Enterococcus sp. were detected according to CLSI Guidelines. Biofilm production was detected by tube method.

Results: 30% specimens were received from Medicine ICU. 60% strains were Gram negative bacilli, 17% strains were Grampositive cocci and 23% strains were Candda species. 90% Gram negative bacilli were β-lactamase producers. 55% strains produced strong biofilm.

Conclusion: To treat ICU patients, antimicrobial susceptibility test must be done. β-lactamases and biofilm production should be detected in microorganisms isolated from clinical samples of ICU patients.

Keywords: Health care Associated Infections, Gram negative bacilli, Gram positive cocci, Candida sp, β-lactamases, Biofilm production.

Introduction

Health care Associated Infections (HAIs) is a global problem. Intensive care units (ICUs) are unique patient care areas in any Health care set up, where severely ill patients are kept together, in an environment of drug resistant microorganisms, several equipments, multiple invasive devices and only few trained health care workers especially in developing countries.[1] The incidence of HAI in ICUs is significantly higher in developing countries compared to developed countries varying between 4.4% to 88.9%.[2] It has been reported that in a single health care set up, the incidence of HAI in ICU is about 2-5 times higher than in General In Patients Departments (IPD). [3] Though ICU has 5% of total

hospital beds, 25% of Health care associated infections (HAIs) occur in ICU [4] which are responsible for increased mortality, morbidity, length of hospital stay and economic loss. The reason being critically ill patients, severely impaired host defence, Medical and Surgical interventions and use of medical devices like endotracheal intubation, central venous catheterization, urinary catheterization & orthopedic implants etc. Presently the ICUs are often called as 'the hubs' of infections. The invasive medical devices act as a portal of entry and nidus for microbes as most commonly they form biofilm on medical devices. It has been also observed that 20-30% of all ICU admissions have HAI. [5, 6] The European Prevalence of Infection in Intensive Care (EPIC)-II study reported that medical devices were the common risk factor but the length of ICU stay was the strongest predictors of HAIs.[6] In EPIC-II study, laboratory provren Blood stream Infection (BSI), pneumonia and clinical sepsis were independently associated with increased mortality ranging from 11% for Surgical Site Infections (SSIs) to 25% for BSIs. [7] Another important point to be noted that in ICUs antibiotics are used more frequently and in some cases for longer period than in any other hospital area. Hence, the ICU patients are usually infected with Multi Drug Resistant Organisms (MDROs). The emergence of MDROs is mainly due to excessive use of antimicrobial agents and 60% of all ICU patients receive antibiotics during their stay.[8]

The most common infections in the ICUs are pneumonia especially Ventillator associated pneumonia (VAP), Catheter associated Urinary tract infections (CAUTI), Catheter related Blood stream infections (CRBSI) and Surgical site infections (SSIs) etc. The ICUs are breeding ground for many drug resistant microorganisms like Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin resistant Staphylococcus aureus (VRSA), Vancomycin resistant Enterococci (VRE), ß-lactamase producing Gram negative bacteria e.g. Pseudomonas aeruginosa, Klebsiella pneumoniae, Burkholderia sp., Stenotrophomonas maltophilia and Fluconazole resistant Candida species etc. [9] The emergence of ESBL, AmpC, MBL, KPC producing Gram negative bacilli are really a big challenge for treating the patients. Even Colistin resistant Gram negative bacilli have been reported.[10] Strict implementation of Infection Control Programme. Bundle care approach and antimicrobial stewardship can significantly reduce the incidence of HAIs in ICU. [11]

Hence, the present study has been undertaken to detect the incidence of different microorganisms isolated from clinical samples of ICU patients and to study the antimicrobial susceptibility profile and biofilm production by these microorganisms.

Material And Methods

The present study was conducted in the department of Microbiology

Ethical Consideration: Approval from Institutional Ethics Committee was taken.

Study period : 6 months.

Type of study: Cross- sectional experimental study.

Sample size: The sample size was calculated as per the following formula [12]--

Sample size (ss) $=Z^2 \times (p) \times((1-p)/c^2$

Where Z=Z value (1.96 for 95% confidence level)

p= prevalence %, expressed in decimal: 0.1 was used

c= margin of error, 0.06 was used

The minimum calculated sample size was 96 (approx). A total number of 100 aerobic microorganisms isolated from clinical samples of ICU patients in the department of Microbiology were studied.

