

Original Research Article

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Prevalence of MRSA, ESBL and Carbapenemase Producing Isolates Obtained from Endotracheal and Tracheal Tubes Secretions of ICU Patient at Tertiary Care Centre

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ABSTRACT

Microbiological infection plays vital role in determining the outcome as well as cost and duration of the hospital stay for patients admitted in ICU setup. Of late nosocomial infections especially ventilator associated pneumonias (VAP) have been recognized to be associated with significant morbidity and mortality in intubated patients. It is of critical importance to do regular surveillance of important pathogen (like Methicillin resistant staphylococcus aureus (MRSA), extended spectrum beta lactamase (ESBL) and carbapenemase producers) and its resistance pattern. Culture and sensitivity patterns if properly determined can greatly improve the outcome of critically ill patients being managed in intensive care units. To find out the prevalence of MRSA, ESBL and carbapenemase producing isolates in endotracheal and tracheostomy tube samples of ICU patients and to know its resistance pattern. In this study we aimed to investigate the pattern of microorganism grown and their sensitivity patterns to propose a proper antibiotic treatment in our hospital ICUs. It was a cross sectional observational study which was carried out over a period of 6 months in the department of microbiology at a tertiary care hospital. Approval of ethical committee was obtained before the study. Total 70 samples consisting of endotracheal tubes and tracheostomy tube secretions received in the department of microbiology for culture and sensitivity from patients admitted in two ICUs (SICU, MICU) were processed as per standard microbiological protocol. Pathogens were identified by vitek 2 compact system. MRSA, ESBL and carbapenemase production was detected by vitek 2 system (Biomérieux) antibioticsusceptibility testing of MRSA, ESBL and carbapenemase producers was done. Antibiotics for testing were chosen as per vitek Id cards. The sensitivity patterns of MRSA, ESBL and carbapenemase producers were analyzed. Out of 70 samples 48(68.57%) were culture positive in which 41(87.27%) were monomicrobial and 7 (12.72%) were polymicrobial (2 organisms in 7 samples). Out of 55 isolated organisms 8 were gram positive cocci (14.54%) and Gram negative organisms were 47 (85.46%). Thus total 9 types of organisms were recovered from 48 culture positive samples. Fourteen *Pseudomonas aeruginosa* (25.54%), 14 *Acinetobacter baumannii* complex (25.54%), 11 *Klebsiella pneumoniae*. (20%) and 8 *Staphylococci* (including *Staphylococcus aureus* 03 and 05 coagulase negative *Staphylococcus* (CONS) (14.54%). Followed by 3 *E. coli* (5.45%), 2 *Enterobacter aerogens* (3.64%), 1 species each of *Serratia marscens*, *Stenotrophomonas* (1.82%). Out of 8 *Staphylococcus* (*S. aureus* and CONS) isolates 5 (62.5%) were MRSA and MRCONS. Most of the MRSA isolates were recovered from the endotracheal tube. Out of all isolates of *Klebsiella* species and *E. coli* 8 species were ESBL producers (57.14%). The maximum ESBL producing isolates were recovered from MICU and were isolated from endotracheal tubes. Isolated MRSA strains were found to be susceptible to vancomycin, linezolid, teicoplanin and rifampicin amongst tested antibiotics. In isolated ESBL producing organisms the highest susceptibility was noted for colistin and tigecycline. The carbapenemase producing isolates 28(59.57%) showed susceptibility to colistin, amikacin and tigecycline. In present study the prevalence of MRSA, ESBL and carbapenemase producing isolates was 62.5%, 57.14%, 59.57% respectively. MRSA isolates were found to be susceptible to antibiotics like vancomycin, rifampicin, linezolid and teicoplanin while ESBL producing isolates were found to be susceptible to antibiotics like imipenem, colistin and tigecycline. Carbapenemase producing isolates were found to be susceptible to antibiotics like colistin, amikacin and tigecycline. Most of MRSA and carbapenemase producers were from SICU and ESBL from MICU. So we need to strengthen our infection control practices so as to reduce these infections.

Keywords

Intensive care units, Antimicrobial agents, Drug resistance/ susceptibility pattern, ESBL, MRSA, Carbapenemase producing isolates.

