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KEY WORDS: MRSA, Multidrug resistance, VISA, Vancomycin MIC creep. Staphylococcus aureus

5-YEAR ANALYSIS OF METHICILLIN RESISTANT, VANCOMYCIN INTERMEDIATE AND VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS- FROM A TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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ABSTRACT

Aim: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most successful modern pathogens. MRSA poses a formidable threat, with persistently high morbidity and mortality. Regional surveillance of the organism is a crucial step to overcome the issues of antimicrobial resistance and treatment failure. This study was conducted to report the prevalence and antibiotic susceptibility pattern of MRSA and Vancomycin Intermediate Staphylococcus aureus (VISA) isolates from various clinical samples at a tertiary care hospital in Punjab, India. **Material and Methods:** This study was conducted in the Microbiology Department at Christian Medical College & Hospital, Ludhiana from 1st January 2019 to 31st December 2023. S. aureus isolated from various clinical samples were processed and organisms were identified as per standard protocols. Antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method following CLSI guidelines. MRSA strains were detected by disk diffusion method using Cefoxitin 30µg disc. All methicillin resistant strains and their susceptibility patters were also confirmed by Microscan Walkaway 96 Plus. Isolates with Vancomycin MIC <2 µg/mL were reported as sensitive, MIC 4-8 µg/mL reported as VISA and MIC >16 µg/mL reported as VRSA. Multi Drug Resistant (MDR) MRSA was identified based on the resistance towards various antimicrobials categories. **Results:** A steady increase in number of MRSA isolates was observed from year 2019 through 2023 with its overall prevalence being 39.32%. Majority of S. aureus strains were methicillin resistant (67.21%). Maximum percentage of MRSA isolates were obtained from pus samples (39.63%). Resistance was not observed among the 1070 strains of MRSA against vancomycin, whereas majority of isolates showed susceptibility to teicoplanin (97.76%) and linezolid (98.88%). VISA was reported in 1.50% MRSA isolates. Among other antibiotics co-trimoxazole was found to be most effective against the MRSA strains (58.16%). MSSA isolates were more susceptible to antibiotics as compared to MRSA isolates. Multidrug resistance was observed among 57.34% MRSA and 21.38 % of MSSA isolates. **Conclusion:** There is increasing prevalence of MRSA and emergence of VISA isolates reported among S. aureus isolates over the past 5 years, which is a major therapeutic challenge. Regular surveillance of prevalence and monitoring of antibiotic sensitivity pattern is required to reduce the prevalence of these notorious pathogen.

INTRODUCTION

Increasing antibiotic resistance is a worrisome trend being observed worldwide. Among gram-positive cocci, Staphylococcus aureus is a well-known cause of community acquired as well as hospital acquired infections. Beginning with the emergence of methicillin-resistant Staphylococcus aureus (MRSA) in UK within two years of Methicillin launch, this organism has become a serious global threat. [1,2] Status of being methicillin resistant itself means that a S. aureus isolate will not be sensitive to Penicillins, Cephalosporins, β-lactamase inhibitors and Carbapenems and can further exhibit resistance to other classes of antibiotics. [3,4]

MRSA causes a variety of infections from insignificant skin diseases to life-threatening infections. The wounds infected with MRSA heal late and are difficult to treat due to the limited range of effective antibiotics that are available. MRSA infection increases the mortality rate, prolonged hospitalization and added health care costs. [5]

For MRSA and multidrug-resistant (MDR) S. aureus infections, vancomycin is considered the main stay of antimicrobial therapy since 1958. However, emerging reports of vancomycin minimum inhibitory concentrations (MIC) creep and even vancomycin resistance in clinical MRSA isolates from different geographical regions are disturbing, because of minimal alternative therapeutic options. [6-9] Only a few studies from our region have previously reported reduced susceptibility to vancomycin after the introduction of the revised CLSI guidelines. Hence, we aimed to determine the recent pattern of antibiotic resistance, assess the vancomycin susceptibility profile and ascertain other therapeutic options for clinical S. aureus isolates in Northern India, which would help in choosing empirical antimicrobial therapy as well as in administering targeted therapy to the patients.

