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ANTIBIOTICS RESISTANCE PATTERN AMONG GRAM POSITIVE COCCI ISOLATED FROM INTENSIVE CARE UNITS IN A TERTIARY CARE HOSPITAL

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ABSTRACT Multi drug resistant Gram positive cocci are implicated in life threatening infections, especially in critical care units. In the current study which was conducted from June 2018 to March 2019, the prevalence and antimicrobial resistance among gram positive cocci isolated from intensive care units of a tertiary care hospital has been detected. Various samples like urine, pus, body fluids, sputum etc. were processed according to standard procedures. Total 81 gram positive cocci were isolated from 1650 samples. Maximum gram positive cocci were coagulase negative Staphylococcus followed by Staphylococcus aureus and enterococci. Coagulase negative Staphylococcus showed highest resistance to cotrimoxazole, erythromycin, ciprofloxacin, and penicillin and maximum sensitivity to gentamicin. Staphylococcus aureus showed maximum resistance to penicillin, tetracycline, co-trimoxazole and ciprofloxacin. All the isolates were sensitive to linezolid and vancomicin. Antimicrobial resistance among enterococci varied from 65% to 85%. Formation of antibiogram and strict adherence to hospital antibiotic policy is crucial to prevent spread of resistance among microorganisms.

INTRODUCTION:

Globally Intensive care units are exploding with multidrug resistant organisms. Both multidrug resistant (MDR) gram negative bacilli and multidrug resistant gram positive cocci are implicated in the epidemic of antimicrobial resistance.

In developing world, gram negative bacilli are more commonly isolated MDR bacteria, compared to gram positive cocci, [1, 2] however these MDR gram positive cocci too, are responsible for increased morbidity, mortality and elevated hospital cost. Common gram positive cocci isolated from ICU settings are methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant enterococcus (VRE), Vancomycin resistant *Staphylococcus aureus* (VRSA), Coagulase negative Staphylococcus (CONS), Penicillin resistant *Streptococcus pneumoniae*.[3]

According to a review given by World health organisation (WHO), low and middle income countries have two – three times higher chances of intensive care unit acquired infections than in high income countries.[4,5] Emergence of multidrug resistant bacteria in India could be due to abundant availability and hassle free sale of antibiotics, irrational and sub optimal dose administration, abrupt stoppage of antibiotic treatment and frequent self treatment by the patients. The repeated and frequent use and misuse of certain agents of several antimicrobial classes may result in selection of organisms which are resistant to a particular antimicrobial. It has been found that twenty to fifty percent of all antibiotics usage was demonstrated as improper.[6]

Complications associated with antimicrobial resistance is not only restricted to development of resistance among microorganism but also to the spread of these resistant strains in the health care facility, especially by not following the hospital infection control policy and unconstraint sale of antibiotics over the counter without prescription.

Blooming number of MRSA are associated with poor clinical outcome and they also impose financial burden. Battle against methicillin resistant *Staphylococcus aureus* is becoming hard as these organisms are resistant to not only available β - lactam antibiotics but also other to classes of antimicrobials, leaving clinician with limited treatment options. [5]

Hitherto, clinicians were treating infections with antibiotics based on their previous experiences. Nonetheless the scenario has changed due to emergence of resistance to essentially all the antimicrobials. This has been made clinicians dependent on data from local hospital antibiogram.[7]

Since the patient in intensive care units are in critical conditions and required urgent initiation of antibiotic treatment, a local antibiogram of that particular hospital will be of immense help for the clinicians to start empirical treatment. [8, 9]

Hence the choice and monitoring of antimicrobial therapy is of maximum importance to avert the springing up of antimicrobial resistance. [6]

Prevalence and resistance pattern of gram positive cocci have not been studied much in contrast to gram negative bacteria. The present study was carried out with the aim of finding prevalence of gram positive cocci and their antimicrobial resistance pattern in various intensive care units of a tertiary care hospital.

MATERIAL AND METHODS

The prospective study was carried out in the department of Microbiology, Chhatrapati Shivaji Subharti Hospital during the period of June 2018 to March 2019. The study included a total of 1650 samples, taken from patients of both sexes, irrespective of age groups, admitted in the intensive care units.

