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Original Article

Microbiology Section

Methicillin Resistance, Vancomycin Intermediate and Vancomycin Resistance *Staphylococcus aureus* Prevalence in a Tertiary Care Hospital of Punjab, India

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ABSTRACT

Introduction: Multidrug resistance *Staphylococcus aureus* (*S.aureus*) is a very notorious pathogen to develop drug resistance. After methicillin, resistance to vancomycin and linezolid were being reported from various areas of the world including India which is a serious issue.

Aim: To determine prevalence of Methicillin Resistant *S. aureus* (MRSA), Vancomycin Intermediate *S. aureus* (VISA) and Vancomycin Resistant *S. aureus* (VRSA) in a tertiary care hospital of Punjab, India.

Materials and Methods: The study was conducted in a tertiary care hospital from June 2016 to May 2017. *S. aureus* isolated from various clinical samples were identified as per standard protocol. Antimicrobial susceptibility was done by Kirby bauer disc diffusion method. MRSA detection was done using 30 µg cefoxitin disc on Mueller Hinton Agar (MHA). Vancomycin resistance was determined by agar dilution method. Vancomycin MIC <2 µg/mL reported as sensitive, MIC 4-8 µg/mL reported as VISA and MIC >16 µg/mL reported as VRSA.

Results: Out of 162 *S. aureus* isolates, 85 (52.4%) were female and 77 (47.5%) were male patients. Maximum 96 (59.2%) *S. aureus* were isolated from pus and pus swab. An 83 (51.2%) were MRSA, 19 (11.7%) were VISA and 4 (2.46%) were VRSA. Ampicillin 130 (80.0%), ciprofloxacin 102 (62.9%), co-trimoxazole 100 (61.7%) and erythromycim 96 (59%) were most resistant whereas tigecycline 162 (100%) was most sensitive followed by linezolid 154 (95%) and tecoplanin 153 (94%).

Conclusion: There is high prevalence of MRSA and an increasing MIC of vancomycin is reported in *S. aureus* which is big challenge for antimicrobial therapy. Performing culture and sensitivity in infectious cases in the hospital will help in reserving these drugs for those cases where all other drugs are resistant.

Keywords: Antimicrobial susceptibility, Infection, Minimum inhibitory concentration, Multidrug resistance

INTRODUCTION

Staphylococcus aureus (*S.aureus*) is the pathogen of greatest concern because it causes both hospital-acquired and community associated infection [1]. It is a very notorious pathogen to develop drug resistance. MRSA is frequently associated with nosocomial infection and its ability to develop resistance to antimicrobial agents is one of the greatest challenges for modern antimicrobial therapy. MRSA infection rate has been increasing over last few decades and a high prevalence rates (>50%) were reported in Asia, Malta, North and South America [2]. In India, incidence varies from 25% to 50% [3]. Methicillin resistance in *S. aureus* is mediated by a chromosome coded gene called *MecA* gene, which alter Penicillin Binding Protein (PBP) present in cell membrane to PBP-2 [4]. Vancomycin is drug of choice for MRSA but overuse of vancomycin has lead to emergence of resistance. It may be low grade resistance known as Vancomycin Intermediate *Staphylococcus aureus* (VISA) or high grade resistance known as Vancomycin Resistance *Staphylococcus aureus* (VRSA). The MIC of VISA is 4-8 ug/mL and VRSA is >16 ug/ml [5]. It is observed that the vancomycin MIC for susceptible strains of *S. aureus* has been gradually increasing over time known as 'MIC creep' which indicates that frequency of VISA and VRSA is likely to increase in future [6]. VISA is due to increase in cell wall thickness of *S. aureus* [7] whereas VRSA is mediated by *VanA* gene carried by transposon Tn 1546 [8]. *VanA* gene is believed to be acquired from Vancomycin Resistance Enterococci (VRE) by horizontal gene transfer [9]. Treatment of VRSA should be based on antimicrobial susceptibility report. Linezolid, telavancin, deptomycin and quinupristin/dalfopristin are the effective drugs [10]. The present study was conducted with the aim to determine prevalence of MRSA, VISA and VRSA among *S. aureus* isolated from different clinical samples in a tertiary care hospital of Punjab, India.

