



## METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN VARIOUS CLINICAL SPECIMENS- PREVALENCE AND ANTIBIOGRAM FROM A MULTI-SPECIALITY HOSPITAL IN PUNJAB

**Dr. Eshani Dewan**

Associate Professor. Dept. of Microbiology. Christian Medical College & Hospital, Brown Road, Ludhiana-141008. Punjab

**Dr. Vandana Verma**

Prof and Head.

**Dr. Maria Thomas**

Assistant Professor.

### ABSTRACT

**Aim:** The emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) has posed a major therapeutic challenge. We report the prevalence and antibiotic susceptibility pattern of MRSA from a tertiary care hospital in Punjab. **Material and Methods:** A 3-year retrospective study was conducted at a multi-speciality hospital in Punjab from 1st January 2020 to 31st December 2022. The samples were processed and organisms identified by standard microbiological techniques. Antibiotic susceptibility was tested by Kirby Bauer disc diffusion method following CLSI guidelines. MRSA strains were detected by Cefoxitin 30µg disc. All methicillin resistant strains and their susceptibility patterns were further confirmed by Microscan Walkaway 96 Plus. Multi drug Resistant (MDR) MRSA was identified based on the resistance towards different antimicrobials categories. **Results:** Out of 886 *S. aureus* reported 66.93% strains were MRSA. A steady increase in number of MRSA isolates was observed from year 2020 to 2022 with its overall prevalence being 42.36%. Maximum percentage (41.48%) of MRSA strains were isolated from pus specimens. Resistance was not observed among the 593 strains of MRSA against vancomycin or linezolid, whereas 98.63% isolates showed susceptibility to teicoplanin. Vancomycin-intermediate *Staphylococcus aureus* (VISA) was reported in 1.35% of MRSA isolates. Among other antibiotics co-trimoxazole was found to be most effective against the MRSA strains (57.14%). MSSA isolates showed a higher susceptibility to all antibiotics as compared to MRSA isolates. Multidrug resistance was observed among 53.65% MRSA and 23.56% of MSSA isolates. **Conclusion:** Regular surveillance of prevalence and monitoring of antibiotic sensitivity pattern is required to reduce emerging rates of VISA and MRSA.

**KEYWORDS :** MRSA, Multidrug resistance, VISA, Vancomycin MIC creep

### INTRODUCTION

The genus *Staphylococcus* includes numerous pathogenic organisms in which *Staphylococcus aureus* is the most important. It is a ubiquitous colonizer of human epithelia and an opportunistic pathogen involved in nosocomial infections.<sup>1</sup> It has overcome most of the therapeutic agents which have been developed in the past years and hence the antimicrobial chemotherapy for this species has always been empirical.<sup>2</sup> The most notable example of this phenomenon was that methicillin was introduced in 1959 to combat *Staphylococci* and other bacteria with penicillin resistance. MRSA (Methicillin Resistant *Staphylococcus aureus*) was first traced in 1961 in UK which spread to every continent soon.<sup>3</sup> Most of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptide antibiotics such as vancomycin.<sup>4</sup> Low level resistance even to vancomycin is emerging at present.<sup>5</sup>

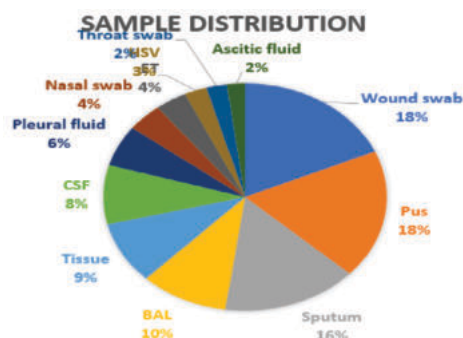
Also, in India, the rate of MRSA in different hospital units show a large variation from 12 to 80.09%.<sup>6</sup> Therefore, knowledge of MRSA prevalence and their antimicrobial profile in a health care set up is crucial to implement control measures for these infections and to minimize the usage of second line antimicrobials. We planned this study to determine the prevalence of MRSA from different clinical samples and their in vitro susceptibility pattern to record the current status of MRSA response to commonly used *Staphylococcal* antibiotics in our tertiary care hospital.

