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PATTERN OF ANTIBIOTIC SUSCEPTIBILITY OF COMMON ISOLATES IN ICU OF A TERTIARY CARE HOSPITAL: 2 YEARS STUDY

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ABSTRACT

Introduction: Antibiotic resistance is a major emerging world-wide problem in the intensive care unit (ICU). The aim of this study was to study the antimicrobial resistance pattern of microbial isolates from patients in intensive care units (ICUs). **Material and methods:** All isolates from different clinical samples were collected and processed by standard microbiological techniques. Antimicrobial susceptibility testing was performed by modified Kirby Bauer method. All gram negative organisms were further tested for ESBL and MBL production. **Results:** Of 451 isolates, 353 (78.2%) were gram negative and 98 (21.7%) were gram positive cocci. The most frequent infections were lower respiratory tract infections (32.9%). The most frequently isolated organisms were *P. aeruginosa* (20.1%) and *Staphylococcus epidermidis* (8.6%). Higher resistance (60-100%) was observed to amoxycillin, ceftazidime, amoxyclav, ciprofloxacin and cotrimoxazole. ESBLs production was found in (45.3%) isolates. 75.0% of *Staphylococcus aureus* and 20.5% of *S. epidermidis* were MRSA positive. 85.7% showed MBL production. **Conclusion:** Surveillance of antibiotic susceptibility patterns of predominant bacteria is necessary to monitor changes in susceptibility patterns and to guide the clinician in choosing empirical or directed therapy appropriately, especially in ICU setting.

KEYWORDS: Intensive Care Unit, antibiotic resistance, MRSA.

INTRODUCTION

ICUs accommodate the most seriously ill patients in a relatively confined environment.[1] Antibiotic resistance is a major emerging world-wide problem in the intensive care unit (ICU) including India. The patient in the ICU has a 5 to7 fold higher risk of nosocomial infection compared with the other patients. This is a consequence of impaired defence mechanism, applying invasive methods and monitoring devices, exposure to broad-spectrum antibiotics and the colonization of resistant microorganisms. The frequent use of broad-spectrum antibiotics results in colonization with resistant Gram-negative bacteria and consequently in serious infections.[2-4] Antimicrobial resistance has emerged as an important determinant of outcome for patients in the ICU.[5] The widespread use of antibiotics put tremendous selective pressure on bacteria which develop new mechanisms to escape the lethal action of the antibiotics. These infections are difficult to treat because of emergence of newer β-lactamases such as extended spectrum β-lactamases (ESBL), AmpC β-lactamases and Carbapenemases.[6]

Increased duration of stay, increased number of indwelling devices and prolonged or inappropriate use of antibiotics in the ICU-leading to selection of multi-resistant 'super-bugs'- among these the methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), extended-spectrum β-lactamase-producing GNB, *P. aeruginosa*, *Acinetobacter*, Glycopeptide intermediate *Staphylococcus aureus* (GISA),

Glycopeptide resistant *Staphylococcus aureus* (GRSA), *Stenotrophomonas maltophila* and *Candida* are notable which all are associated with significantly increased morbidity and mortality.[1,7-8] Continuous spreading of antimicrobial resistance is common in ICU that may lead to be a clinical disaster. If this resistance spreads, monitoring the use of antimicrobials and review of sensitivity patterns are imperative. Study of antimicrobial sensitivity patterns in ICUs and critical care units (CCUs) are crucial and far more important for giving effective treatment and control in the spread of resistance. The present study was therefore, designed to study the antimicrobial resistance pattern of microbial isolates from patients in intensive care units (ICUs) of a tertiary care hospital.