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Introduction

Intensive care units are specialized wards in hospitals to offer close monitoring and personalized care to very sick patients in whom there is imminent risk to life. ICUs despite their apparent impact on patient outcome have become high risk areas for health care associated infection. The patient in the ICU has a 5-7 fold higher risk of a health care associated infection compared with the average patient and 20-25% of all health care associated infection develops in ICUs (Wenzel *et al.*, 1983). Critically ill patients admitted in ICUs are always at higher risk of developing infections with various antibiotic resistant organisms (Like MRSA, ESBL and CARBAPENEMASE producers) due to prolonged hospitalization, serious illness, compromised hosts, usage of invasive devices and procedures, higher rates and longer duration of antibiotic usage and exogenous transmission of bacteria, usually by hospital personnel (Dasgupta *et al.*, 2015; Shanmuga Vadivo *et al.*, 2014). Frequent and indiscriminate use of broad – spectrum antibiotics without culture sensitivity tests results in colonization with multi resistant organisms (Like MRSA, ESBL & CARBAPENEMASE producers) and consequently in serious infection (Alireza *et al.*; Kiran *et al.*, (2015); Dautzenberg *et al.*, 2015). ESBLs were first identified in the early 1980s; since then, ESBLs have been identified worldwide and have been found in a number of different organisms, including *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis* and *Salmonella* spp. The advent of ESBL producers has represented a great threat to the use of many classes of antibiotics, particularly cephalosporins (The microbiological profile of nosocomial infections in the intensive care unit). It is well known that poor outcomes occur when patients with serious infections due to ESBL producing organisms are treated with antibiotics to which the organism is frankly

resistant. The mortality rate in such patient is significantly higher than that in patients treated with antibiotics to which the organism is susceptible (The microbiological profile of nosocomial infections in the intensive care unit).

MRSA strains have emerged in the last decade as one of the most important nosocomial pathogen in ICU patients. Infected and colonized patients provide the primary reservoir and transmission is mainly through staff. The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization and intravascular catheterization. More than 70% of *Staphylococcus aureus* isolated in ICUs is MRSA. Its detection is important for prevention of its transmission as well as for effective treatment of patients (Sista *et al.*, 2004).

Of late carbapenemase producing organisms have emerged as a challenge for treating physicians in intensive care units. Carbapenem resistant organisms especially carbapenem resistant *Klebsiella pneumoniae* (CRKP) is one of the common bacteria responsible for sepsis in ICUs (Mouloudi *et al.*, 2014). Invasive procedures like central lines and urinary catheters are very important for management of critically ill patients but they are also responsible for life threatening consequences due to severe resistant infections. Antibiotic overuse and misuse partly due to incorrect diagnosis; and irregular consumptions due to wrong prescription or poor compliance all contributes to the worldwide drug resistance among the hospital acquired organisms (Radji *et al.*, 2011).

One of the important nosocomial infection is ventilator associated pneumonia (VAP) seen in patients who have been intubated for mechanical ventilation. Since these patients are already critically ill occurrence of VAP

seriously jeopardizes the chances of recovery. The mortality rates from VAP vary widely depending upon the demography and status of healthcare in that particular country and have been reported up to 50% (Baker *et al.*, 1996). Common organisms involved in ventilator associated pneumonia include Gram negative organisms like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* species, and Gram-positive organisms such as *Staphylococcus*. Multibacterial infections and changing bacterial infections are also common in patients who have been admitted in ICU for prolonged periods. These changing flora complicate therapy by acquiring drug resistance and altering their sensitivity pattern (Montravers *et al.*, 2015). Therefore updated knowledge of local epidemiological and susceptibility profile is recommended for guiding clinicians regarding empirical choice of antibiotics and has become mandatory along with adequate clinical diagnosis and bacterial confirmation (Aarts *et al.*, 2007). Rapid identification of the bacteria and antimicrobial therapy based upon the culture and sensitivity patterns of the involved organisms is the gold standard treatment of any infection. For this purpose automated bacterial identification systems have been in use since decades.

Only few of them are being used now a day. Some of the rapid identification systems which are currently in use are ATB, MicroScan and VITEK. The newer version of VITEK namely VITEK 2 system (bioMérieux, Marcy l'Etoile, France) is significant as it gives results within 3 hours (Cuziat *et al.*, 1997). This early detection is based upon its mechanism of detection which is fluorescence based and more sensitive in detecting metabolic changes thereby continually monitoring reactions and in the process provides much faster detection and identification (Gayral *et al.*, 1997).