Our objectives were to observe the trend of MRSA during the past three years and to study their antimicrobial profile. The findings of the study will help us to recommend the empirical therapy schedule to clinicians and implement a stringent hospital infection control program.

### MATERIAL AND METHODS

A retrospective study was conducted in the Microbiology Department at Christian Medical College & Hospital, Ludhiana over a period of 3 years (1<sup>st</sup> January 2020 to 31<sup>st</sup> December 2022). A total of 14,000 clinical samples comprising of pus, wound swab, sputum, cerebrospinal fluid (CSF), throat

swab, nasal swab, endotracheal secretions (ET secretions), bronchoalveolar lavage (BAL), tissue, pleural fluid, ascitic fluid and high vaginal swab (HSV) were collected from outpatients and inpatients from different wards [Chart 1]. All the samples were aseptically handled and processed.



[Chart 1]

The morphotyping was done for all the samples based on the Gram staining method to determine the likely organism present. Subsequently, the clinical specimens were inoculated onto blood agar plates, MacConkey agar and glucose broth for further subculturing and incubated at 37°C for 24 hours. *S. aureus* from the samples was identified by standard microbiological techniques based on colony morphology, gram stain, catalase, slide and tube coagulase and Hugh Leifson's oxidative fermentation test based on standard methods.<sup>7,8</sup> *Staphylococcus aureus* ATCC-25923 of known coagulase production was included as control strain.

Antimicrobial susceptibility testing was performed using Muller-Hinton agar plates by disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>9</sup> 0.5 Mc Farland suspension of the isolate was made and culture was done on the MHA plate. MRSA was detected by Cefoxitin disc diffusion test. A 30µg Cefoxitin disc was placed and incubated at 37°C for 24 hrs. The zone of inhibition of *S. aureus* ≤ 21 mm was considered as methicillin resistant.

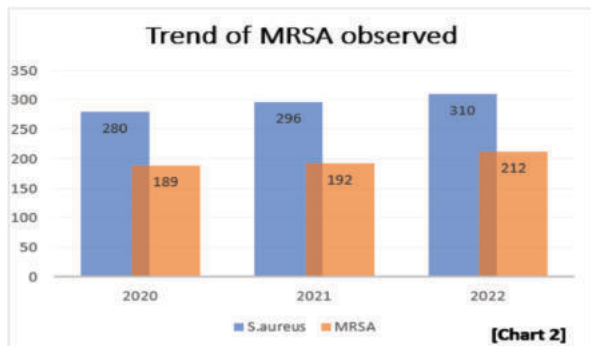
Further, the antibiotic susceptibility pattern of MRSA was

determined by the modified Kirby Bauer disc diffusion method. The antibiotics used 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). Strains of *Staphylococcus aureus* for which vancomycin MICs were ≤ 2 µg/ml were considered to be sensitive, those for which MICs were between 4 µg/ml and 8 µg/ml were considered to be Vancomycin-intermediate *Staphylococcus aureus* (VISA) and those for which MICs were ≥ 16 µg/ml were considered to be resistant (VRSA) according to the CLSI guidelines 2018.<sup>9</sup> *S. aureus* ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing. An isolate was labelled as Multi drug Resistant (MDR) MRSA if it was non-susceptible to at least 1 agent in ≥ 3 antimicrobials categories.

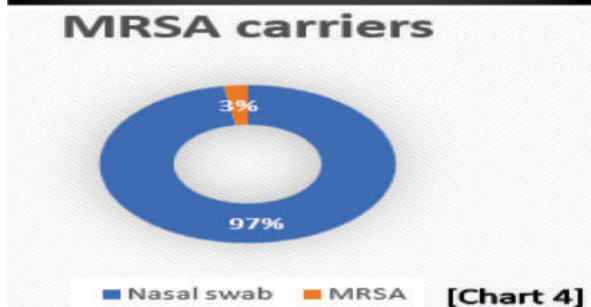
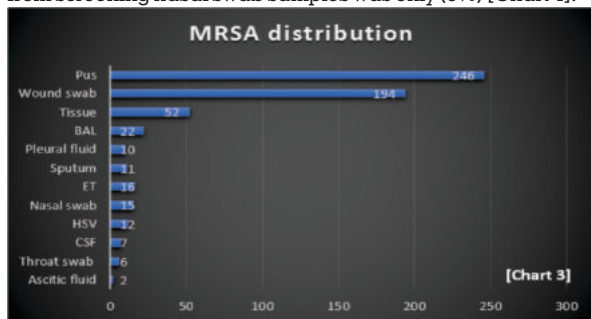
**RESULTS**