MATERIALS AND METHODS

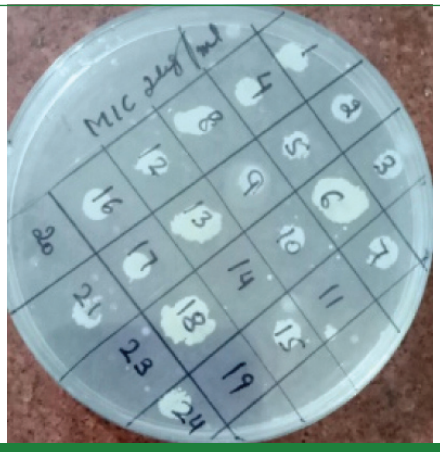
This prospective study was undertaken at tertiary care hospital from June 2016 to May 2017 after obtaining approval of Institutional ethical and research committee (Ref No /AIMSR/ MC/Estt/2016-2018/868). Informed consent from each patient was taken before collecting patient's data. Various clinical samples like blood, urine, pus, Endotrachial Tube (ET) secretions, Cerebrospinal Fluid (CSF), swabs (throat, ear, vaginal) and body fluids were collected and processed for bacterial culture using suitable culture media like glucose broth, BHI broth, blood agar and McConkey agar and incubated overnight at 37°C. All positive culture was observed for type of growth whereas negative culture (except urine) was subjected to subculturing. Positive culture were further identified by colony morphology, Gram staining and biochemical reactions. For Gram positive cocci in cluster, first catalase test was done and if catalase was positive coagulase test was done as per standard protocol [11]. Gram positive cocci which were both catalase and coagulase positive were identified as *S. aureus*.

Antimicrobial susceptibility was done by Kirby bauer disc diffusion method using a panel of HiMedia antibiotic discs- ampicillin (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), co-trimoxazole (1.25/23.75 µg), erythromycin (15 µg), linezolid (30 µg), tigecyclin (15 µg), teicoplanin (30 µg) and vancomycin (30 µg) [5]. MRSA detection was done using 30 µg cefoxitin disc on MHA and results were interpreted according to Clinical and Laboratory Standard Institute (CLSI) guideline [5]. *S. aureus* showing cefoxitin zone size <21 mm were considered MRSA [Table/Fig-1].

S. aureus strain with vancomycine zone size <17mm by disc diffusion method were further tested for MIC by agar dilution method. Muller Hinton Agar (MHA) plates containing vancomycin concentrations

[Table/Fig-1]: Cefoxitin screen test positive (MRSA).

of 0.5, 1, 2, 4, 8, 16 and 32 µg/mL were prepared in house using vancomycin powder in pure form (obtained from Sigma-Aldrich, India). After dividing the plates in multiple quadrants, 0.5 McFarland bacterial suspensions were inoculated onto these plates with the help of micropipette. First all resistant strains were tested for MIC of 0.5, 1 and 2. Strains with MIC>2ug/mL were tested for higher MIC i.e., 4, 8, 16 and 32ug/ml. *S. aureus* ATCC 25923 was included in all the test plates as control organisms. Plates were incubated at 35°C for 24 hour. Each spot was noted for the presence of growth or no growth. The least concentration of antibiotic that was able to inhibit visible growth of the organism was taken as MIC of that strain. Vancomycin MIC<2 µg/mL reported as sensitive, MIC 4-8 µg/mL reported as VISA and MIC>16 µg/mL reported as VRSA [Table/Fig-2-4] [5].

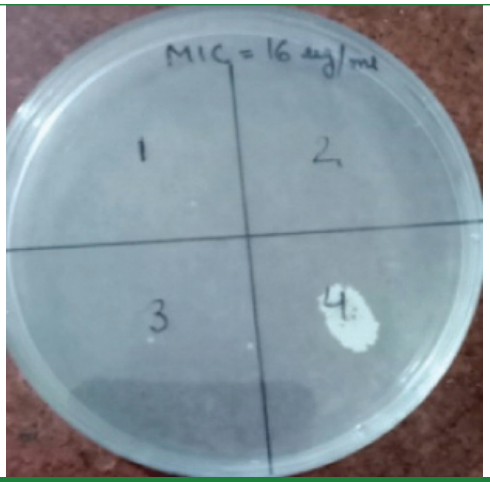


[Table/Fig-2]: Vancomycin agar dilution method with MIC-2 µg/mL.

[Table/Fig-3]: Vancomycin agar dilution method with MIC-8 µg/mL.

STATISTICAL ANALYSIS

The clinical data for all the patients infected with *S.aureus* was collected and analysed.



[Table/Fig-4]: Vancomycin agar dilution method with MIC-16 µg/mL.

RESULTS

A total 162 *S. aureus* were isolated from various clinical samples. Among these 85 (52.4%) were from female compared to 77 (47.5%) from male patients.

Majority of *S. aureus* 70 (43.2%) were isolated from age group 16-40 years followed by 41 (25%) from 41-60 years, 36 (22%) from >60 years and 15 (9.2%) from 0-15 years age group. Maximum number of *S. aureus* were isolated from pus and pus swab (96) , urine (30), blood (21), sputum and ET secretions (07), body fluids (05) and others samples (03) [Table/Fig-5].

Type of samples	Number	Percentage
Pus and Pus Swab	96	59.2%
Urine	30	18.5%
Blood	21	12.9%
Sputum and E T secretion	07	4.3%
Body fluids	05	3.0%
Others	03	1.8%

[Table/Fig-5]: Distribution of *S. aureus* among various clinical samples.