Aim of our study was to determine the aerobic bacterial profile and antibiotic sensitivity and resistance pattern of isolates from endotracheal secretions in a tertiary care referral centre. Each isolate was identified by using automated VITEK 2 compact system.

Materials and Methods

This Cross – sectional observational study was conducted after the approval from Ethical Committee. Seventy samples of endotracheal and tracheostomy secretions were included in the study which were received from ICU (MICU, SICU) patients for culture and sensitivity between Jan 2016 to June 2016 in the Microbiology Laboratory of MGM medical college Aurangabad. Gram's staining of received samples was done to rule out whether it was a colonizer /pathogen using Qscore (Barret Score) (Winn *et al.*, 2006). Received samples were inoculated on 5% sheep blood agar and MacConkeys agar and incubated overnight at 35°C. Each growth was gram stained to see whether it was gram positive /gram negative cocci /bacilli (Patricia, 2014). The card is selected. Each organism suspension was prepared from the growth of pure cultures. Bacterial cells were suspended in 2.5 ml of a 0.45% sodium chloride solution. The suspension used in the VITEK 2 system was adjusted to 0.5 Mcfarland standard by densicheck (BiomeriueX). Isolated organisms were subjected for identification of MRSA, ESBL and CARBAPENEMASE and susceptibility testing was done using Vitek 2 compact system (As per manufacturer's instruction).

Results and Discussion

The present study was carried out in a tertiary care hospital in Aurangabad. The samples received in the department of Microbiology were processed for culture and sensitivity by using Vitek 2 compact system. It identified

MRSA, ESBL and CARBAPENEMASE producers were identified with Vitek 2 compact system and recorded by it.

Out of 70 samples received in microbiology lab from the patients admitted in ICUs 15 (31.42%) were culture negative and 48 (68.57%) were culture positive. Upon arrival of the sample in the microbiology lab the relevant history, sign and symptoms of the patient were noted so as to make a correlation with the clinical condition of the patient.

Out of the culture positive cases 41 (85.41%) were monomicrobial and 7 (14.59%) were polymicrobial (2 organisms in 7 samples). Polymicrobial growth was most commonly associated with those patients who were being treated for diseases requiring prolonged stay in the hospital. Moreover polymicrobial growth was also associated with invasive procedures like central lines or catheterization.

The analysis of the organisms isolated from endotracheal and tracheal tube secretions showed that the commonest organism isolated were *Pseudomonas* spp, *Acinetobacter* each (25.54%) and *Klebsiella* (20%)(Table 1).

The analysis of origin of the microbiology sample revealed that the 51 samples were received from endotracheal secretion while 19 samples were from tracheal tube secretions. The presence of predominantly endotracheal samples was probably because of artificial ventilation required in intensive care units (Table 2).

Amongst isolated organisms gram positive cocci were 8 (14.54%) and Gram negative organisms were 47(85.46%) (Table 3). The analysis of organisms isolated from the ICU patients revealed that out of 8 (14.54%) staphylococcus (aureus and coagulase negative *Staphylococci*) isolated 5 were

Methicillin resistant *Staphylococcus aureus* (MRSA) (3) and 2 were methicillin resistant coagulase negative *Staphylococci* (MRCONS). Out of these 5 samples 3 (5.45%) belonged to SICU and 2 (3.63%) belonged to MICU. So out of all MRSA isolated 60% belonged to SICU and 40% belonged to MICU.

Out of the total 5 MRSA, MRCONS isolates, the highest susceptibility was noted for Vancomycin (100%), Rifampicin and Linezolid (80 % susceptibility for each one of them) followed by Azithromycin (40%) and erythromycin (20%).

Regarding isolation of Gram Negative Bacilli (GNB) from samples of ICU patients, Out of isolates of *E. coli*, and *Klebsiella* spp. 8 (57.14%) were ESBL producers.

Out of 3 ESBL producers of *E. coli*, 2 isolates were obtained from MICU and 1 isolate was found from the samples from SICU and out of 5 ESBL producers of *Klebsiella* spp., 3 were obtained from MICU and 2 isolates were found from SICU. The final analysis revealed that most of the extended spectrum beta lactase producing organisms was found in Endotracheal tube samples from Medical intensive care unit.

