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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BLOOD SAMPLES OF ICUS PATIENTS

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ABSTRACT: Introduction: Bloodstream infections (BSI) refer to the presence of microorganisms in blood, which constitute one of the most serious situations among infectious diseases; as they are a threat to every organ in the body. Septicemia is a condition in which bacteria circulate and actively multiply in the bloodstream and may produce their products (e.g. toxins) that cause harm to the host Similarly, the presence of viruses, parasites and fungi in blood can be described as 'viremia', 'parasitemia' and 'fungemia' respectively. **Material and Method:** This study was conducted over two years, during which 3,145 BSI suspected patients' blood culture samples were analyzed using the Bact/Alert 3D automated system. *Staphylococcus aureus* isolates were initially identified based on colony morphology and Gram stain followed by final confirmation and antimicrobial susceptibility testing by Vitek 2 Compact system, employing GP and P628 cards. **Result:** In our study, out of 3,145 patients blood samples 315(10.01%) samples showed growth on culture media. Out of 315 culture positive samples 61 (19.36%) was identified as *S. aureus*. Prevalence of *S. aureus* bacteremia was recorded as 1.93%. *S. aureus* isolates were highly sensitive to teicoplanin, doxycycline, minocycline (100%), vancomycin, tigecycline (96.72%), linezolid (91.80%), and rifampicin (90.16%). However, they showed resistance to benzylpenicillin (100%), oxacillin (96.72%), ciprofloxacin (90.17%), levofloxacin (86.89%), erythromycin (67.22%) and cotrimoxazole (57.38%). **Conclusion:** The most effective antimicrobial agent against *S. aureus* was teicoplanin, doxycycline, minocycline, and vancomycin. For antibiotics utilized both inside and outside of hospital settings, we advise a strict drug policy, regular surveillance of hospital-related infections and monitoring of antibiotic sensitivity patterns. The guidelines from the National Centre of Disease Control (NCDC) for the appropriate use of antibiotics should be followed.

INTRODUCTION: Bacteremia refers to the presence of bacteria in blood without any multiplication [1]. Microbial invasion of the bloodstream can cause shock, multiple organ failure, disseminated intravascular coagulation, and mortality in the short term [2].

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Bacteremia is a threat that runs through every organ in the body, whether it is temporary, continuous, intermittent, or transient [3]. Bacteremia is becoming more common in children under the 5 years of age. Diagnosis of bacteremia is also to identify the causative organism in some infections.

Isolation of bacteria from blood cultures generally signifies a serious invasive infection that necessitates immediate antibiotic therapy [4]. The most common sources of bacteremia include the genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infections (5%), miscellaneous sites (10%), and unidentified sources

(10%) [3]. Methicillin resistant *S. aureus* infections are mostly nosocomial and are becoming more common in several places throughout the world [5]. Methicillin was originally used in humans in the 1960s to treat infections caused by penicillin-resistant *S. aureus*, but MRSA appeared just a few years later [5, 6]. Therefore, screening of antimicrobial resistant is important to set up an appropriate antimicrobial therapy. Hospital acquired infection is common infection in all hospitals of various ICU departments. Present study was conducted to determine the prevalence of *staphylococcus aureus* and their antibiotic sensitivity pattern from blood samples of various ICUs of Adesh Institute of Medical Sciences and Research (AIMSR) Hospital.

MATERIAL AND METHODS: A two-years prospective study was conducted on blood samples collected from patients suspected of bacterial infections such as nosocomial infection, or skin infection, food poisoning, admitted to various ICUs of Adesh Hospital, Bathinda. These included the Intensive Care Unit (ICU), Pediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU), and Cardiac Care Unit (CCU). Patient details were documented using a structured proforma. During the study, a total of 3,145 patients blood samples from all age groups were analyzed. Blood samples were collected by well trained staff at the bedside using sterile syringes before antimicrobials started.

For children and newborns (below 12 years), 1.5–3.0 mL of blood was drawn into pediatric yellow-top bottles, while for patients older than 12 years, 2.5–5.0 mL samples were collected in green-top adult bottles. Cultures were processed immediately on automated BACT/ALERT 3D system. The instrument signaled positive results when microbial growth was detected, whereas samples without growth after 5 days were reported as no growth after 5 days of incubation.

