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INTENSIVE CARE UNIT: A BREEDING GROUND FOR ANTIBIOTIC RESISTANT BACTERIA

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ABSTRACT

Objective: The present study was undertaken to detect the prevalence of multiple antibiotic resistant bacteria isolated from ICU patients and to study their antibiotic susceptibility profile. **Method:** The organisms were isolated from different clinical specimens and antibiotic susceptibility tests were done as per Clinical Laboratory Standard Institute (CLSI) guideline. The different tests for detection of Methicillin Resistant Staohylococcus aureus (MRSA) and Gram negative bacilli producing Extended spectrum beta-lactamases(ESBL), AmpC β-lactamases and Metallobetalactamases(MBL) were performed by standard methods. **Results**: A total number of 240 bacterial strains were studied. Out of which 105(43.8 %) were Gram positive isolates and 135(56.2%) were Gram negative isolates. 57(54.3%) MRSA strains were isolated. Amongst Gram negative isolates 29(21.5%) strains were only ESBL; 13(9.6%) only AmpC β-lactamase, 18(13.3%) both ESBL and AmpC β-lactamase and 21(15.6%) were MBL producers. 19 MBL producing strains were resistant to all antibiotics studied as per CLSI guideline. **Conclusion:** Accurate detection of multidrug resistant bacteria by any Clinical Microbiology Laboratory should be done for prompt and effective antimicrobial therapy to ICU patients and successful management of infections.

Keywords: MRSA, Multidrug resistant organisms.

INTRODUCTION

Intensive care units (ICUs) are unique patient care areas where severely ill patients are housed together in an environment of multiple invasive devices, drug resistant micro-organisms and few trained health care workers, especially in developing countries. Though ICU represents only 5% of the hospital beds, 25% of Hospital acquired infections occur in ICUs[1]. In ICU patients the mortality ranges from 11% for surgical site infections (SSIs) to 20% for blood-stream infections (BSIs)[2]. Though Carbapenems, Vancomycin and Linezolid are reserved antibiotics and are used as a last resort for treating severe infections with multiple drug resistant bacteria but bacteria have developed resistance to these drugs also.

The ICUs are breeding ground of several drug resistant micro-organisms e.g. *Methicillin Resistant Staphylococcus aureus (MRSA*), newer B-lactamases i.e. Extended Spectrum Beta-Lactamases(ESBL), AmpC β-lactamase and Metallobetalactamases(MBL) producing Gram negative bacilli, *Vancomycin resistant Enterococci (VRE), Vancomycin resistant*

Staphylococcus aureus (VRSA) and *Vancomycin intermediate sensitive Staphylococcus aureus (VISA*) etc. ICUs usually have a higher prevalence of infection with Multidrug Resistant Organisms
(MDRO) than do non-ICU settings. MDROs are defined as micro-organisms predominantly bacteria, that are resistant to one or more classes of antimicrobial agents. The key to success in controlling infections in ICU patients are prompt and accurate detection of these micro-organisms.[3]
Hence, the present study was undertaken to detect the prevalence of multiple antibiotic resistant bacteria isolated from ICU patients and to study their antibiotic susceptibility profile. In this study simple phenotypic methods for detection of MRSA and newer β-lactamases were used, which can be routinely and easily carried out in any Clinical Microbiology Laboratory.

MATERIAL AND METHODS

A total number of 240 bacterial strains isolated from clinical samples of ICU patients were studied from January 2008 to January 2011 and were identified by conventional methods[4]. The clinical samples collected were blood, endotracheal tube aspirate, pus and wound swab, urine, urinary catheter tip, body fluids etc. Antibiotic sensitivity testing was done by using Kirby Bauer disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines[5]. All antibiotic disks and culture media used were procured from HiMedia Pvt Ltd.(India). *Staphylococcus aureus* strains were tested for Methicillin resistance by using Cefoxitin (30 µg) disc as per CLSI guidelines[5]. Inducible Clindamycin resistance was detected by D-Zone test as per NCCLS guideline 2004[6].
To detect *VRE, VRSA* and *VISA,* Vancomycin disk were put and for confirmation Vancomycin E test strip (AB biomeriux) was used[4].
In 135 Gram negative bacterial isolates antibiotic susceptibility test was done with Ceftazidime, Cefotaxime, Cefoxitin, Co-trimoxazole, Aztreonam, Ciprofloxacin, Amikacin and Imipenem as per CLSI guideline[5].
In Gram negative bacterial isolates, for detection of ESBL producing strains combined disk method using Ceftazidime (CA 30 µg) & Ceftazidime-Clavulanic acid (CAC 30/10 µg) disk for Enterobacteriaceae as per CLSI guideline and for *Pseudomonas aeruginosa* combined disc using Piperacillin (Pc 100µg) & Piperacillin-Tazobactum (PIT 100/10µg) was used. Confirmation of ESBL production was determined by ESBL E-test strip (AB Biomeriux)[4].