MATERIALS AND METHODS

The present prospective study was conducted in the Microbiology Department of a teaching tertiary care hospital during July 2012-June 2014 and the study was approved by the Intuitional Ethics committee. All isolates obtained from different clinical samples (e.g. Urine, Pus, Blood, Sputum, BAL, Tracheal secretions, CSF, Peritoneal and Pleural fluid) were identified based on their characteristic appearance on the media and the patterns of biochemical reactions using conventional bacteriological methods[9] and evaluated for antibiotic susceptibility by the Kirby–Bauer disc diffusion method on Muller–Hinton agar (Himedia) according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013).[10] The susceptibility of the isolated bacteria were tested against ampicillin (10 µg), piperacillin (100 µg), ceftriaxone (30µg) ceftazidime (30 µg), cefepime (30 µg), cefotaxime (30 µg), gentamicin (30 µg), amikacin (30 µg), ciprofloxacin (30 µg), trimethoprim/sulfomethoxazole (1.25/1.23 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg) and nitrofurantoin (100 µg).

The Gram-negative isolates (including members of the family Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp.) were subjected to test phenotypically for ESBL and MBL production. Quality control was assured by concurrent testing with the American Type Culture Collection (ATCC) strains including *E. coli* ATCC 25921, *P. aeruginosa* ATCC 27852 and *Staphylococcus aureus* ATCC 25923.

Detection of ESBL: The Gram-negative isolates showing resistance (or decreased zone diameter according to the ESBL screening method of the Clinical and Laboratory Standards Institute) to third generation cephalosporins (i.e. ceftazidime, ceftriaxone, cefotaxime, aztreonam, cefpodoxime) were tested for ESBL production by using the phenotypic disc confirmatory test (PDCT).

The phenotypic disc confirmatory test (PDCT): This test was performed as a disc diffusion test, as recommended by the CLSI. The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA by using a sterile cotton swab. (a) Ceftazidime (CA) disc (30 µg) and ceftazidime- clavulanic acid (CAC) disc containing 20+10 µg of the antibiotics were placed at a distance of 30 mm from each other and (b) cefotaxime (CE) disc (30 µg) and cefotaxime-clavulanic acid (CEC) disc (20+10 µg) were placed at a distance of 30 mm from each other.[10]

Detection of Metallo – β - Lactamase (MBL): The imipenem resistant isolates were tested by the imipenem-EDTA double disk synergy test (DDST) as described by Lee et al. The test organism was inoculated onto MHA plates as recommended by CLSI. An imipenem 10 µg disc was placed 10mm edge to edge from a blank disc which contained 10 µl of EDTA (750 µg), with overnight incubation at 37°C. An enhancement in the zone of inhibition in the area between the imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the disc was interpreted as a positive result.[10-11]

Detection of MRSA: The detection of MRSA done by cefoxitin (30 µg) disc diffusion tests. Mueller-Hinton agar (MHA) plates were overlaid with a saline suspension with the isolate (turbidity matching 0.5McFarland standard) and cefoxitin (30 µg) discs were placed after 10 minutes (HiMedia, India). After 24 hours incubation at 35ºC, the plates were read using the CLSI cut-off points as reference: ≤19 mm for cefoxitin considered as MRSA. *S. aureus* ATCC 25923 (*mecA* negative) and ATCC 43300 (*mecA* positive) were used as controls for all the tests.[12]

Detection of vancomycin resistance: Minimum inhibitory concentration (MIC) for vancomycin for VRE was determined

by E-test as per the procedure of CLSI.[4] An isolate is considered susceptible to vancomycin if the MIC is ≤ 4µg/ml and resistant if MIC ≥ 32 µg/ml.[4] Quality control was assured by concurrent testing with the American Type Culture Collection (ATCC) strains including two strains of *Enterococcus faecalis* ATCC 29212 and ATCC 51299 were used as sensitive and resistant controls, respectively. The MIC values of vancomycin for the control strains must be within the ranges provided by the CLSI prior proceeding to test organisms.[10]

RESULTS

During July 2012 to June 2014, 425(46.4%) of the 915 specimens were culture positive and 490 (53.5%) were culture negative. 451 isolates were isolated from 425 culture positive specimens. 26 (2.84%) specimen were positive for two different bacteria. Out of these, 17 specimens from LRTIs, 7 specimens from wound infection and 2 from UTI were positive for two different types of bacteria. Out of 451 isolates, 353 (78.2%) were gram negative organisms and 98 (21.7%) were gram positive cocci. Of these 451 isolates, 157 (34.8%) were isolated from respiratory specimens, 128 (28.3%) were from urine, 107 (23.7%) isolates were from pus, 43 (9.53%) from blood and 16 (3.54%) isolates were from miscellaneous sample (Table 1). The isolation patterns of organisms as well as infection pattern are given in Table 1.