Inclusion criteria:

All the samples (Pus, urine, tracheal aspirates, Pleural fluid, CSF, sputum, other body fluids) received in the clinical microbiology laboratory of Chhatrapati Shivaji Subharti Hospital.

Exclusion criteria:

Blood and stool sample were not included in the study, fungi and anaerobic bacteria were also excluded from the study.

Samples were inoculated on to Blood agar, chocolate agar and MacConkey agar. Urine sample were inoculated on cystine lactose electrolytes deficient agar (CLED).

Direct microscopy was done for all samples. The media were incubated at 37°C for 24 to 48 hrs. The isolates were identified by gram staining, colony morphology and standard biochemical tests. [10] Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method. Following antibiotic disks were used for gram positive cocci. Penicillin G (10 μg), Ampicillin (10 μg), Cefoxitin (30 μg), Cotrimoxazole (1.25/232.75 μg), Tetracycline (30 μg), Erythromycin (15 μg), Clindamycin (02 μg), Moxifloxacin (5 μg), Chloramphenicol (30 μg), Gentamicin (10 μg),

Teicoplanin (30 µg), Linezolid (30 µg), Novobiocin (30 µg), Bacitracin (5 µg), Optochin (0.04 Unit), Gentamicin high level (120 µg), Streptomycin high level (300 µg). MIC for Vancomycin was detected by broth dilution method.

Methicillin resistance in *Staphylococcus aureus* was detected by using 30 µg cefoxitin disk, by modified Kirby-Bauer disk diffusion method as per the standard guidelines. If the diameter of zone of inhibition was > 22 mm then it was consider methicillin resistant *Staphylococcus aureus* (MRSA), and if the zone of inhibition diameter was > 25 mm in case of coagulase negative Staphylococcus, then they were considered as methicillin resistant coagulase negative Staphylococcus (MR-CoNS).

RESULTS

A total of 1650 sample were processed in the present study from June 2018 to March 2019.

Out of which 81 (5.0 %) sample grew gram positive cocci. Fifty were from male patients and thirty one from female patients. Gram positive cocci were isolated throughout the study periods and maximum cases were seen during the month of July and August.

Table 1: Age wise distribution of patients with Gram Positive Cocci growth (n= 81)

S.no.	Age(in years)	No. of Isolates	Percentage
1.	<10	12	14.81
2.	11-20	4	4.94
3.	21-30	4	4.94
4.	31-40	8	9.88
5.	41-50	11	13.58
6.	51-60	17	20.99
7.	> 61	25	30.86
Total	81	100	

Table 2: ICUs wise distribution of Gram Positive Cocci (n =81)

ICUs	Isolates	Percentage
SICU	33	40.75
MICU	21	25.93
RICU	12	14.81
SPL.ICU	08	9.89
PICU	04	4.94
NICU	02	2.45
BICU	01	1.23
Total	81	100
P value=<0.0007(S)		

Table 3: Bacteriological Profile of Gram positive cocci (n = 81)

Bacteria Isolated	Number of Isolate	Percentage(%)
Coagulase negative staphylococcus (CoNS)	40	49.38
Staphylococcus aureus	24	29.63
Enterococci	17	20.99
Total	81	100

Table 4: Percentage of resistant pattern of Gram positive cocci isolated from ICUs samples to various antibiotics (%) (n=81)

Antibiotics	Staph.Aureus	CoNS	Enterococcus
Penicillin	92	65	85
Ampicillin	Nt	Nt	80
Erythromycin	20	75	80
Clindamycin	20	70	80
Co-trimoxazole	85	75	Nt
Tetracycline	86	43	Nt
Ciprofloxacin	78	70	Nt
Moxifloxacin	65	60	Nt
Chloramphenicol	20	35	85
Gentamicin	15	15	Nt
Linezolid	0	30	0
Vancomycin	0	20	0
Teicoplanin	Nt	Nt	0
HLG	Nt	Nt	65
HLS	Nt	Nt	65

DISCUSSION

It is an established fact that intensive care units are the breeding places for development of antimicrobial resistance among microorganisms. Due to vulnerable patients admitted in intensive care units and large usage of antibiotics in these settings, ICUs are becoming hotbed for antimicrobial resistance. Main goal of clinicians and clinical microbiologists is to decrease the antimicrobial resistance among microorganisms, hence to improve clinical outcome and health care costs.