MATERIAL AND METHODS

This retrospective and prospective study was undertaken in the Microbiology Department at Christian Medical College & Hospital, Ludhiana from 1st January 2019 to 31st December 2023, after obtaining approval of Institutional research committee (Ref. CMC/2307). A total of 27,207 clinical samples like pus, wound swab, sputum, cerebrospinal fluid (CSF), throat swab, nasal swab, endotracheal secretions (ET secretions), broncho alveolar lavage (BAL), mini-BAL, tissue exudates, swabs (throat, conjunctival, vaginal), body fluids (pleural, peritoneal, ascitic, expressed prostate secretions), suction tip and central line will be collected from outpatients and inpatients from different wards.

All the samples were aseptically handled and processed for bacterial culture using suitable culture media like glucose broth, blood agar and MacConkey agar and incubated overnight at 37°C. All positive cultures were observed for type of growth whereas negative cultures were subjected to subculturing. Positive cultures were further identified by colony morphology, Gram staining and biochemical reactions. For Gram positive cocci in cluster, first catalase test was done, if positive it was followed by slide and tube coagulase tests as per standard protocol. [10,11] Gram positive cocci which were both catalase and coagulase positive were identified as S. aureus. Staphylococcus aureus ATCC-25923 of known coagulase production was included as control strain.

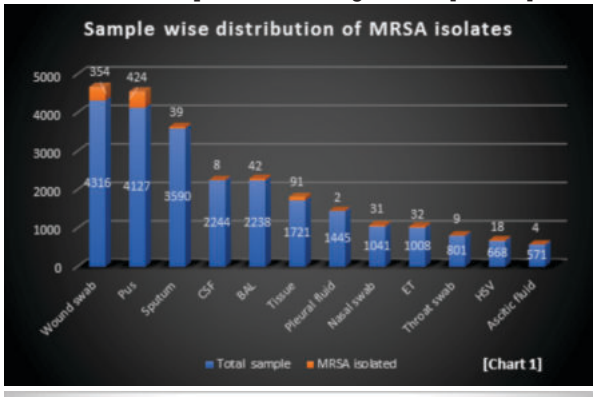
Antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards

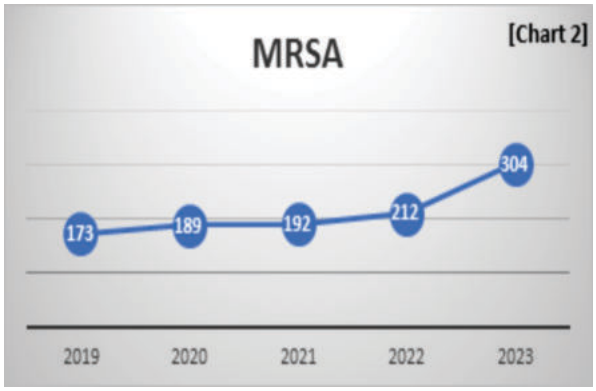
Institute (CLSI) guidelines.[12] 0.5 Mc Farland suspension of the isolate was made and culture was done on the Mueller Hinton Agar (MHA) plate. Hi-Media antibiotic discs were penicillin-G (PEN) (10 unit); erythromycin (ERY)(15µg); clindamycin (CLI)(2µg); ciprofloxacin (CIP) (5µg); trimethoprim-sulfamethoxazole (SXT) (1.25/23.75µg).; cotrimoxazole (COT)(25µg); vancomycin (VAN)(30µg); linezolid (LNZ)(30µg) and teicoplanin (TEC)(30µg). MRSA detection was done using 30 µg cefoxitin disc on MHA at 37°C for 24 hrs. S. aureus showing cefoxitin zone size <21 mm was considered as MRSA.

All methicillin resistant strains and their susceptibility patters were also confirmed by automatic bacterial identification and susceptibility analysis system (Microscan Walkaway 96 Plus) using the Pos MIC 24 panel. Strains of Staphylococcus aureus for which vancomycin MICs were ≤ 2 µg/ml were considered to be sensitive, those for which MICs were between 4-8 µg/ml were considered to be intermediate sensitive (VISA) and those for which MICs were ≥16 µg/ml were considered to be resistant (VRSA) according to the CLSI guidelines 2018. S. aureus ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing. An isolate was labelled as MDR MRSA if it was non-susceptible to at least 1 agent in ≥ 3 antimicrobials categories.