Selection criteria

Inclusion criteria: 100 aerobic microorganisms isolated from different clinical samples of ICU patients in the

department of Microbiology were studied. All the samples collected after 48 hours of ICU stay of the patient were included in the study

Exclusion criteria: Anaerobic bacteria isolated from different clinical samples were not included in the study.

The different clinical samples of ICU patients, like blood, urine, pus, endotracheal secretion, CSF and body fluids etc., received in the Department of Microbiology were cultured on blood agar, Mac Conkey's agar and Sabouraud's Dextrose agar (SDA). The organisms grown on culture media were characterized by conventional tests.[13] Any fungus grown on SDA were identified by microscopy and conventional tests for Candida species, like growth on CHROM agar, germ tube test and Chlamydospore formation etc. [14] On C HROM agar C.albicans form light green, C.tropicalis form blue, C..krusei form purple to fuzzy and C. glabrata form cream coloured colonies.

Antibiotic susceptibility test: All bacterial strains were subjected to antibiotic susceptibility test by Kirby-Bauer disc diffusion method [16] according to Clinical and Laboratory Standard Institute (CLSI) Guidelines.[17] Using sterile swab lawn culture of test strain (turbidity adjusted to 0.5 Mc Farland) was done on Mueller Hinton agar (MHA) plate. With all aseptic precaution the antibiotic discs were put on MHA plate. Six antibiotic discs were put on 90 mm diameter MHA plate. The antibiotic discs for Gram negative rods (GNR) and Gram positive cocci were put as per CLSI Guidelines. For Candida strains antifungal discs of Fluconazole and Voriconazole were used as per CLSI guidelines. [18]

Detection of ß-lactamase producing GNRs [17] : Extended spectrum ß-lactamases (ESBLs) producing strains were detected by Combine disc method using Ceftazidime (CAZ-30µg) and Ceftazidime plus Clavulanic acid (CAC) discs If the zone of inhibition with CAC was ≥ 5mm in diameter compared to CAZ disc alone the strain was reported as ESBL positive. AmpC ß-lactamases producing strains were detected by Cefoxitin (CX-30µg) and Cefoxitin/Cloxacillin discs,. An increase in zone size of ≥ 5mm around Cefoxitin plus Cloxacillin discs compared to Cefoxitin disc alone was considered positive for AmpC ß-lactamases production. MBL producing strains were detected by Imipenem (IPM-10 µg) and Imipenem plus EDTA discs. The test was considered positive if after incubation, the increase in inhibition zone with Imipenem plus EDTA disc was ≥ 7mm than the Imipenem disc alone.[19].

In Staphylococcal strains detection of Methicillin resistance was done by Cefoxitin (CX) discs and Inducible Clindamycin Resistance was detected by using Erythromycin and Clindamycin discs keeping them 15mm apart as per CLSI guidelines. [17] Methicillin Resistant Staphylooccus strains were detected by Cefoxitin (CX-30µg) disc [17] and if the zone of inhibition was ≤ 21 mm the strain was reported as Methicillin resistant. Cefoxitin is surrogate marker of mecA mediated methicillin resistance. High level Aminoglycoside Resistance (HLAR) in Enterococcus sp. were detected by High level Streptomycin and High level Gentamicin discs according to CLSI guidelines.[17]

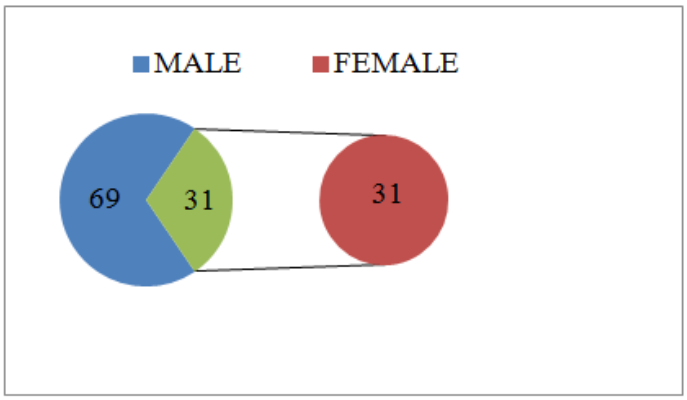
Detection of Biofilm formation: Biofilm production was detected by Tube method. [20] 10 ml Trypticase Soy Broth (TSB) with 1% glucose was inoculated with a loopful of test strain and was incubated at 37^0 C for 24 hours. The growth along with the broth was decanted. The tubes were washed with Phosphate buffer saline (pH 7.3). The excess stain was washed with deionized water and the tubes were dried. The test result was interpreted as strong, moderate, weak and absent for biofilm production.