Out of total 8 ESBL producing *E. coli* and *Klebsiella* 6 were found in samples from endotracheal tube secretions and 2 were found in tracheostomy tubes.

The final analysis of ESBL producing organism revealed that the highest susceptibility of the ESBL producing organisms was found to be towards colistin, tigicycline followed by imipenem. While ESBL producing *E. coli* were 66.66% and *Klebsiella* were 80% resistant to piperacillin/tazobactam. Out of the culture positive ESBL isolates of *E. coli* organism the

highest susceptibility was noted for amikacin (100%) followed by colistin (66.66%), tigicycline (66.66%) and imipenem(66%). Whereas for *Klebsiella* isolates, the highest susceptibility was noted for colistin (80%) and tigicycline (80%) followed by imipenem (60%), levofloxacin and amikacin.

Analysis of carbapenemase producing organisms revealed that *Acinetobacter* (42.85%) was the most common organism producing carbapenemase in various samples received from intensive care units. Out of 14 *Acinetobacter* 12 (85.71%) were carbapenemase producer and among 14 *Pseudomonas* 8 (28.57%) were carbapenemase producers (Table 9). Likewise carbapenamase producing isolates on susceptibility testing revealed the similar pattern of susceptibility. They are found to be most sensitive to tigicycline, colistin and rifampicin. The susceptibility pattern of *Acinetobacter*, *Pseudomonas*, *E. coli* *Klebsiella* for colistin is 91.66%, 87.5%, 100%, 80% and for tigeicycline is 100%,

87.5%, 100% and 80% respectively. While none of carbapenemase was susceptible to imipenem. The maximum isolation of carbapenemase producing organisms was from SICU (Table 11).

The analysis of resistance patterns of various organisms depending upon their sample origin revealed that the most of the organisms belonging to both ET and TT were carbapenemase producing followed by ESBL producing and MRSA (Table 12).

The analysis of the data in our study revealed that the most common drug effective for the treatment of MRSA was rifampicin, lizolid and teicoplanin while the ESBL and carbapenemase producing gram negative organisms like *E. coli* and *Klebsiella* were found to be susceptible to colistin, tigicyclin and rifampicin and in emergency situation these susceptibility patterns are important while starting empirical antibiotic therapy in ICU patients belonging to our hospital

Table.1 Organisms isolated from ET and TT of patients admitted in ICU

S.No.	Organism	Number (%)
1	<i>Staphylococcus aureus</i>	3 (5.45%)
2	CONS	5 (9.09%)
3	<i>Enterobacter aerogens</i>	2 (3.64%)
4	<i>E-coli.</i>	3 (5.45%)
5	<i>Klebsiella Spp</i>	11 (20%)
6	<i>Stenotrophomonas</i>	1 (1.82%)
7	<i>Serratia</i>	2 (3.64%)
8	<i>Pseudomonas spp</i>	14 (25.45%)
9	<i>Acinetobacter spp</i>	14 (25.45%)
	Total	55 (100%)

Table.2 Origin of samples in studied cases

Sample	No of samples
Endotracheal Tube	51 (72.85%)
TT	19 (27.15%)

Table.3 Percentage of GNB and GPC

GNB	47(85.46%)
GPC	8 (14.54%)

Table.4 MRSA isolated in different types of ICUs

Location	Number of MRSA, MRCONS
SICU	3
MICU	2

Table.5 Susceptibility pattern of MRSA isolates

	Erythromycin	Azithromycin	Levofloxacin	Rifampicin	Linezolid	Teicoplanin	Vancomycin
MRSA (n=5)	1(20%)	2(40 %)	None	4 (80 %)	4 (80%)	3 (60%)	5(100%)

Table.6 ESBL producing *E. coli* and *Klebsiella* spp. isolated in different ICUs

Type Of ICU	<i>E. coli</i> (n=3)	<i>Klebsiella</i> spp (n=5)
MICU	2	3
SICU	1	2

Table.7 Sample wise distribution of ESBL producing *E. coli* and *Klebsiella* spp. isolated in different ICUs

Type Of Specimen	<i>E. coli</i> (n=3)	<i>Klebsiella</i> spp (n=5)
Endotracheal Tube Secretions	2	4
TT	1	1