Out of 162 *S. aureus* isolates, 83 (51.2%) were MRSA and 79 (48.7%) were MSSA. Vancomycin MIC for *S. aureus* is shown in [Table/Fig-6]. Among these 23 vancomycin resistant strains, 19 (11.7%) were VISA with MIC 4-8 ug/mL [Table/Fig-6], whereas 4 (2.46%) were VRSA with MIC>16 ug/mL. Among 4 VRSA strains 1 strain had MIC>32ug/ml [Table/Fig-6]. Vancomycin resistance was seen only in MRSA strains whereas all MSSA were vancomycin sensitive. Antibiotic susceptibility pattern of *S. aureus* showed maximum resistance to ampicillin 130 (80.0%) followed by ciprofloxacin 102 (62.9%), co-trimoxazole 100 (61.7%) and erythromycin 96 (59.2%). tigecycline 162 (100%) was most sensitive followed by linezolid 154 (95%) and tecoplanin 153 (94%) [Table/Fig-7].

MIC value	<2 µg/mL	4-8 µg/mL	16 µg/mL	32 µg/mL	64 µg/mL
No. of Strains	139 (97.8%)	19 (11.7%)	3 (1.8%)	1 (0.6%)	0 (0.0%)

[Table/Fig-6]: MIC of vancomycin for *S. aureus* strains.

DISCUSSION

A total of 162 *S. aureus* were isolated from various clinical samples. In our study, overall male to female ratio was 1:1.2. Similar ratio with more isolation of *S. aureus* from females was also observed in a study from Bihar [12]. Maximum isolation of *S. aureus* were from pus and swabs as this is one of the leading cause of skin and soft tissue infection and surgical site wound infection. Another study also reported maximum isolation from pus and wound swabs [13]. In our study, 102 *S.aureus* were isolated from IPD as compare to 60 from OPD patients and among these maximum patients were from surgery followed by orthopaedic. This is because most pus samples

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	32 (20.0%)	130 (80.0%)
Amikacin	97 (59.8%)	65 (40.1%)
Cefoxitin	79 (48.7%)	83 (51.2%)
Ciprofloxacin	60 (37.0%)	102 (62.9%)
Clindamycin	106 (65.4%)	56 (34.5%)
Co -trimoxazole	62 (38.2%)	100 (61.7%)
Erythromycin	66 (40.7%)	96 (59.2%)
Gentamicin	102 (62.9%)	60 (37.0%)
Linezolid	154 (95.0%)	08 (4.9%)
Tigecycline	162 (100%)	0 (0.0%)
Teicoplanin	153 (94.4%)	9 (5.5%)
Vancomycin	139 (85.8%)	23 (14.2%)

[Table/Fig-7]: Antibiotic susceptibility pattern of *S. aureus*.

were received from surgery and orthopaedic departments. Another author also reported more isolation from orthopaedic followed by surgery patients. The proportion of MRSA varied among countries ranging from 0.4 per cent in Sweden to 48.4 per cent in Belgium [3]. The incidence of MRSA varies from 25% in western part to 50% in Southern India [3]. In our study, the overall MRSA prevalence was 51.2% (83). Another study from Punjab reported 46% [14], from Uttar Pradesh 54.8% [15], from Hyderabad 79.6% [16], and from Southern India 31.2% [17] MRSA. Vancomycin is one of the drugs of choice for MRSA treatment but overuse of this drug has lead to development of resistance. VISA was first reported by Hiramatsu K et al., from Japan in a four-month-old infant in 1997 [18]. However, a fully resistant strain of *S. aureus* to vancomycin was reported only in 2002 from Michigan, US [19]. Tiwari HK et al., were the first to report VISA strain from Indian subcontinent [20]. We reported VRSA from 23 (14.2%) stains. Out of these, 19 (11.7%) strains were having MIC between 4-8 ug/ml [Table/Fig-6] whereas 4 (2.46%) strain were MIC >16ug/ml [Table/Fig-3] thus complete Vancomycin resistance was observed in 4 (2.46%) whereas, 19 (11.7%) strains were VISA. Another study by Thati V et al., reported 1.9% VRSA and 4.46% VISA isolates by MIC [16]. Our study is showing higher numbers of VISA and VRSA probably because of the time lapse between these two studies. Most *S. aureus* strains were resistant to amoxicillin, ciprofloxacin and co-trimoxazole [Table/Fig-7]. All *S. aureus* strains including MRSA and VRSA were sensitive to tigecycline 162 (100%) whereas sensitivity for linezolid was 154 (95%) and teicoplanin was 153 (94%).

LIMITATION

Limitations of our study were that we could not perform molecular analysis for knowing genetic mechanism of drug resistance. Future Recommendations of our study is to do time to time analysis of antimicrobial susceptibility pattern of *S.aureus* and to include molecular analysis of resistant strains.

CONCLUSION

Our study showed high prevalence of MRSA and emergence of *S. aureus* isolates with high MIC to vancomycin. This is because of extensive use of antimicrobial agents. There is a need to enforce the judicious use of higher antibiotics and continuous national wide surveillance programs to map the susceptibility pattern of these antibiotics in our country.

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