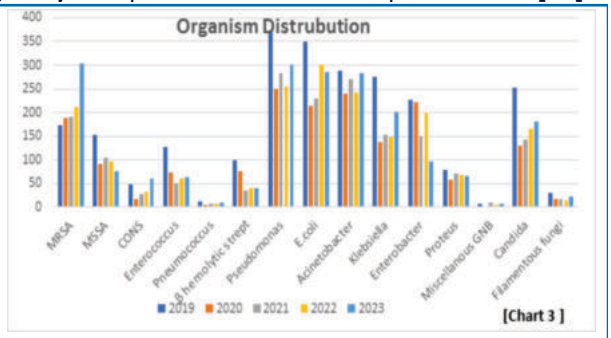
RESULTS

During the 5-year study duration a total of 9,829(36.13%) organisms were isolated from 27,207 clinical samples. Among the culture positive isolates 6357(64.68%) were Gram negative bacteria, 2493(25.36%) were Gram positive bacteria followed by 979(9.96%) fungal pathogens. Most common isolate was *Staphylococcus aureus* 1592 (16.20%) among which 1070 were detected as MRSA strains (67.21%). Maximum percentage (39.63%) of MRSA were isolated from pus specimens [Chart 1]. A continuing increase in MRSA isolates was observed from January 2019 through December 2023 with its overall prevalence being 39.32%. [Chart 2].





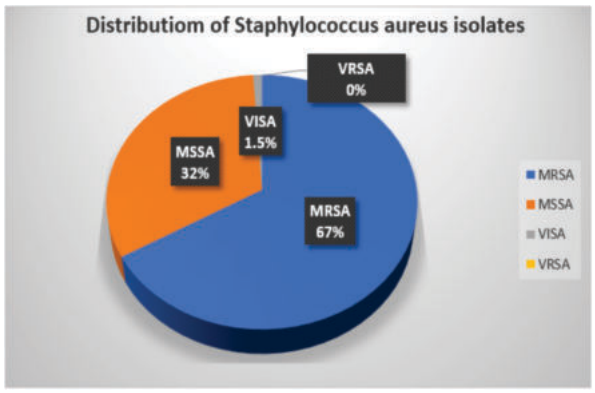
The most frequent isolates during the study period after Staphylococcus aureus were Pseudomonas spp. (14.87%), Acinetobacter spp. (14.06%), E. coli (14.03%), Klebsiella spp. (9.32%), Enterobacter spp. (9.09%) and Candida spp. (8.88%) [chart 3].



MRSA were isolated most among male patients over 60 years of age (62.54%). The results of antibiotic susceptibility tests were studied for the S. aureus isolates. As compared to the MSSA isolates, MRSA were significantly more resistant to almost all the conventional antimicrobials. None of the 1070 strains of MRSA showed resistance against vancomycin and majority of the isolates were sensitive to linezolid (98.88%) and teicoplanin (97.76%). Among other antibiotics co-trimoxazole was found to be most effective for the MRSA strains (58.16%), followed by clindamycin (56.24%), ciprofloxacin (34.09%) and erythromycin (25.33%). [Table 1]. Sixteen clinical isolates were confirmed as VISA with MIC range between 4-8 µg/ml. We have observed a slow but steady increase in VISA isolates over the study period [Chart 4]. Multidrug resistance was observed among 57.34% MRSA and 21.38 % of MSSA isolates.

	MSSA (%)	MRSA (%)
Penicillin-G (PEN)	76.45	100
Erythromycin (ERY)	52.93	74.67
Ciprofloxacin (CIP)	59.23	65.91
Clindamycin (CLI)	26.44	43.76
Cotrimoxazole (COT)	31.29	41.84
Trimethoprim-sulfamethoxazole (SXT)	59.92	100
Vancomycin (VAN)	0	0
Teicoplanin (TEC)	0	2.24
Linezolid (LNZ)	0	1.12

Table-1: Antibiotic resistance pattern of Staphylococcus aureus (MRSA and MSSA) isolates



DISCUSSION

S. aureus is a fine example of a modern pathogen, which has been able to morph and adapt with much tenacity to the changing landscape of new age medical interventions and the available antimicrobial agents. The increase in isolates of S. aureus with resistance to methicillin (MRSA) and decreased susceptibility to vancomycin (VISA) has created grave concern for development of new anti-staphylococcal agents that kills resistant mutants. Frequent use of vancomycin as the drug of choice for treatment of infections caused by multidrug-resistant MRSA has putatively led to selection of these isolates with reduced susceptibility to vancomycin. The global prevalence of MRSA, VRSA and VISA isolates is 14.69% 1.5%, 1.7%, and 4.6%, respectively.[13,14] In India, the rate of MRSA in different hospital units show a large variation from 12

to 80.09%.[15] Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in a health care setup is crucial to implement control measures for these infections and minimize usage of second line antimicrobials.