Approval of the Research: The study were carried out after clearance of institutional research Committee, Adesh University Bathinda, and Ethics committee for Biomedical and Health Research, Adesh University, Bathinda. (Ref no. AU/EU_BHR/2K23/501), and last permission from Adesh hospital medical superintendent for Hospital data collection. (ref. No AIMSR/Admn.MS/025).

Identification and Antimicrobial Sensitivity Testing (AST) of the Bacterial Isolates: Samples flagged positive by the BACT/ALERT 3D system were sub-cultured onto blood agar, and nutrient agar plates using streaking techniques. The plates were incubated aerobically at 37 °C for 24–48 hours, and growth was observed after 24 hours. Gram-positive isolates were initially identified based on colony morphology and Gram stain characteristics (Duguid *et al.*, 2008). Final species-level identification was performed using the Vitek 2 Compact automated system with GP identification cards. AST was carried out using the Vitek 2 Compact automated system with the P628 card, following CLSI guidelines, 2023 [7]. ATCC strain 29213 used as a control of *S. aureus*.

RESULT: *S. aureus* isolates were identified on the basis of colony characteristics on blood agar (BA) and Nutrient agar plates. *S. aureus* colony showed round, convex, size 1-3 mm in diameter with a sharp border colony surrounded by zone of clear beta-hemolysis on blood agar **Fig. 1**. On nutrient, it showed golden yellow colour pigment the production of staphyloxanthin, a carotenoid pigment **Fig. 2**, On gram staining from the colonies, Gram positive cocci (1 μm), arranged in clusters were observed **Fig. 3.** In the present study, out of total 3145 blood samples processed; 315 samples were identified to be positive which showed growth on the culture media. Therefore, in the present study, culture positivity was seen in 10.01%. 2830 samples (89.99%) were sterile as no growth detected with the Bact/Alert 3D blood culture system. Out of 315 culture positive samples, 84 (26.2%) gram-positive bacteria, 228 (72.45%) gram-negative bacteria, and 3 (0.95%) *Candida* spp. were isolated. Among gram positive bacteria, 61 (72.61%) were of *S. aureus*. 77% *S. aureus* isolated were from males and 23% from females. Clearly it shows that rate of bacteremia was slightly higher in males as compared to females. Maximum *S. aureus* isolates were obtained from patients with age group of 51-60 years (18.03%) as compared to other age groups. Age wise distribution of *S. aureus* isolates shown in **Table 1**. In this study, maximum *S. aureus* was obtained from CCU (39.34%) followed by MICU (27.90%), ICU (18.03%), NICU, PICU and SICU (4.91%) each.