Table 1. Isolates from different clinical sample in ICU Patient

ISOLATES	LRTI	WOUND	UTI	SEPTICEMIA	MENINGITIS	PERITONITIS
P. aeruginosa	41	31	11	6	1	1
E. coli	19	19	34	6	3	2
***Klebsiella* spp.**	32	12	28	3	1	-
***Acinetobacter* spp**	30	9	14	6	-	-
***Citrobacter* spp**	2	2	14	2	1	-
Enterobacter spp	2	1	2	1	-	-
***Serratia* spp**	2	1	1	1	-	-
S. maltophila	1	1	1	-	-	-
Proteus vulgaris	-	1	5	-	-	-
Proteus mirabilis	-	1	1	1	-	-
CONS	9	11	9	9	-	1
S. aureus	17	12	2	4	1	-
Enterococcus	2	6	6	4	3	2
Total (451)	157	107	128	43	10	6

The most frequent infections were lower respiratory tract infections 140 (32.9%), urinary tract infection 126 (29.6%) and wound infections 100 (23.5%). The most frequently isolated organisms among gram negative were 91 *P. aeruginosa* (20.1%), followed by 83 *E. coli* (18.4), 76 *K. pneumoniae* (16.8%) and 59 *Acinetobacter* spp (13.0%). Among the gram positive, 39 *Staphylococcus epidermidis* (8.6%), 36 *Staphylococcus aureus* (7.9%) and 23 *Enterococci* spp. (5.1%) were frequently isolated. Most frequently isolated bacteria from LRTI among gram negative were *P. aeruginosa, Acinetobacter spp*., *K. pneumoniae* and among gram positive were *Staphylococcus aureus.*

Very high rate of resistance (60-100%) was observed among *P. aeruginosa, E.coli, K. pneumoniae, Acinetobacter* spp. and *Staphylococcus aureus* isolates to amoxycillin, ceftazidime, amoxyclav, ciprofloxacin and cotrimoxazole. Higher resistance to macrolides was found for *S .aureus* than for *S. epidermidis.* Colistin, imipenem and amikacin were the most effective *in vitro* drugs against gram negative isolates and linozolid and vancomycin were the most effective against gram positive isolates (Table 2). High rate of resistance (60-80%) to third generation cephalosporins was observed among isolates of *Pseudomonas aeruginosa*, *E. coli* and *K. pneumoniae*.

