Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Blood Samples

Mahesh Chandra[1], Amandeep Kaur[2]

[1]M. Sc. Medical Microbiology Student, Department of Microbiology, Adesh Institute of Medical Science and Research, Bathinda.
[2]Professor, Department of Microbiology, Adesh Institute of Medical Science and Research, Bathinda.

Corresponding Author: Amandeep Kaur

ABSTRACT

Introduction: *Staphylococcus aureus (S. aureus)* is a Gram-positive cocci arranged in grape like clusters, non-motile, non-sporing, non-capsulated, that causes nosocomial infections, severe blood infections, bacteremia, food poisoning, cutaneous infections, toxic shock syndrome.

Objectives: To isolate and identify *S. aureus* from blood samples.
To perform antimicrobial susceptibility testing of *S. aureus*.
To find prevalence of bacteremia caused due to *S. aureus*.

Material & Methods: The study was carried out from October 2021 to March 2022, A total 1,270 samples were received in Bacteriology section from patients admitted in various ICUs & EMR of AIMSR hospital. *S. aureus* was identified on the basis of colony characteristics, gram staining morphology and biochemical tests. Antimicrobial susceptibility testing was done by automated method, Vitek 2 compact machine using P628AST card.

Results: The prevalence of *S. aureus* in culture positive blood samples was 2.83%. Maximum *S. aureus* were obtained from CCU department and minimum from NICU & PICU. *S. aureus* isolation was slightly more in males 25(69.4%) than females 11 (30.6%) and was found to be same in the age group of 21-40 & 41-60 years (30.60%) as compared to other age groups. *S. aureus* isolates were highly sensitive to teicoplanin, vancomycin, tigecycline, doxycycline, minocycline (100%). Sensitivity towards linezolid was recorded as (96%), Tetracycline (86.1%) and Daptomycin (77.7%).

However, they showed resistance to Benzylpenicillin, & Oxacillin (100%), Ciprofloxacin & Levofloxacin (83.3%), Erythromycin (69.5%), Cotrimoxazole (52.8%), Clindamycin (41.7%), Gentamicin (38.9%), Daptomycin (22.3%), Tetracycline (13.9%), Rifampicin (5.6%), Linezolid (3%).

Conclusion: *S. aureus* bacteremia causes a health burden, particularly in low and middle income countries.
S. aureus infections are a significant clinical problem in medical practice as the organism shows resistance to the commonly used first line drugs.

Keywords: Staphylococcus aureus, Gram positive cocci, Bacteremia, methicillin resistant S. aureus, antimicrobial susceptibility testing.

INTRODUCTION

Bloodstream infections (BSIs) are one of the most common severe diseases, causing significant morbidity and mortality among hospitalized patients around the world.[1] These infections are among the most frequent hospital infections, causing significant morbidity and mortality around the world.[2] BSIs are exceedingly frequent in newborns and children, and they have become common causes of death and morbidity.[3] Bacterial isolation from blood cultures usually indicates a significant invasive infection that requires antibiotic treatment right away.[4] *S. aureus* is a common cause of infection in the population and in hospitals, causing skin infections to

deep tissue infections &life-threatening pneumonia, endocarditis, septic arthritis and septicemia. [5] Coagulase increases *S. aureus* pathogenicity by blocking phagocytosis. It creates a fibrin clot wall around the lesion.[6]. Cloxacillins are antibiotics that are used to treat beta-lactamase-producing bacteria. Vancomycin is a drug that is used to treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). In hospitals, some vancomycin and teicoplanin resistance have emerged.[7]

MATERIALS AND METHODS

A study was carried out for six months from October 2021 to March 2022. Blood samples were received from patients suspected of having blood stream infection, nosocomial infection etc., admitted in Adesh hospital Bathinda in various ICUs & EMR departments. All the patient's identification details like Hospital CR. Number, Lab. Number, patient name, age, gender &Department name with specimen collection date were recorded on a formatted proforma. A total no of 1,270 blood samples were received during the study period from various ICUs (ICU, NICU, PICU, CCU) and EMR of AIMSR hospital

Blood samples were collected from bedside of the patients by the trained nursing staff using sterile syringe. A sample of 1.5-3.0 ml was taken from children and newborns and 2.5 ml- 5.0 ml for age group more than 12 years old blood sample was collected and added to respective blood culture bottles. The blood sample was quickly processed for culture using an automated Bact/Alert system. When adequate bacterial growth happened, the system automatically issued a signal, if no bacterial growth occurred within 5 days of culture, it was reported as sterile.

Identification of the bacterial Isolates

Blood samples which were alarmed as positive by the Bact/Alert system were further inoculated on blood agar,

MacConkey agar & nutrient agar plates used streaking culture methods.[8] Plates were incubated at 37°C for 24-48 hrs. The isolates which were beta- hemolytic, small, lactose fermenting colonies and produced yellow pigment on nutrient agar were subjected to gram staining and appropriate biochemical reactions for identification of *S. aureus*.[9]

Antimicrobial susceptibility testing

Antibiotic susceptibility test was performed by Vitek 2 compact automated machine, using P628 card recommended by Clinical Laboratory Standard Institute (CLSI) guidelines.[10] An isolated colony of *S. aureus* was picked and added into sterile saline solution provided by the BioMerieux to make a suspension equivalent to a 0.5 McFarland standard turbidity was adjusted by using a DensiCHEK plus (BioMerieux) and further processed as per the manufacture's instruction P628 AST panel was used for susceptibility testing of *S. aureus*.