Table.8 Susceptibility pattern of ESBL producing *Klebsiella* spp. and *E. coli*

ESBL	COL	Tig	LE	AK	PIT	CTR	IPM
<i>E. coli</i> (n=3)	2	2	2	3	1	None	2
<i>Klebsiella</i> Spp (n=5)	4	4	2	2	1	1	3

Table.9 Carbapenemase producing organisms

Type of organism	Number	Percentage%
<i>Acinetobacter</i>	12 (42.85%)	85.71%
<i>Pseudomonas</i>	8 (28.57%)	57.14%
<i>Klebsiella</i>	5 (17.85%)	45.45%
E-coli	1 (3.57%)	33.33%
Others	2 (7.14%)	66.66%
Total	28 (100%)	

Table.10 Susceptibility pattern of carbapenemase producing *Acinetobacter*, *Pseudomonas*, *Klebsiella* spp. and *E. coli*

Carbapenemase Producing isolates	Rif	COL	Tig	LE	AK	IMP	CTR
<i>Acinetobacter</i> (n=12)	10	11	12	9	8	None	1
<i>Pseudomonas</i> (n=8)	6	7	7	7	6	None	2
<i>E. coli</i> (n=1)	1	1	1	None	1	None	None
<i>Klebsiella</i> Spp (n=5)	4	4	4	3	2	None	1

Table.11 Carbapenemase producing organisms isolated in different ICUs

Type of ICU	
MICU	12
SICU	16

Table.12 Organisms producing carbapenemase/ ESBL/ MRSA

Sample	Organisms producing carbapenemase/ ESBL/ MRSA	
ET	Carbapenemase producing	22
	ESBL	6
	MRSA	3
TT	Carbapenemase producing	6
	ESBL	2
	MRSA	2

Figure.1 Culture positive and culture negative samples

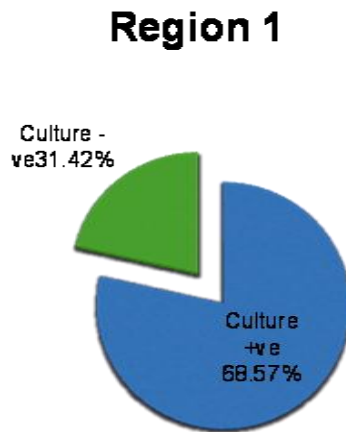
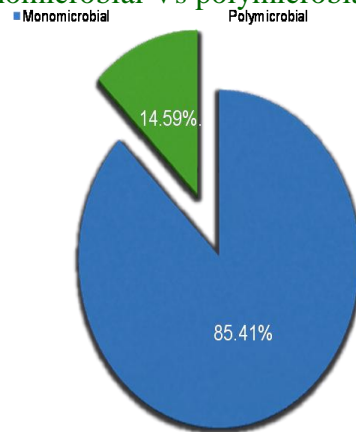


Figure.2 Monomicrobial Vs polymicrobial growth



Out of 70 samples 48 (68.57%) were culture positive in which 41 (87.27%) were monomicrobial and 7 (12.72%) were polymicrobial (2 organisms in 7 samples).

There were 42 (60%) male and 28 (40%) females. Out of 55 isolated organisms 8 were gram positive cocci (14.54%) and Gram negative organisms 47 (85.46%). Thus total 9 types of organisms were recovered from 48 culture positive samples. The culture positivity in Pratirodh Koirala et al., study was higher (90%) compared to our culture positivity rate of 68.57%, might be due to our hospitals good infection control practices. Our study results of culture positivity is in concordance with results of Ashis Kumar Saha (59.6%) and incidence of male positivity was 69.17%, female positivity was (26.25%) (Ashis et al., 2016) In our study the predominant organisms isolated were Gram negative organisms 47 (85.46%) from all the ICUs including *Pseudomonas* spp (25.54%), *Acinetobacter* spp (25.54%), *Klebsiella* spp. (20%) and *Staphylococci* (14.54%) followed by, *E. coli* (5.45%), *Enterobacter aerogens* (3.64%), *Serratia marscens* (3.64%) and *stenotrophomonas* (1.82%), which matches to a study by Abdullahi Alireza et al., (2013) study, in their study Gram negative were the most common isolated organisms including *Acinetobacter* (24.2%) was the commonest isolated organism from endotracheal aspirates, followed by *Pseudomonas* (16.7%) and *staphylococci*(12%). A study by Tandia et al., also matches to our study, the commonest isolated organism was *Pseudomonas*, followed by *staphylococci* in their study.

Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India conducted a comprehensive research about the prevalence of MRSA across major hospitals and medical institutes of India they found the incidence of MRSA to be 53 % at all India institute of medical sciences, 60% at Jawahar Lal Nehru institute of postgraduate medical education and research puducherry, 84% at regional institute of medical sciences imphal. These results were similar to the results of our study in which we found the

incidence of MRSA to be 65.5% (Joshi et al., 2013). Barai et al in Dhaka also had a MRSA prevalence of 77% (Barai et al., 2010).

Out of the all isolates of MRSA, the highest susceptibility, about 80%, was noted for linezolid and rifampicin. Many studies have concluded similar sensitivity patterns for MRSA sensitivity. Ali khaled et al., (2013) in their study found MRSA to be uniformly sensitive to linezolid. Al-zoubi et al., (2015) conducted a study on the sensitivity patterns of MRSA isolates, they found 85.8 % of the isolated MRSA sensitive to rifampicin. CLSI recommends that all resistant isolates for linezolid should be confirmed by MIC testing. However this was not done in our study. If at all if it would have been done, the susceptibility to linezolid would have been still higher like Kassim et al., in their study, 1103 *S. aureus* MICs were analyzed and found that susceptibility to linezolid, vancomycin and clindamycin, was comparable between the two guidelines.

The growth and sensitivity patterns of ESBL also revealed these organisms to be sensitive to rifampicin, colistin and tigicycline. Bishara et al., (2005) found ESBL producing *E. coli* and *Klebsiella* to be equally susceptible to colistin and etrapenem and 82% resistant for *Klebsiella* to Piperacillin /tazobactam. This sensitivity to colistin and resistance pattern of ESBL for Piperacillin /tazobactam (80%) also matches to our study for ESBL *Klebsiella*. Hoban et al., (2007) found tigeicycline and minocycline to be most active against extended-spectrum beta-lactamase producers and multidrug-resistant *Acinetobacter baumannii* and these were resistant to cefepime, ceftazidime, ceftriaxone, levofloxacin, and piperacillin–tazobactam.

The carbapenemase producing organisms in our study were found to be susceptible to rifampicin, colistin and tigicycline. These

findings were in conformity with many studies conducted on the growth and sensitivity patterns of carbapenemase producing organisms. Dheepa muthusamy *et al.*, (2015) found a good number of carbapenem resistant organisms to be sensitive to polymyxins like colistin and polymyxin B. Hence, they recommended that polymyxins like colistin and polymyxin B to be considered as the next drug of choice for carbapenem resistant strains. In this study these isolates were 100% resistant to Imipenem this result of imipenem was in concordance with our study. As in our study in Amina Kandeel's study she stated 100% sensitivity of carbapenemase producers to Colistin followed by tigecycline. But in contrast to this a number of other studies by Soren *et al.*, and Lee *et al.*, have found rifampicin to be highly effective in treating infections caused by Gm-ve ESBL producing organisms (Soren *et al.*, 2015; Lee *et al.*, 2013).

It is concluded in the present study that the prevalence of MRSA, ESBL and Carbapenemase producing isolates was 62.5%, 57.14% and 59.57% respectively. Most of the MRSA isolates and Carbapenemase producers were recovered from SICU. The maximum ESBL producing isolates were *Klebsiella* spp. and were recovered from MICU and from endotracheal secretions. So as to reduce the prevalence of these pathogens we need to strengthen the infection control practices in ICUs, screening of health care worker for MRSA nasal carriage and their treatment, to avoid excessive antibiotic usage, prolonged hospitalization and unnecessary intravascular catheterization. MRSA isolates were found to be most sensitive to antibiotics like Vancomycin, Rifampicin, linezolid. ESBL producing organisms were found to be susceptible to drugs like colistin, tigecycline, amikacin and Imipenem, while

carbapenemase producing organisms were found to be most susceptible to antibiotics like, colistin, tigecycline and rifampicin. The present results can be used as a guide for choosing an appropriate therapy, particularly when treating suspected MRSA, ESBL and Carbapenemase infections in our ICU patients. These guidelines can decrease morbidity and mortality in patients admitted in ICU.

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