- ESBL DETECTION

For ESBL detection, Combined disk method and ESBL E-test were used.
Combined disk method
Broth cultures of test strains adjusted to McFarland 0.5 standard and inoculated on Mueller Hinton(MH) agar plates. Ceftazidime (CAZ) 30 µg and Ceftazidime plus Clavulanate (CAC) 30µg plus 10µg were used. After overnight incubation at 37^0C increase in zone diameter of ≥5mm with CAC disk as compared to CAZ disk alone was considered positive for ESBL detection.
ESBL E-test[4]
Lawn culture of test strain was done on a MH agar plate & ESBL E-test strip was placed. After overnight incubation at 37^0C, MIC ratio of ceftazidime/Ceftazidime Clavulanic acid (TZ/TZL) ≥ 8 or deformation of ellipse or phantom zone present was considered positive for ESBL production.

- DETECTION OF AmpC β-LACTAMASE

For detection of AmpC β-lactamase producing stains substrate inducer combination of Imepenem(10 µg) / Ceftazidime(30 µg) disks and for confirmation disk potentiation test using

3 aminophenyl boronic acid (100 µg/ml) was used[7].

Confirmatory test : Disk potentiation test

Two ceftazidime(30µg) disks with centre to centre distance of 30mm were placed on lawn cultured MH plate. 3-aminophenylboronic acid (APB) was dissolved in DMSO at a concentration of 100mg/ml. 10µl of this APB solution was added to one of the ceftazidime disk. After overnight incubation at 37^0C, an increase in zone size of ≥5mm around the Ceftazidime - APB disc compared to ceftazidime only disk was recorded as a positive result.

- DETECTION OF METALLOBETALACTAMASES (MBL)

All Imipenem resistant strains were tested for MBL production by disk potentiation (DP) test using Imipenem and Imipenem-EDTA disk and were confirmed by MBL E test strip (AB biomeriux)[8,9].

Disk Potentiation Test (DP): The IMP-EDTA combined disk test was performed for detection of metallobetalactamases. Test strains (turbidity adjusted to 0.5 McFarland standard) were inoculated on to MH agar plate. Two imipenem disk (10 µg) were placed on the plate wide apart and 10 µl of 50mM zinc sulphate solution was added to each of the imipenem disks. Then 10µl of 0.5M EDTA solution was added to one of the disk and the plates were incubated at 35^0 C for 16-18 hrs.If the increase in inhibition zone with the Imipenem and EDTA disk was ≥7 mm than the imipenem disk alone, it was considered as MBL positive.

MBL E-Test : Confirmatory test:

The MBL E-test was done and interpreted using test strains and Quality control strains according to manufacturer's instructions. Overnight broth culture of test strain (turbidity adjusted to 0.5 McFarland standard) was used to inoculate MH agar plate. The MBL E-test strip was put on that inoculated MH plate with a sterile forceps and plates were incubated at 37^0C for 18-20 hrs. After incubation, MIC ratio of Imipenem /Imipenem-EDTA (IP/IPI) of ≥8 or deformations of ellipse or phantom zone indicate MBL production.

The *Candida albicans* strains were detected by growth on Sabouraud's Dextrose agar (SDA), Gram's staining, germ tube formation, chlamydospore formation, growth on CROME agar and Hi-Candida identification kit. The antifungal drug sensitivity test with Fluconazole and Voriconzole for *Candida albicans* was done as per CLSI guideline[10].

The control strains used in this study were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 90028.

RESULTS

Out of 240 bacterial stains isolated from ICU patients, 105 (43.8%) were Gram positive cocci and 135 were Gram negative bacilli. From 2 ICU patients *Candida albicans* was isolated. Maximum number of bacterial strains isolated were from Neonatal ICU (NICU) 123(51.3%), followed by Medicine ICU (MICU) 58(24.2%), Paediatrics ICU (PICU) 32(13.3%) and Operation theater ICU (OT ICU) 27(11.3%). The prevalence of MRSA, ESBL, AmpC, both ESBL & AmpC and MBL producing strains from different ICUs is shown in Figure 1. Amongst 96 *Staphylococcus aureus* strains isolated, 57 (59.4%) were *MRSA* and most of the *MRSA* strains 44(77.2%) were isolated from blood. All (100%) *MRSA* strains were resistant to Penicillin & sensitive to Vancomycin and Linezolid. 20(35.1%) *MRSA* strains were resistant to 08 antibiotics commonly used for treating Staphylococcal infections as per CLSI

guideline[5]. Amongst 57 *MRSA* isolates, resistance to Ciprofloxacin, Erythromycin, Clindamycin and Pristinamycin was found to be 51(89.5%), 46(80.7%), 26(45.6%) and 39(68.4%) respectively. 11(19.3%) and 14(24.6%) *MRSA* strains were even resistant to rifampicin and mupirocin respectively.