FIG. 1: *S. AUREUS* SHOWING BETA HEMOLYSIS ON THE BLOOD AGAR

FIG. 2. *S. AUREUS* SHOWING GOLDEN YELLOW PIGMENT ON NUTRIENT AGAR

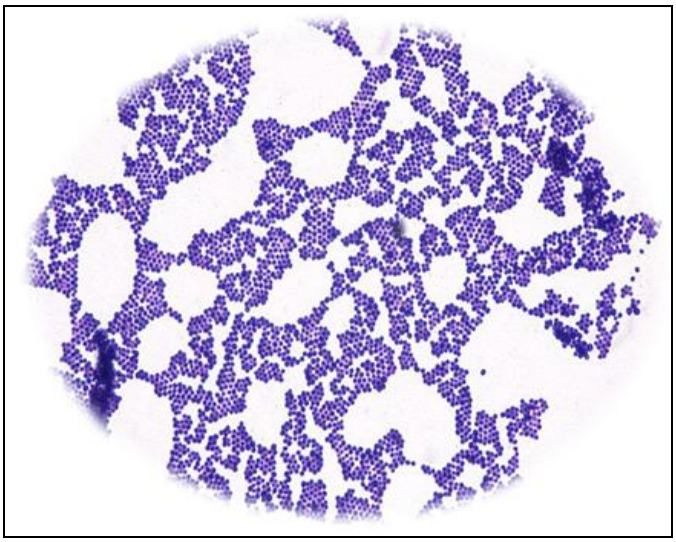

FIG. 3: MICROSCOPIC VIEW OF *S. AUREUS* SHOWING GRAM POSITIVE COCCI IN CLUSTERS

TABLE 1: AGE WISE DISTRIBUTION OF BACTEREMIA DUE TO *S. AUREUS*

Sr. no.	Age Group	No of isolates	Percentage (%)
1	0-10	04	6.55%
2	11-20	05	8.20%
3	21-30	05	8.20%
4	31-40	10	16.39%
5	41-50	08	13.11%
6	51-60	11	18.03%
	61-70	10	16.39%
8	71-80	05	8.20%
9	81-90	03	4.93%
10	91-100	0	0%
	Total	61	100%

Antimicrobial Sensitivity Pattern of *S. aureus*: In the present study S. aureus isolates showed maximum susceptibility towards teicoplanin, doxycycline, minocycline (100%), vancomycin and tigecycline (96.72%), linezolid (91.80%), rifampicin (90.16%), while moderate sensitivity recorded towards was daptomycin (80.32%), tetracycline (83.60%), clindamycin (62.29%) and gentamicin (55.73%). However lesser antibiotic sensitivity was recorded for cotrimoxazole (42.62%), erythromycin (32.78%).

Antimicrobial Resistance Pattern of *S. aureus*: In the present study isolates of *S. aureus* were maximum resistant to benzylpenicillin (100%), oxacillin (96.72%), ciprofloxacin (90.17%), andlevofloxacin (86.89%). while moderate resistant was recorded for erythromycin (67.22%) andcotrimoxazole (57.38%). Lesser antibiotic resistance was seen for clindamycin (37.70%), gentamicin (44.27%), daptomycin (19.67%), tetracycline (16.40%). **Table 2**. Showing antibiotics susceptibility pattern of *S. aureus*.

TABLE 2: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *S. AUREUS*. (N=61)

Sr. no.	Antibiotic tested	Total no of Sensitive isolates (%)	Total no of Resistant isolates. (%)
1	Benzylpenicillin	(00) 00%	(61) 100%
2	Oxacillin	(02) 3.28%	(59) 96.72%
3	Gentamicin	(34) 55.73%	(27) 44.27%
4	Ciprofloxacin	(06) 9.83%	(55) 90.17%
5	Levofloxacin	(08) 13.11%	(53) 86.89%
6	Erythromycin	(20) 32.78%	(41) 67.22%
7	Clindamycin	(38) 62.29%	(23) 37.71%
8	Linezolid	(56) 91.81%	(05) 8.19%
9	Daptomycin	(49) 80.33%	(12) 19.67%
10	Teicoplanin	(61) 100%	(00) 0%
11	Vancomycin	(59) 96.72%	(02) 3.28%
12	Tetracycline	(51) 83.60%	(10) 16.40%
13	Tigecycline	(59) 96.72%	(02) 3.28%
14	Rifampicin	(55) 90.17%	(06) 9.83%
15	Cotrimoxazole	(26) 42.62%	(35) 57.38%
16	Doxycycline	(61) 100%	(00) 0%
17	Minocycline	(61) 100%	(00) 0%