RESULTS

Out of total 1270 blood samples processed; 195 samples showed growth, culture positivity was 15.3% and 84.7% were culture negative. 836 (65.82%) samples were received from males and 434 (34.18%) from female patients. Out of total bacterial isolates. 36 isolates were identified as *S. aureus*. From males, 25 (69.5%) and females, 11 (30.5%) isolates were obtained. Maximum isolates were obtained from patients with age group of 21-60 years (30.60%) as compared to other age groups. (Table 1)

Table 1. Age wise distribution of bacteremia due to S. aureus

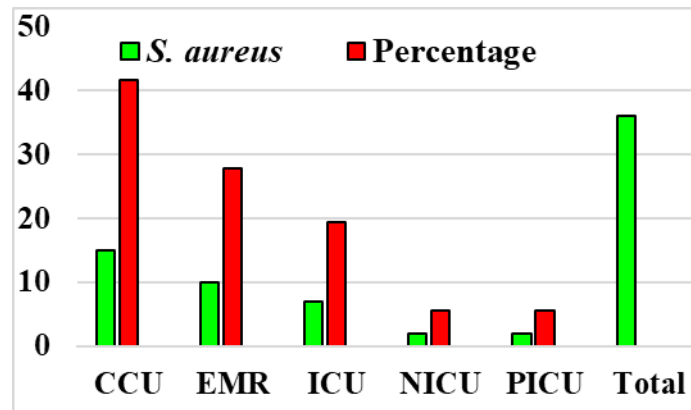| Sr no.	Age Group	No of isolates	Percentage (%)
1	0-20	04	11.12%
2	21-40	11	30.60%
3	41-60	11	30.60%
4	61-80	08	22.23%
5	80-100	02	5.45%
6	Total	36	100%

Figure 1: *S. aureus* obtained from various ICUs departments.

S. aureus isolates showed 100% susceptibility towards Teicoplanin, Vancomycin, Tigecycline, Doxycycline, Minocycline antibiotic sensitivity was recorded for Cotrimoxazole (47.2%), Erythromycin (30.5%), Ciprofloxacin (16.6%), Levofloxacin (16.7%). Isolates of S. aureus were 100 % resistant to Benzylpenicillin and Oxacillin, Ciprofloxacin, & Levofloxacin (83.3%). while moderate resistance was seen for Erythromycin (69.5%) & Cotrimoxazole (52.8%). Lesser antibiotic resistance was observed for Clindamycin (41.7%), Gentamicin (38.9%), Daptomycin (22.3%), Tetracycline (13.9%), Rifampicin (5.6%), Linezolid (3%) as shown in Table 2

Table 2: Antibiogram of S.aureus (N= 36)

Antibiotics	No. of Sensitive isolates (%)	No. of Resistant isolates (%)
Benzylpenicillin	(00) 00%	(36) 100%
Oxacillin	(00) 00%	(36) 100%
Gentamicin	(22) 61.1%	(14) 38.9%
Ciprofloxacin	(06) 16.6%	(30) 83.3%
Levofloxacin	(06) 16.6%	(30) 83.3%
Erythromycin	(11) 30.5%	(25) 69.5%
Clindamycin	(21) 58.3%	(15) 41.7%
Linezolid	(35) 97.0%	(01) 3.0%
Daptomycin	(28) 77.7%	(08) 22.3%
Teicoplanin	(36) 100%	(00) 0%
Vancomycin	(36) 100%	(00) 0%
Tetracycline	(31) 86.1%	(05) 13.9%
Tigecycline	(36) 100%	(00) 0%
Rifampicin	(34) 94.4%	(02) 5.6%
Cotrimoxazole	(17) 47.2%	(19) 52.8%
Doxycycline	(36) 100%	(00) 0%
Minocycline	(36) 100%	(00) 0%

DISCUSSION

In the present study prevalence of *S. aureus* bacteremia was reported to be 2.83%. This is similar with Kante. et al and Jhajhria. et al who have reported 4.5% and 3.5% *S. aureus* bacteremia respectively.[8,11,] Wasihum et al and Mittal et al reported prevalence of *S. aureus* in bacteremia to be 10.5% &13.3% respectively. Therefore, prevalence reported by these studies is higher as compared to present study.[12,13]

In the present study, *S. aureus* bacteremia was observed more in males (69.5%) than in females (30.5%) mainly due to more admission rate of males as compared to females in this hospital ICUs & EMR. The study by Zeki et al and Jhajhria et al correlate with the results of this study as they have also reported more prevalence of *S. aureus* bacteremia in males as compared to females.[14,11]

In the present study maximum *S. aureus* were obtained from age group 21-40 years & 41-60 years (30.6%) followed by other groups was 61- 80 years (22.3%), 0-20 years (11.2%) and 80-100 years (5.45%). Afrasyabi et al reported that, *S. aureus* bacteremia was highest (53.3%) in age group 21-40 years, (31.7%) in age group 41-60 years and equally in age group 0-20 & 61-80 years (6.0%).[15] Mittal et al reported that maximum numbers of positive blood cultures were from age group of 18-60 years.[13] The results of present study are similar to study by Afrasyabi et al and Mittal et al.