All the culture media, antibiotic discs and antifungal discs were procured from Hi Media Pvt Ltd, Mumbai.

Statistical Analysis: It was done by chi-square (χ^2) test, standard error of difference etc.

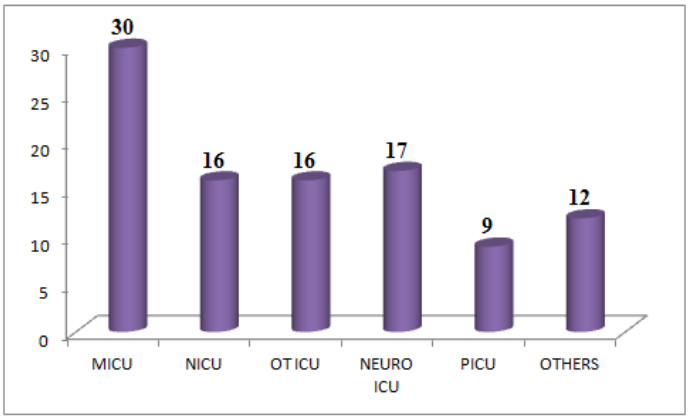
Results

Figure 1: Incidence of male and female patients from different ICUs.



The figure1 shows that in the present study, microorganisms were isolated from 69 (69%) male patients and 31 female patients of different ICUs. . 33% microorganisms were isolated from 31-60 years age group followed by 25% microorganisms from >60 yeas age group.

Figure 2: Incidence of isolation of microorganisms from different ICUs



Others included CVTS ICU-6, orthopedics ICU-3 and 1 each from Renal ICU, Surgery ICU, Trauma ICU respectively.

In our study, microorganisms isolated from 30% specimens were received from Medicine ICU (MICU) followed by 17% specimens from Neuro ICU. 9% specimens were received from Pediatrics ICU (PICU).When the incidence of isolates from different ICUs were statistically analyzed by χ^2 test it was found that number of isolates from MICU were more and statistically significant.

33% strains were isolated from urine followed by 26% from endotracheal tube aspirate and 21% from blood sample. 4% strains were isolated from drain tube from operation site. Total 11% strains were isolated from Surgical Site Infection. 66% strains were isolated from patients with medical devices. 27% strains were isolated from catheter associated urinary tract infection (CAUTI), 26% strains were isolsated from patients with ventilator associated pneumonia (VAP) and 13% strains were isolated from central line related blood stream infection (CLRBSI).

Figure 3: Incidence of microorganisms isolated from ICU patients (n=100)

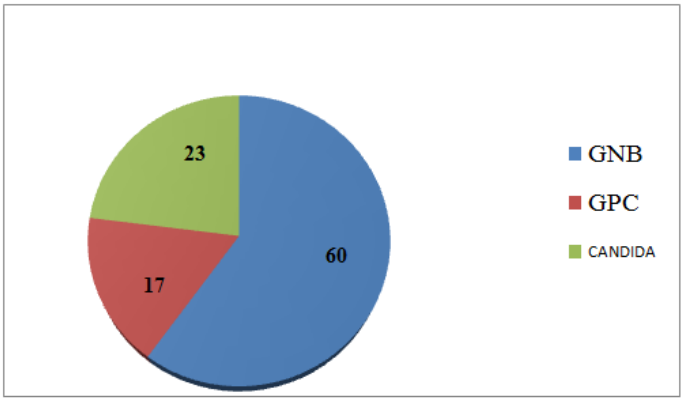


Figure 3 shows the incidence of different types of microorganisms isolated from ICU paients. 60%microorganisms were Gram negative Bacilli (GNB). Out of this 60 Gram negative bacilli 54(90%) were β-lactamase producers. 2 strains produced only ESBL, 3 strains produced only AmpC β-lactamase and 20 strains produced only MBL. 29 strains produce β-lactamases in combination.8 strains produced ESBL and AmpC β-lactamases, 7 strains produced ESBL and MBL, 4 strains

produced AmpC β-lactamases and MBL and 10 strains produced all 3 types of β-lactamases i.e. ESBL plus AmpC β-lactamases plus MBL.