DISCUSSION: In this study, total of 3,145 patients blood samples were processed during the study period. Culture positivity was seen in 315 (10.01%) samples and 2,830 (89.98%) samples were found sterile. In the present study prevalence of *S. aureus* of from 3,145 patients' blood samples was reported to be 1.93% (n=61). similar studies were done by Nazir *et al*., 2018, Jhajhria *et al*., 2018, Sweta *et al*., 2016 reported (3.36%, 3.5%, 2.0%) respectively [2, 8, 9]. Other studies done by Mittal *et al*., 2021, Cugati and Saikumar 2017 reported (13.3%, 7.0%) respectively [3, 10]. These studies reported high prevalence of *S. aureus* as compared with present study. In the present study, *S. aureus* bacteremia was observed more in males 47 (77%) than in females 14 (23%) mainly due to more admission rate of males as compared to females. Present study correlate with Jhajhria *et al*., 2018, reported, 64.2% *S. aureus* were isolated obtained from males and 35.8% were isolated from females [9]. In this study, Maximum *S. aureus* isolates were obtained from patients with age group of 51-60 years (18.03%) followed by other age groups, showing on **Table 1**. The results of present study are similar to study by Mittal *et al*., 2021 and Afrasyabi *et al*., 2013 [3, 11]. In the present study *S. aureus* isolates were highly sensitive to teicoplanin, doxycycline and minocycline (100%) each, vancomycin and tigecycline (96.72%), rifampicin (90.16%), linezolid (91.80%), tetracycline (83.60%), daptomycin (80.32%), clindamycin (62.29%), gentamicin (55.73%). Lesser sensitivity was for cotrimoxazole (42.62%), erythromycin (32.78%). In this study, *S. aureus* were resistant to benzylpenicillin (100%), oxacillin (96.72%), ciprofloxacin (90.17%), levofloxacin (86.89%), erythromycin (67.22%). Similar studies done by kumhar *et al*., 2002 reported, *S. aureus* was sensitive to vancomycin (82.3%), erythromycin (34.5%), oxacillin (30.4%), co-trimoxazole (29.1%), gentamicin (27.9%) [12].

Kante *et al*., 2014 reported, *S. aureus* sensitive to erythromycin (100%), vancomycin (100%) [13]. Wasihun *et al*., 2015 reported, *S. aureus* was found highly sensitive to oxacillin (70.4%), cotrimoxazole (66.7%), doxycycline (53.7%), erythromycin (44.4%), ciprofloxacin (38.9%), gentamicin (33.3%) [14]. According to Jhajhria *et al*., (2018), *S. aureus* showed complete sensitivity to vancomycin and linezolid (100%), while moderate sensitivity was observed to ciprofloxacin and teicoplanin (57.1%), gentamicin (42.8%), cotrimoxazole (35.7%), and erythromycin (7.1%) [9].

Similarly, Mittal *et al*., (2021) reported that *S. aureus* isolates were fully sensitive to vancomycin and linezolid (100%), followed by teicoplanin (95%), gentamicin (70%), clindamycin and tetracycline (40%), and ciprofloxacin (20%) [3]. In the present study, *S. aureus* showed an erythromycin sensitivity of 32.78%, which is comparable to the findings of Kumhar *et al*., (34.5%), and Zeki *et al.*, (38%) [12, 15].

Conversely, much higher susceptibility rates were reported by Kante *et al*., (100%) and Nazir *et al*., (82%) [8, 13]. Gentamicin sensitivity in this study (61%) was close to the observation of Mittal *et al.*, (70%) [3], while lower rates were noted by Kumhar *et al*., (28%) and Wasihun *et al*., (33%) [12, 14]. The ciprofloxacin sensitivity recorded (20%) was in agreement with Mittal *et al*., [3]. For clindamycin, the present study documented a sensitivity of 62.29%, which corresponds with Zeki *et al*., (55.5%), Nazir *et al*., (42%), and Mittal *et al*., (40%) [3, 8, 15], though Sweta *et al*., observed a higher rate of 100% [2]. Cotrimoxazole sensitivity (42.62%) in this study was similar to that of Nazir *et al*. (54%) and Zeki *et al*., (50.7%) [8, 15], while Wasihun *et al*., reported complete sensitivity (100%) [14].

CONCLUSION: The occurrence of *S. aureus* resistance can differ based on location, the group being studied, and the epidemiology within hospitals. This study recorded *S. aureus* isolates were resistant to benzylpenicillin (100%) and oxacillin (96.72%). Therefore, it is essential to assess the local occurrence of these MRSA strains and their resistance characteristics in order to establish antibiotic guidelines and support doctors in effectively managing these cases. Targeted antimicrobial treatment should commence based on the outcomes of culture tests. The elevated levels of methicillin resistance at our facility highlight the necessity for improved screening and infection control measures moving forward. Confirmation The necessity of healthcare providers using antibiotics correctly in hospitals to avoid antibiotic resistance is also emphasized in this research.

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