Table 2. Antibiotic Resistant Pattern of Common Isolates in ICU Patient

Antibiotics	***P. aeruginosa***	***E .coli***	***Klebsiella***	***Acinetobacter***	***Citrobacter***	***S. aureus***	***Enterococci***	***S. epidermidis***
AMP	*91(100)*	*80(96.3)*	*76(100)*	*59(100)*	*19 (90.4)*	*34 (94.4)*	*21(91.3)*	*21(53.8)*
PC	*80(87.9)*	*65(78.3)*	*63(82.8)*	*54(91.5)*	*14(66.6)*	*26(72.2)*	*14(60.8)*	*19(48.7)*
G	*39(42.8)*	*32(38.5)*	*33(43.4)*	*37(62.7)*	*08(38.0)*	*11(30.5)*	*13(56.5)*	*14(35.8)*
AK	*21(23.0)*	*16(19.2)*	*17(22.3)*	*22(37.2)*	*5(23.8)*	*08(22.2)*	*09(39.1)*	*12(30.7)*
CF	*53(58.2)*	*52(62.6)*	*60(78.9)*	*48 (81.3)*	*8(38.0)*	*22(61.1)*	*11(47.8)*	*13(33.3)*
CAZ	*79(86.8)*	*66(79.5)*	*65(85.5)*	*51(84.4)*	*12(57.1)*	*22(61.1)*	*--*	*16(41.0)*
CTR	*79(86.8)*	*63(75.9)*	*65(85.5)*	*51(84.4)*	*12(57.1)*	*21(58.3)*	*--*	*16(41.0)*
CS	*78(85.7)*	*62(74.6)*	*65(85.5)*	*51(84.4)*	*12(57.1)*	*20(55.5)*	*--*	*15(38.4)*
I	*22(24.1)*	*13(15.6)*	*14(18.4)*	*20(33.9)*	*1(4.7)*	*--*	*--*	*--*
PTZ	*30(32.9)*	*33(39.7)*	*25(32.8)*	*33(55.9)*	*5(23.8)*	*07(19.4)*	*11(47.8)*	*12(30.7)*
AMC	*91(100)*	*62(74.6)*	*59(77.6)*	*57(96.6)*	*7(66.6)*	*21(58.3)*	*11(47.8)*	*15(38.4)*
CAC	*40(43.9)*	*33(39.7)*	*25(32.8)*	*27(45.7)*	*6(28.5)*	*17(47.2)*	*--*	*14(35.8)*
CFS	*40(43.9)*	*33(39.7)*	*25(32.8)*	*26(44.0)*	*6(28.5)*	*15(41.6)*	*--*	*14(35.8)*
COT	*91(90.6)*	*64(79.7)*	*76(100)*	*59(100)*	*17(80.9)*	*22(61.1)*	*--*	*08(20.5)*
VA	*--*	*--*	*--*	*--*	*--*	*3(8.3)*	*02(8.6)*	*00 (00)*
CN	*--*	*--*	*--*	*--*	*--*	*28(77.7)*	*--*	*16(41.0)*
LZ	*--*	*--*	*--*	*--*	*--*	*00(00)*	*00(00)*	*00(00)*
E	*--*	*--*	*--*		*--*	*05(13.8)*	*04(17.3)*	*17(43.5)*
NF	*5(100)*	*13(16.4)*	*9(11.8)*	*8(38.0)*	*1(5.0)*	*00*	*02(8.6)*	*02(22.2)*

AMP –Ampicillin, PC – Piperacillin, G – Gentamicin, AK – Amikacin, CF – Ciprofloxacin, CAZ – Ceftazidime, CTR – Ceftriaxone, CS - Colistin, I – Imipenem, PTZ – Piperacillin-Tazobactum, AMC – Amoxycillin-Clavulinic acid, CAC – Ceftazidime-clavulinic acid, CFS – Cefoperazone-sulbactum ,COT - Cotrimoxazole, VA – Vancomycin, CN – Cefoxitin, LZ – Linezolid, E – Erythromycin, NF - Nitrofurantoin

Very high rate of resistance (60-100%) was observed among *P. aeruginosa, E.coli, K. pneumoniae, Acinetobacter* spp. and *Staphylococcus aureus* isolates to amoxycillin, ceftazidime, amoxyclav, ciprofloxacin and cotrimoxazole. Higher resistance to macrolides was found for *S .aureus* than for *S. epidermidis.* Colistin, imipenem and amikacin were the most effective *in vitro* drugs against gram negative isolates and linezolid and vancomycin were the most effective against gram positive isolates (Table 2). High rate of resistance (60-80%) to third generation cephalosporins was observed among isolates of *Pseudomonas aeruginosa*, *E. coli* and *K. pneumoniae*.

Among gram negative isolates, production of ESBLs were found in 160 (45.3 %) of frequently isolated organism. The ESBLs producing strains were, 39 *P. aeruginosa* (42.8%), 44 *E. coli* (53%),43 *K. pneumoniae* (56.5%), 23 *Acinetobacter* spp (38.9%) and 6 (28.5%) *Citrobacter* spp (Figure 1). However, in this study only 39 (24.37%) of ESBLs producing isolates were susceptible to tazobactum. In this study, high sensitivity to Colistin (100%), Polymyxin B (100%) and Imipenem (85-90%) was seen among ESBLs producing bacteria while only 30-35% Amikacin sensitivity was seen among ESBLs producing bacteria.