A total of 14,000 samples were processed of which 5334 (38.10%) were positive. Out of the total culture positive samples 65.92 % isolates were gram negative bacteria, 24.86 % were gram positive bacteria, followed by 9.22% which were fungal pathogens. Among the gram-positive cocci, 66.82% isolates were confirmed as *Staphylococcus aureus*. Out of the *S. aureus* reported 593(66.93%) strains were MRSA and the remaining 484(33.07%) strains were MSSA.

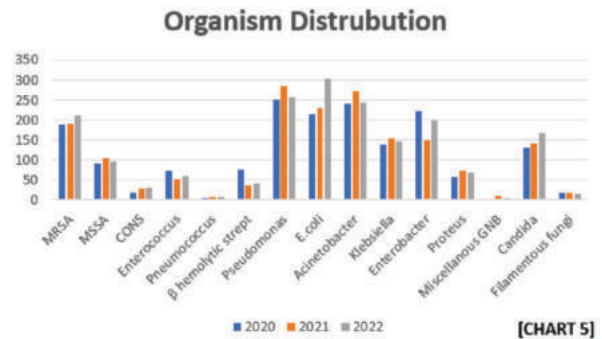
A steady increase in number of MRSA isolates was observed over the year January 2020 to December 2022 with its overall prevalence being 42.36%. [Chart 2]



Maximum percentage (41.48%) of MRSA was isolated from pus specimens [Chart 3]. The identification of MRSA carriers from screening nasal swab samples was only (3%) [Chart 4].

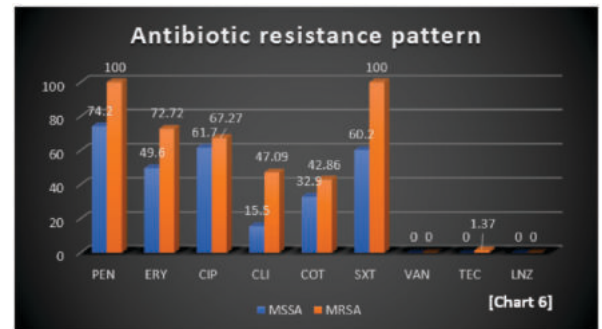


The most frequent isolates were *Staphylococcus aureus* (16.61%), *Pseudomonas* spp. (14.79%), *Acinetobacter* spp. (14.15%), *E. coli* (13.97%), *Enterobacter* spp. (10.68%), *Klebsiella* spp. (8.25%) and *Candida* spp. (8.23%) [chart 5].



Among the MRSA isolates, male patients were 67.63%. and 32.37% patients were in the age group of above 60 years.

Resistance was not observed among the 593 strains of MRSA against vancomycin and linezolid, whereas 98.63% isolates showed susceptibility to teicoplanin. Among other antibiotics co-trimoxazole was found to be most effective for the MRSA strains (57.14%), followed by clindamycin (52.91%), ciprofloxacin (32.73%) and erythromycin (27.28%). MSSA isolates showed a higher susceptibility to all antibiotics as compared to MRSA isolates [chart 6]. Eight 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 period of the study. Multidrug resistance was observed among 53.65% MRSA and 23.56% of MSSA isolates.