T

able1: Isolation of different microorganisms from specimens of ICU patients (n= 100)

Microorganism	Number
Pseudomonas aeruginosa	20
Klebsiella pneumoniae	16
Acinetobacter baumanii complex	12
E.coli	10
Enterococcus fecalis	5
Coagulase positive Staphylococci	10
Candida sp.	23
Others*	4

Others* include: Citrobacter fruendi -1, Enterbacter clocae – 1, Coagulase negative Staphylococcus (CONS)-2

Table 1 shows isolation of different microorgaisms from clinical specimens received from ICU patients. 23% strains isolated were Candida species followed by 20% strains were Pseudomonas aeruginosa and 16% strains were Klebsiella pneumoniae. Out of 10 Coagulase positive Staphylococci 8 were MRSA and 3 were inducible clindamycin resistant.

Table 2: Antibiotic susceptibility profile of Gram positive cocci isolated (n=17)

Gram +vecocci	Susceptibility to Antibiotics (No. Of strains)						
	Penicillin	Erythromycin	Cloxacillin	Vancomycin	Linezolid	HLS	HLG
Coagulase +ve Staph (10)	2	1	1	8	10	-	-
CONS (2)	0	1	0	2	2	-	-
E. faecalis (5)	0	1	0	3	5	1	1

Table 2 shows the antibiotic susceptibility profile of Gram positive cocci isolated. 2 (20%) Coagulase positive Staphylococci and 2(40%) E.faecalis were Vancomycin resistant. All 17 (100%) Gram positive cocci were sensitive to linezolid. 4 (80%) E. faecalis strains were High Level Aminoglycoside Resistant (HLAR). Among 10 Coagulase positive Staphylococci, 7 (70%) strains were Methicillin Resistnt Staphylococci (MRSA). OUT of 2 CONS isolated, 1 was (Methicillin Resistant Coagulase negative Staphylococci (MRCONS). Out of 17 Gram positive cocci strains isolated 8 (47.1%) strains were inducible clindamycin resistant.

Table 3: Antibiotic susceptibility profile of Gram negative bacilli isolated (n=60)

Gram negative Bacilli (60)	Susceptibility to Antibiotics (No. of strains)					
	Amikacin	Ciprofloxacin	Ceftazidime	Imipenem	Meropenem	Colistin
Pseudoimonas aeruginosa (20)	9	9	7	4	4	18
Klebsiella pneumoniae (16)	8	7	5	7	8	15
Acinetobacter baumanii complex (12)	2	2	3	2	2	10
E.coli (10)	4	1	6	6	5	10
Others*(2)	1	1	0	1	1	2

Others* include: Citrobacter fruendi -1, Enterbacter clocae – 1

Table 3 shows antibiotic susceptibility profile of 60 Gram negative bacilli isolated from clinical samples of ICU patients. 55(91.7%) strains were sensitive to colistin followed by 24 (40%) strains which were sensitive to amikacin.

Table 4 : Incidence of different Candida species isolated and their antifungal drug susceptibiity profile (n=23).

Candida species (n=23)	Number	Sensitive strains no.	
		Fluconazole	Voriconazole
Candida albicans	8	5	7
Candida tropicalis	11	6	8
Candida krusei*	4	-	4

Candida krusei* are intrinsically resistant to fluconazole. Hence, fluconazole sensitivity was not tested for Candida krusei.

Table 4 shows the incidence of Candida species isolated from different specimens received from ICU patients. Out of total 23 Candida species isolated 8 (34.8%) were Candida albicans, 11 (47.8%) were Candida tropicalis and 4 (17.4%) were Candida krusei. Hence, 15 (65.2%) Candida nonalbicans species were isolated. 5 Candida tropicalis strains were resistant to fluconazole.

Figure 4: Incidence of biofilm producing microorganisms isolated from specimens received from ICU patients