In the present study, *S. aureus* isolates were highly sensitive to Teicoplanin, Vancomycin, Tigecycline, Doxycycline, Minocycline (100%) and Rifampicin 94.4%, while Linezolid (97%), Tetracycline (86.1%), Daptomycin (77.7%), Gentamicin (61.1%), & Clindamycin (58.3%). Lesser sensitivity was for Cotrimoxazole (47.2%), Erythromycin (30.5%), Ciprofloxacin (16.6%), Levofloxacin (16.7%). Benzylpenicillin and Oxacillin (100%) completely resistant.

In a study by Kumhar et al most of the isolates of *S. aureus* were found susceptible to Vancomycin (82.3%), Erythromycin (34.5%), Oxacillin (30.4%), Co-trimoxazole (29.1%), Gentamicin (27.9%).[16]

In a study done by Kante et al most of the isolates of *S. aureus* were found highly sensitive to Erythromycin (100%) and Vancomycin (100%).[8]

Wasihun et al reported in a study, that most of the isolates of *S. aureus* were found sensitive to Oxacillin (70.4%), Cotrimoxazole (66.7%), Doxycycline (53.7%), Erythromycin (44.4%), Ciprofloxacin (38.9%), Gentamicin (33.3%).[12]

Another study by Jhajhria et al, most of the isolates of *S. aureus* were found highly sensitive to Vancomycin & Linezolid (100%), Oxacillin (71.4%), Ciprofloxacin & Teicoplanin (57.1%), Gentamicin (42.8%), Cotrimoxazole (35.7%), Erythromycin (7.1%).[11] Mittal et al reported that, most of the isolates of *S. aureus* was found to be highly sensitive to Vancomycin & Linezolid (100%), Teicoplanin (95%), Gentamicin (70%), Clindamycin& Tetracycline (40%), Ciprofloxacin (20%).[13]

Zeki et al reported that most of *S. aureus* were found to be highly sensitive to Linezolid and Vancomycin (100%), Gentamicin (80%), Clindamycin55.5%, Cotrimoxazole (50.7%), ciprofloxacin (46.0%), Erythromycin (38.0%).[14]

Sweta et al reported that, most of the isolates of *S. aureus* were found highly sensitive to Vancomycin, Teicoplanin, Linezolid and Clindamycin (100%) [3]

The results of present study are in concordance with Kante et al and Mittal et al. reporting that vancomycin, teicoplanin and linezolid to be the most effective antibiotics against *S. aureus*.[8,13]

Sensitivity of *S. aureus* towards erythromycin reported by Sweta et al, Kumhar et al and Zeki et al is 34.5%, 38% and 40% respectively which is similar (30.5%) to the sensitivity reported in the present study.[3,16,14] However, Kante et al and Nazir et al had reported *S. aureus* to be highly sensitive towards Erythromycin i.e., 100% and 82% respectively.[8,17]

The sensitivity for Gentamicin recorded in present study is almost similar to Mittal et al i.e. 61%.[13]

On the contrary, many authors have reported very low sensitivity of *S. aureus* towards gentamicin. Kumhar et al & Wasihun et al have reported it to be 28% and 33% respectively.[16,12]

The results of present study are in line with Mittal et al who recorded sensitivity of *S. aureus* for ciprofloxacin to be 20%.[13]

Sensitivity of *S. aureus* towards clindamycin reported by Zeki et al, Nazir et al and Mittal et al is 55.5%, 42% and 40% respectively, which is similar (58.3%) to the sensitivity reported in the present study. [14,17,13] However, Sweta et al reported very high sensitivity of *S. aureus* (100%) towards clindamycin.[3] The sensitivity for cotrimoxazole recorded in present study is almost similar to Nazir et al and Zeki et al

i.e 54% and 50.7% respectively, which is similar (47.2%) to the sensitivity reported in the present study.[17,14] However, Wasihun et al have reported very high sensitivity of *S. aureus* towards cotrimoxazole i.e., 100%.[12]

CONCLUSION

Antimicrobial resistance rates may vary depending on geographic locations, study subjects, and hospital epidemiology. Therefore, in order to develop an antibiotic policy and instruct doctors on how to properly treat such instances, it is unquestionably necessary to ascertain the local incidence of these sensitive bacteria and their resistance profiles. According to the results of the culture, specific antibiotic therapy should be started. Antibiotic stewardship and infection control procedures need to be improved due to high antibiotic resistance rates in hospitals.

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