19(33.3%) *MRSA* strains belonged to $iMLS_B$ phenotype i.e. inducible Clindamycin resistant. Out of 5 *Coagulase negative Staphylococci* isolated 2 (40%) were Methicillin resistant but 4 *Enterococcus faecalis* strains isolated were Vancomycin sensitive.

Amongst 135 Gram negative bacterial isolates 57(42.2%) were *Pseudomonas aeruginosa*, 30(22.2%) were *E.coli*, 43(31.9%) were *Klebsiella pneumoniae* and rest 5(3.7%) include others i.e. *Proteus vulgaris*(2), *Citrobacter freundii*(1), *Acinetobacter baumanii*(2). 29(21.5%) were only ESBL producers 13(9.6%) were only AmpC β-lactamase producers, 18(13.3%) were both ESBL and AmpC producers and 21(15.6%) strains were MBL producers. 19(90.5%) MBL producing strains were resistant to all 08 antibiotics used as per CLSI guideline[5] and 2 were only sensitive to Amikacin. Confirmation of MBL production by E-test is shown in Figure 2. Out of 21 MBL producing strains 12(57.1%) strains were *Pseudomonas aeruginosa*. Maximum 7(53.8%) strains producing AmpC β-lactamases were *Klebsiella pneumoniae* and 11(37.9%) ESBL producing strains were *E.coli*. All (100%) MBL producing strains were sensitive to Polymyxin B. Amongst 18 strains producing both ESBL and AmpC β-lactamase, 7(38.9%) were resistant to all 8 antibiotics used as per CLSI gudeline[5], but were sensitive to Imipenem and Amikacin. None of the MBL producers also produced ESBL or AmpC β-lactamase.

143 (59.6%) bacterial strains were isolated from blood samples, followed by 27(11.3%) from endotracheal tube aspirate. In 2 cases, *Candida albicans* was isolated from blood and from urine respectively. *Candida albicans* was isolated on two repeat culture from the same sample. The two *Candida albicans* strains were sensitive to fluconazole and Voriconazole[10].

DISCUSSION

According to World Health Organization(WHO) guidelines, 2002 more than 1.4 million people at any time suffer from Health care associated infections (HAI)[11]. The highest rates of HAIs occur in ICUs, and the crude mortality rates of HAI in the ICUs of the developed countries range from 12 to 80%[12]. Though no nationwide surveillance data are available, Rosenthal et al in 2006 had reported that the rates of HAI in ICUs range from 12.3% in few ICUs in India to 88.9% in a Turkish ICU[13]. The ICUs are the highest consumers of antimicrobials in any Health care set up. Moreover, ICUs have an unusually high rate of antimicrobial resistance[14].

Antibiotic resistance in ICU acquired infections is responsible for increase in mortality, morbidity and prolonged hospital and ICU stay[15].

Ventillator-associated pneumonia (VAP), Cather-associated urinary tract infection(CA-UTI), Catheter-related bloodstream infections(CRBSIs), Surgical site infections(SSI), *Clostridium difficile*-associated diarrhoea(CDAD), Paranasal sinusitis especially maxillary sinusitis are common infections occurring in ICUs. In the present study, out of 23 bacterial strains isolated from urine, 13(56.5%) were isolated from CA-UTI. To consider CA-UTI, no minimum time period for which the catheter is to be in place is required[16]. CA-UTI account for 30-40% of health care associated infections[17]. Richards et al had reported that 95% of UTI cases in hospitals are catheter associated[18].

Our hospital is a tertiary care hospital, which is situated in a rural set-up and patients from surrounding villages of Vidarbha and the adjoining states come to our hospital for treatment. Lack of awareness, indiscriminate use of antibiotics before attending the hospital, might be the contributory factors for high prevalence of *MRSA* and Gram negative bacilli with production of newer β-lactamases i.e. ESBL, Amp C and MBL production, leading to multiple drug resistant organisms.

Infection in the ICU patients can be successfully managed by prompt institution of effective antimicrobial therapy. But in an era of multidrug resistance, selecting the appropriate antibiotic is a significant challenge. Thus, every health care set up with ICU facility must have Clinical Microbiology Laboratory where different organisms isolated can be studied for antimicrobial resistance mechanisms and prompt communication of urgent Microbiology reports, help to modify the antimicrobial prescribing guidelines.