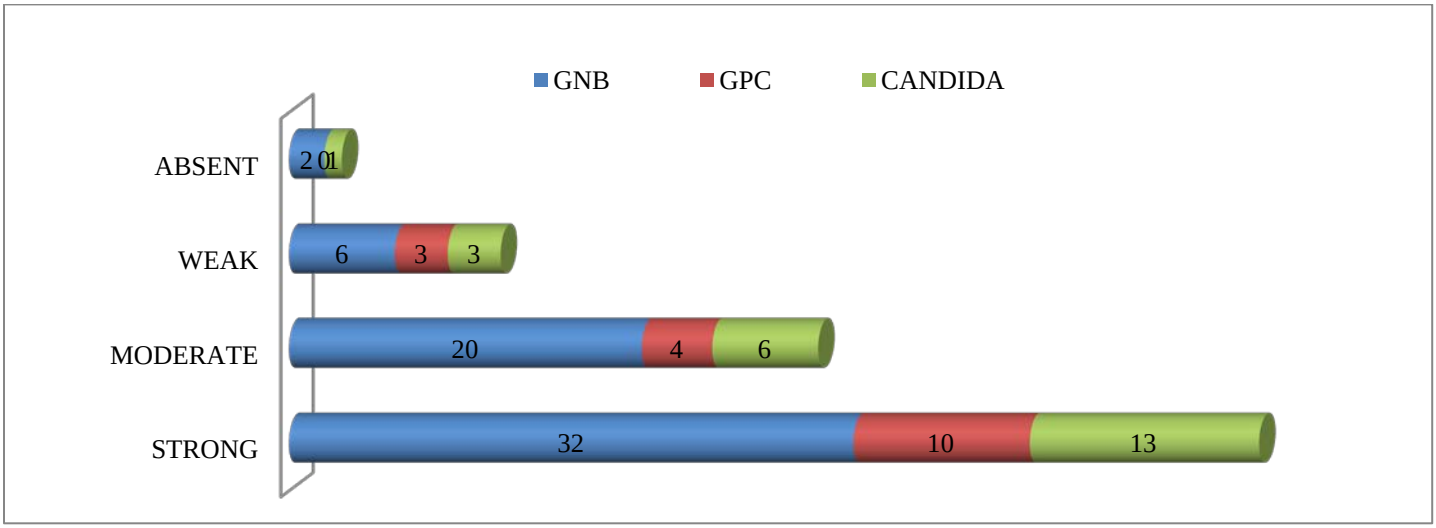


Figure 4 shows incidence of biofilm producing microorganisms isolated from clinical specimens of ICU patients. 55% strains produced strong biofilms, 30% strains produced moderate biofilms and 12% strains produced weak biofilms. 3strains were biofilm nonproducers.

Statistical analysis: Using Standard error of difference, it is inferred that the observed difference between biofilm producers and biofilm nonproducers is not due to chance and the incidence of biofilm producers is really more compared to nonbiofilm producers among ICU isolates

Discussion

Various research workers have report the incidence of ICU acquired infections varies from 2.3% to 49.2%. This variation is because of patient selection prolong stay in ICU, type of ICU, invasive medical devices, existing infection control practices and surveillance techniques followed. The EPIC-2 study showed that 62% of infections were with Gram negative isolates and the most common Gram negative bacterial isolates were Pseudomonas species (20%). [6] Our study correlated well with EPIC-2 study that 60% of our isolates were Gram negative bacilli. The commonest isolate was Pseudomonas aeruginosa (20%). Similarly Dasgupta et al. from West India [21], Datta etal. from North India [22], Mythri and Kashinath from South India [23] and Choudhuri from East India [24] showed that major ICU infections were caused by Gram negative organisms. Among 60 Gram negative bacilli 55 (91.7%) strains were sensitive to Colistin. The detection of Candida species in 23% isolates is a cause of concern. Edgeworth et al. have reported that fungal pathogens are also becoming more common with nosocomial infections.[25]

The emergence of of multidrug resistant organisms(MDROs) has mainly worsen the condition of ICU associated infections. The increase in β-lactamases producing Gram negative organisms such as ESBL, AmpC β-lactamases and MBL are also responsible for antibiotic resistance and inappropriate antimicrobial therapy. The biofilm formation was quite common in ICU infections, as in the present study, 55% isolates were strong biofilm producers and only 3% isolates were biofilm nonproducers.

Conclusion

Hence , to conclude emperical therapy should never be given to ICU patients and antibiot ic ther apy should be always given after antibiotic susceptibility test. β-lactamase and biofilm production are to be detected in isolates from ICUs for effective treatment of ICU patients and to prevent their spread in health care set up.

Clinical Significance

Scope of the study is immense. The clinical significance of this study is effective antimicrobial therapy can be given to the patients having ICU associated infections. As most of the microorganisms isolated from ICU patients are resistant to commonly used antimicrobials, antibiotic susceptibility profile and detection of β- lactamase and biofilm production must be done in Clinical Microbiology Laboratory.

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