**DISCUSSION**

India faces a unique challenge in tackling antimicrobial resistance due to its diverse geography and vast population, low healthcare spending and inappropriate/overuse of antimicrobials.<sup>10</sup> In India, the significance of MRSA had been recognized relatively late and it emerged as a problem in the 1980s and in the 1990s. In this study, MRSA accounted for 63.20% of all *S. aureus* infection. Similar reports were seen from other studies in India and internationally.<sup>5</sup> The prevalence of MRSA was higher in males (67.63%) as compared to females (32.37%). Maximum MRSA strains were isolated from patients >60 years of age. These findings were in concordance with a number of other studies.<sup>11,12</sup>

The predominance in pus (41.48%) could be due to exposure of wound to microorganisms in the environment and *S. aureus* present on skin as commensal makes wounds more prone for infection. Similar findings were reported from Varanasi (42%) and Andhra Pradesh (64%).<sup>13,14</sup>

Another significant observation in this study was only 3% of individuals were identified as carriers of MRSA from screening samples. This could be attributed to stringent infection control practices at our hospital and increased awareness about the same. Contemporary literature shows highly variable carrier rate ranging from 0% to 29%.<sup>2,15</sup>

A steady increase in number of MRSA isolates was observed from year 2020 to 2022 with its overall prevalence being 42.36%. The observed prevalence was lower than other studies from Punjab, where rates were 46% and 51%, respectively.<sup>16,17</sup> According to a study by Indian Network for Surveillance of Antimicrobial Resistance, the north zone, has the second-highest (41%) MRSA prevalence.<sup>18</sup> However, a much higher MRSA prevalence of 54.85% and 59.3% was observed by Anupurba *et al.* and Tiwari *et al.*, respectively.<sup>19,20</sup> Above studies show considerable variations between institutions. We observed susceptibility to ciprofloxacin was low in both MSSA (38.30%) and MRSA (32.73%). But the significant and clinically relevant observation of this study is the moderate resistance shown by MRSA to other conventional antibiotics. The other contemporary reports state higher resistance rates for aminoglycosides and fluoroquinolones. In study Qureshi *et al.* on spectrum of antimicrobial resistance among MRSA, ciprofloxacin resistance was as high as 98.9%.<sup>21</sup>

High resistance to erythromycin was seen as such antibiotics are usually used at random to cure generalized and pyogenic infection. MSSA isolates showed a higher susceptibility to antibiotics as compared to MRSA isolates. Joshi *et al.* and Vidhani S *et al.* also found the same.<sup>18,22</sup>

All MRSA strains were sensitive to vancomycin and linezolid, while most of them were susceptible to teicoplanin. This is in accordance with other studies.<sup>19,22</sup>

The eight MRSA isolates (1.35%) for which MIC for vancomycin was between 4-8 µg/ml, indicated the emergence of vancomycin resistance, as VISA strains can spread if vancomycin is given to the patient for a prolonged period. These strains were also multiple drug resistant. Emergence of VISA may be due to buildups of selective pressure of vancomycin. Many recent reports also recorded the emergence of low level and intermediate vancomycin resistance. Similar percentages of VISA were reported from other studies in Northern India.<sup>23,24</sup>

The percentage of MDR strains among MRSA was found to be 53.65%. Though these MDR strains are not found with additional virulence properties, they add burden to hospital personal in infection control and have limited therapeutic options. In reports from other parts of the country, the burden of such strains has ranged from 23.2% to 63.6%.<sup>25</sup> Reporting of high rates of MDR MRSA leads to the possibility of exploitation of vancomycin by clinicians. But bearing in mind the slow but alarming increase in VISA isolates and limited drugs being available for its treatment, prudent use and continuous monitoring of MIC levels should be continued diligently so that we may not fall back into pre-antibiotic era. All strains with the vancomycin MIC 4-8µg/ml should be earmarked and sent to the laboratory for further characterization. This helps to identify the potential areas which are already under the major threat of VRSA/VISA emergence.

## CONCLUSION-

Our study is a preamble to understand the nature of MRSA isolates in this part of India. Overuse of vancomycin can lead vancomycin MIC creep. This is of great concern as this may be associated with an increasing probability of treatment failure. So, we emphasize the need for continuous surveillance and monitoring of MIC levels of vancomycin in MRSA isolates. Stringent hospital infection control program and robust antimicrobial stewardship which includes development of antibiotic policies based on local microorganism flora and the sensitivity patterns, prescription audit and pharmacovigilance in order to prevent and control the spread of these notorious pathogens. We plan a follow up study to analyse the MRSA and VISA rates to assess our efforts.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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