This study presents a comprehensive data of resistance pattern among S. aureus isolates in a tertiary care hospital in the Northern part of India. During the course of our study out of 27,207 samples, 9,829(36.13%) were found to be culture positive. Out of these 2493(25.36%) were Gram positive, among them 1592 (16.20%) isolates were found to be Staphylococcus aureus which was the most common pathogen in our study, this finding is consonant with a number of national and international studies.[16,17,18,19]

The prevalence of MRSA infection is not homogenous in different parts of India. The prevalence rate of MRSA among various clinical samples was found to be 39.32% in the current study. This finding synchronizes with MRSA prevalence of 37.3% reported by ICMR AMRSN and 40% as reported by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group study.[18,19] The prevalence of MRSA observed in our institute was much lower than other contemporary studies from Punjab.[20,21] This can be attributed to our stringent infection control practices and a continuous surveillance program for MRSA and other multidrug-resistant organisms (MDROs). In spite of these efforts through the duration of 5 years a continuous increase in MRSA isolates in the clinical specimen was observed. The number of MRSA increased from 6.93% in 2019 to 15.19% in 2023. Lohan et al., from North India and Mallick et al., from Central India observed the similar patterns. This year Thomnsen et al., published a 12-year study of AMR surveillance trends in MRSA which highlighted an upward trend in the burden of MRSA.[22-24]

We observed the higher rate of MRSA isolation among male patients who were >60 years of age (62.54%). These findings are corroborated by other similar reports.[5,16,22] Rate of isolation of S. aureus with regard to clinical specimens was the highest in pus samples (39.63%). This is a common finding globally and could be due to exposure of wound to microorganisms in the environment or endogenous infections as S. aureus is a common skin commensal making the wound more prone to infection.[17,21,22]

Considering the antibiogram pattern of MRSA, in line with previous studies,[5,19,20] our present study also observed that MRSA isolates were significantly more resistant to various antibiotics, such as penicillin, trimethoprim sulfamethoxazole, fluoroquinolones, erythromycin and clindamycin. Even though multiple drug resistance(57.34%) was seen among majority of MRSA isolates, full-blown resistance to vancomycin in S. aureus was not observed. A 100% sensitivity of MRSA to vancomycin suggests its prudent use and continuous monitoring of MIC levels by the Hospital Infection Control Committee (HICC) and formulation of strict antibiotic policy in our Institute. Burden of such strains varies considerably geographically. Tiwari, et al., from Varanasi (72.1%), Arora, et al., from Amritsar (73%) and Alfeky et al., from Egypt (79%) reported a higher incidence of these strains.[20,25,26] On the other hand lower rates of MDR-MRSA isolates were documented by Anupurba et al., from Varanasi (32.0%) and Hadyeh et al., from Palestine (28.6%).[27,28]

CONCLUSION-

Antimicrobial resistance is a phenomenon inevitably related to microbial evolution and antibiotic overuse/misuse which accelerate the emergence of MDR strains. MRSA is a persistent and ever-growing problem for healthcare institutions. Present study stresses upon the need of continuous monitoring of MRSA and their antibiogram in tertiary care setting. Irrational use of antibiotics, absence of antimicrobial stewardship program, lack of surveillance and reporting system, failure to observe infection control practices like hand washing and barrier nursing could be some of the reasons for this problem and should be tackled judiciously. Although no isolate exhibited resistance to vancomycin in the current or previous studies we recommend continuous monitoring by clinical microbiology laboratories for increasing prevalence of VISA MIC creep, reduced vancomycin susceptibility (RVS) and even vancomycin resistance in clinical MRSA isolates[29]. The need of the hour, therefore, is to regularly evaluate the vancomycin MICs and the physicians must carefully adjust the treatment doses to improve the clinical outcome among the patients

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