In the current study majority of the isolates were obtained from male patients (62.5 %) compared to female (37.5 %). This is consistent with the study conducted by Metri B C. et al., where they found 60.6 % isolates from male patients.[11] Similarly Zhanel G G et al., found that most of the isolates detected were from male patients.[12] On the contrary, A Al - Jawad et al., found maximum isolates from female patients.[13]

Majority of the isolates were from patients aged 60 and above which is probably due to deteriorating immunity in old age. Roy A et. al., also found maximum isolates from the patient above 60 year of age.[7]

The most common gram positive cocci isolated in present study was coagulase negative Staphylococcus (CoNS) followed by *Staphylococcus aureus* and enterococci. This is in accordance with the study conducted by Bhatia A et al., Zaveri J R et al., and Savanur S S et al., where they found coagulase negative Staphylococcus as the most common gram positive isolate.[6, 14, 1] In contrast, Roy A et. al., [7] found *Staphylococcus aureus* as predominant gram positive cocci in their study.

CoNS are established skin colonizer and are common contaminants in the sample received in the microbiology laboratory. This could be the reason for maximum CoNS isolates detected in our study.

In our study, CoNS showed highest resistance to cotrimoxazole, erythromycin, ciprofloxacin, and penicillin. Maximum sensitivity was shown to gentamicin. Resistance to linezolid and vancomycin was 30 % and 20 % respectively. Bhatiya A et al. found that almost all the CoNS isolates showed resistance to penicillin. They also found quite high resistance to ciprofloxacin, erythromycin and levofloxacin.[6]

In current study, *Staphylococcus aureus* showed maximum resistance to penicillin, tetracycline, co-trimoxazole, and ciprofloxacin. Ahmad S et al., detected maximum resistance among *Staphylococcus aureus* to ciprofloxacin, amoxicillin/clavulanic acid, erythromycin and ceftriaxone which varied from 80 to 100 %.[15] On the contrary Roy A et al., detected low antimicrobial resistance pattern in their study in Imphal, i.e. resistant to ciprofloxacin and co - trimoxazole varied from 15% to 18%.[7]

This diverse difference in antimicrobial resistance among different studies depends on several factors like follow up of infection control practices, practice of rational antibiotic usages and availability of required facilities in the health care settings.

Eighty three percent of staphylococcus aureus and 62.5 % of CoNS were resistant to methicillin in the current study (MRSA p value = <0.0035 and MR-CoNS p valve =<0.0031, which shows that the results obtained were significant). This is similar to the study conducted by Metri B C et al., where they detected 82.5 % of MRSA.[11]

For the treatment of methicillin resistant staphylococcus (MRSA), vancomycin is commonly used drug, but resistance is emerging against vancomycin as well. However, in our study we found all the *staphylococcus aureus* isolates susceptible to vancomycin which was tested for MIC by broth dilution method. Metri B C et al., and Ahmad S et al., too found all the strains of *Staphylococcus aureus* and CoNS susceptible to linezolid and vancomycin.[11, 15] Savanur S S et al., detected 100% resistance to Vancomycin and clindamycin amongst Staphylococcus, contrary to our study.[1]

In present study, there was quite high antimicrobial resistance detected among enterococcus species. It varied from 65% to

85%. Because of the intrinsic resistance to various antibiotics like ampicillin , aminoglycosides and glycopeptides, enterococcus species are of increasing importance in the hospital infections including intensive care unit.[3]

In their study Savanur S S et al., found that enterococcus showed maximum resistance to cephalosporin 84.6%, cotrimoxazole 76% and amoxicillin 61%. [1]

Limitation:
Anaerobic bacteria and fungal pathogens were not including in this study.

CONCLUSION:
As the study was conducted in a tertiary care centre where, often referred patients are admitted in severe sepsis and would have exposed to prior antibiotic administration. This could be the reason for high resistance seen in our study. To tackle the unrestrained antimicrobial resistance, every health care setting should draw up its antibiotic policy and prepare antibiogram to help clinicians in selecting most rational antimicrobial for empirical treatment of the patients. Also antimicrobial stewardship program should be strengthened and should get full support from hospital administration.

Conflict of interest: None declare

Ethics approval: The study has been approved by Institutional ethical and research committee.

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