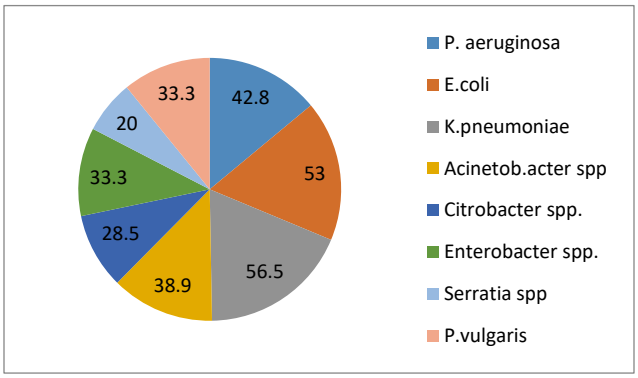

Figure 2. Distribution of ESBLs producing strains

Among the *Staphylococcus* spp 75.0% (27/36) of *Staphylococcus aureus* and 20.5% (8/39) of *S. epidermidis* were MRSA positive.13.88% (5) of *Staphylococcus aureus* and 13.1 % (3) of *Enterococc*i spp. were vancomycin resistant. There was no resistance against linozolid.

Among the 353 gram negative isolates, 70 (19.8%) showed imipenem resistance by the disc diffusion method. Of these, 42 (60.0 %) were non-fermenters and 28 (40.0%) were Enterobacteriaceae. Among Imipenem resistant non fermenter isolates 36 (85.7%) showed MBL production by the imipenem (IMP)-EDTA combined disc test, which include 20 (55.5%) *Pseudomonas aeruginosa* and 16 (44.4%) *Acinetobacter* spp. Among Imipenem resistant Enterobacteriaceae, 22 (78.5%) were positive for MBL which include 10 (45.4%) *E. coli*, 11 (50%) *Klebsiella pneumoniae* and 1 (4.5%) *Citrobacter* spp.

DISCUSSION

The emergence of antimicrobial resistance in ICUs is of great concern as it increases the likelihood of drug interactions/side effects and cost of therapy due to use of newer antibiotics. Resistance may also be responsible for prolonged hospital stays and can affect prognosis. The problem of resistance in a hospital is difficult to understand without the knowledge of antimicrobial use pattern[8,12,13] so monitoring the use of antimicrobial and review of sensitivity pattern are important.

Organisms were isolated in 46.4 % out of cultures investigated, compared to 36.8% by Sheth et al and 64.7 % in Indonesian ICU study.[13] The most common infections in our study were LRTI, urinary tract infection and wound infections which are similar to other Indian studies.[2,3,11.12]

This result revealed that *P. aeruginosa*, *Klebsiella* spp., *E. coli, Acinetobacter* spp and *Staphylococcus aureus* were most predominant isolates in ICU of KIMS, Narketpally, Nalgonda. In Asian countries including India, the most frequent pathogen isolated from infections in the ICU are *P. aeruginosa, Klebsiella* spp., *E. coli, Enterococcus* and *Staphylococcus aureus.*[12,16] In Thailand the predominance causative pathogens in ICU, were the imipenem resistant *P .aeruginosa*, ceftazidime-resistant *Acinetobacter baumannii*, third-generation-cephalosporin-resistant *K. pneumoniae*, and quinolone-resistant *E. coli.*[17] Another study performed at ICU of a tertiary care centre in Saudi Arabia showed that the most frequent pathogens are *Acinetobacter baumannii*, *P. aeruginosa, E. coli, K. pneumonia.*[18] Recently, similar studies were conducted in hospitals and several ICUs in Asian countries including Philippines,[20] India,[11,20-23] Iran,[24-25] China,[26] Malaysia,[27]

Singapore[28] and Nepal,[29] demonstrated that the most frequent microorganism derived from ICU samples were *P. aeruginosa, Klebsiella* spp. and *Staphylococcus.* For example, in 12 ICUs in seven Indian cities, overall 87.5% of all *Staphylococcus aureus* health care associated infections were caused by methicillin-resistant strains, 71.4% of Enterobacteriaceae were resistant to ceftriaxone and 26.1% to piperacillin-tazobactam, 28.6% of the *P. aeruginosa* strains were resistant to ciprofloxacin, 64.9% to ceftazidime and and 42.0% to imipenem.[2,16]