'Bundled approach or interventions' comprise of 'small straightforward set of best practices' which should primarily aim at the prevention of cross transmission and control or elimination of reservoirs or sources of infection should be followed. These 'Bundled approach when followed collectively can significantly reduce infection rates and improve patient's outcome in ICU[19]. Standard precautions, contact precautions, antimicrobial stewardship programmes etc., SMART i.e. (Specific, Measurable, Achievable, Relevant & Time bound) objectives & educating the health care workers, especially the nursing staffs are need of the hour[19]. Proper hand hygiene between every patient contact itself reduces the infection rate.

Hence to conclude, detection of multidrug resistant bacteria by simple phenotypic methods should be done by any Clinical Microbiology Laboratory for effective antimicrobial therapy to ICU patients. Active efforts should be taken by all health care personnel to prevent antibiotic resistance as WHO has already mentioned ''No action today, No cure tommorow''.

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FIGURES:

Figure 1: Prevalence of drug resistant bacteria

Figure 1: Prevalence of drug resistant bacteria isolated from different ICUs

TOP SIDE ↑

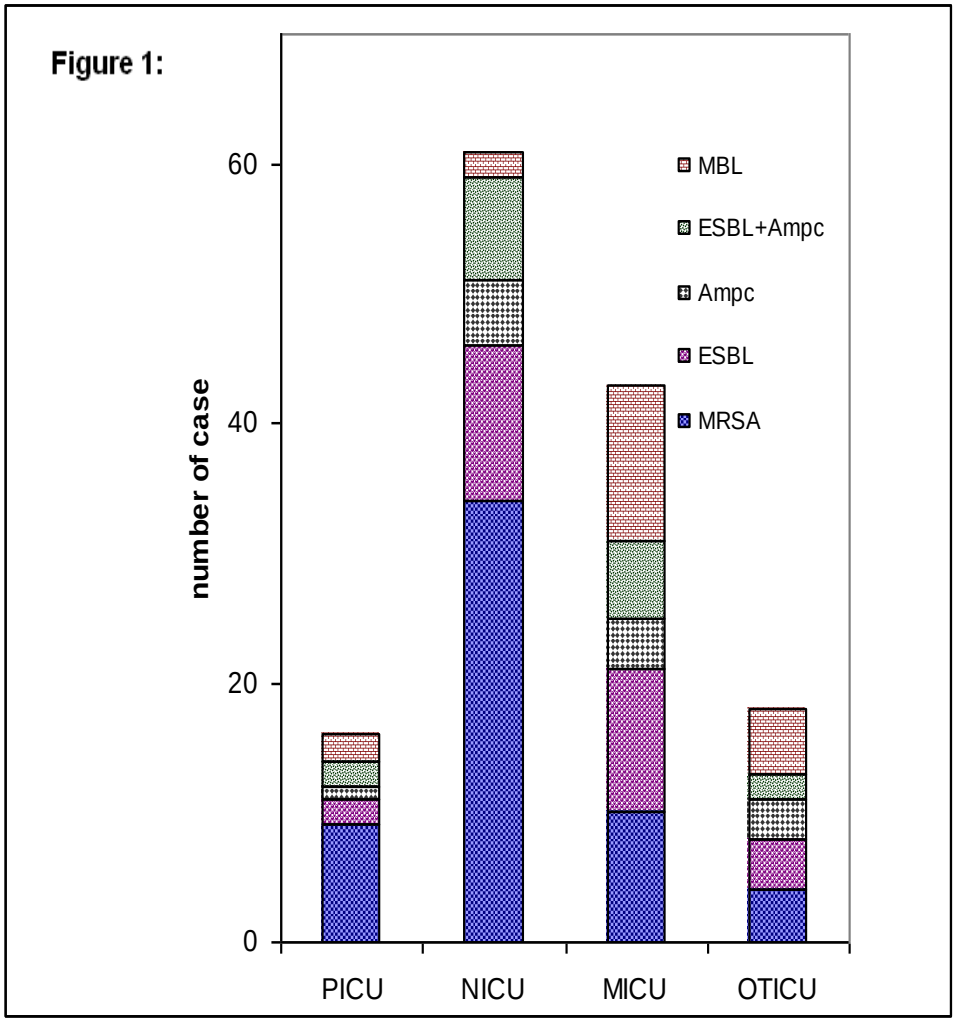

Fig 1: Prevalence of drug resistant bacteria isolated from different ICUs

Figure 2: MBL E-Test positive (E.coli)

TOP SIDE ↑

Figure 2: MBL E-Test positive (E.coli)