High rate of resistance (60-80%) to third generation cephalosporins was observed among isolates of *Pseudomonas aeruginosa*, *E. coli* and *K. pneumoniae*. Production of ESBLs was found in 30-40% of frequently isolated organism. The ESBLs rate of *P. aeruginosa, E.coli, K. pneumonia, Acinetobacter* spp. and *Citrobacter* spp. were 43%, 53%, 56%, 47% and 30% respectively. Third generation cephalosporin such as ceftazidime were extensively used in our hospital before therefore, the resistance observed here may be due to ESBLs, which may appear under the selective influence of the extensive use of antibiotics.

Tazobactum is expected to inhibit ESBLs. Piperacillin-tazobactum should be good choice for inhibiting ESBL producing microorganisms. However, in this study only 26.6% of ESBLs producing isolates were susceptible to tazobactum. This is probably a result of the widespread distribution of non TEM/SHV ESBLs, such as PER-I which is resistant to tazobactum.

In this study, Colistin (100%), Polymyxin B (100%) and Imipenem (85-90%) was highly sensitive for ESBLs producing bacteria while Amikacin was sensitive for 30-35% of ESBLs producing bacteria.

In this study 77.1% of *Staphylococcus aureus* and 41.0 % of *S. epidermidis* were MRSA positive. 8.3% of *Staphylococcus aureus* and 8.6 % of *Enterococci* spp. were vancomycin resistant. The association between intensity of care and risk for MRSA acquisition is well described. ICUs with more 'at-risk' patient populations are more prone to higher rates of MRSA acquisition for a number of reasons including more staff to patient contact, higher use of medical devices compared to units with less acute patients and more selective pressures induced by antibiotic therapy.[23] There was no resistance against linozolid. This may be due to limited use of linozolid against these bacteria.

Lower respiratory tract infections (LRTI) are the most common bacterial infections among patients in intensive care units (ICUs) occurring in 10-25% of all ICU patients and resulting in high overall mortality, which may range from 22-71%.[1,2] Most common bacterial agents of LRTI in the ICU are *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Citrobacter*, *Escheric hia coli.*[3-5] The commonly isolated organisms in this study among gram negative were *P. aeruginosa E.coli. K. pneumoniae*, *Acinetobacter* spp. and among gram positive were *Staphylococcus aureus* 36(8.90%)*, Staphylococcus epidermidis* 39 (7.88%) and *Enterococci* spp. which are similar to other studies.[2,3,28]

CONCLUSION

Antimicrobial resistance has emerged as an important determinant of outcome for patients in the ICU. The escalating problem of antimicrobial resistance has substantially increased overall health care cost. This increase is a result of prolonged hospitalization and convalescence associated with antibiotic treatment failures. The need to develop new antimicrobial agents and the implementation of broader infection control and public health interventions aimed at curbing the spread of antibiotic resistance pathogens. ICUs are unique because they have seriously ill patients in confined environment where antibiotic use is extremely common. Effective strategies for the prevention of antimicrobial resistance in ICUs have focused on limiting the unnecessary use of antibiotics and strict implementation of infection control practices. Clinicians treating critically ill patients should consider antimicrobial resistance as an important part of their routine treatment plans. Careful, focused attention to this problem at the local ICU level, using a multidisciplinary intervention, will have the greatest likelihood of limiting the development and dissemination of antibiotic-resistant infections. The prescribing of antibiotics in the ICU is usually empiric. Therefore, the ongoing surveillance

of antibiotic susceptibility patterns of predominant bacteria is a fundamental effort to monitor changes in susceptibility patterns and to guide the clinician in choosing empirical or directed therapy appropriately, especially in ICU setting. Appropriate antibiotic utilization in ICU is crucial not only in ensuring an optimal outcome, but also in preventing the emergence of multi drug resistance bacteria.

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