Section: Microbiology

Original Research Article

AN OBSERVATIONAL STUDY TO FIND OUT METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA), VANCOMYCIN INTERMEDIATE STAPHYLOCOCCUS AUREUS (VISA) AND VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS (VRSA) AMONG CLINICAL ISOLATES FROM SURGICAL SITE INFECTIONS AT SAWAI MAN SINGH (SMS) MEDICAL COLLEGE, JAIPUR

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ABSTRACT

Background: *Staphylococcus aureus* is a leading cause of surgical site infections (SSIs) resulting into increased morbidity and mortality. Irrational and inappropriate use of antimicrobials has led to increasing prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) and emergence of Vancomycin resistant strains of *Staphylococcus aureus*. The study was aimed to determine MRSA, VISA (Vancomycin-Intermediate *Staphylococcus aureus*) and VRSA (Vancomycin-Resistant *Staphylococcus aureus*) among clinical isolates from SSIs at a tertiary care hospital. **Materials and Methods:** An observational study was conducted in the Department of Microbiology, SMS Medical College, Jaipur from January 2024 to December 2024. From a total of 647 pus samples from clinically suspected cases of SSI received at the Bacteriology Laboratory, 145 non-duplicated *Staphylococcus aureus* were isolated using standard microbiological protocol. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method. Vancomycin Screen Agar (VSA) and Minimum inhibitory concentration (MIC) by broth microdilution method (BMD) were done for vancomycin susceptibility as per CLSI guidelines. **Results:** Out of 647 clinically suspected cases of SSI screened, 550 (85%) were culture positive. Among these, 145 were *Staphylococcus aureus* (26.3%) followed by 110 *Escherichia coli* (20%), 99 *Pseudomonas species* (18%), 88 *Klebsiella species* (16%), 33 *Acinetobacter species* (6%), 26 *Enterococcus species* (4.7%) and so on. Out of 145 *Staphylococcus aureus* isolates, 81 were detected as MRSA (55.87%). Among the isolates, maximum resistance was seen with Ciprofloxacin (89.7%) followed by Erythromycin (71%), Clindamycin (46.2%), Gentamicin (19.4%). All the *S. aureus* isolates were 100% sensitive to Tigecycline, Vancomycin, Teicoplanin and Linezolid. All the isolates showed MIC <2µg/ml by BMD. No VISA/ VRSA were detected in the study. **Conclusion:** In view of increasing burden of MRSA and multidrug resistant pathogens in hospital settings, there is need for frequent monitoring of antimicrobial susceptibility pattern and strict infection control practices.

INTRODUCTION

Surgical site infections (SSIs) are one of the most commonly reported health care-associated infections (HAIs) that lead to increased morbidity, mortality, prolonged hospital stays, antimicrobial resistance and financial strain on health care system [1]. Surgical site infections are defined as infections

occurring at the surgical wound within 30 days after surgery or within one year if an implant is left in place [2].
As per reports from Centre for Disease Control and Prevention (CDC), SSIs account for ~20% of all HAIs and is associated with a 2- to 11-fold increase in the risk of mortality, with 75% of SSI-related deaths directly caused by the infection [3].
Despite advancements in aseptic techniques, sterilization methods, surgical procedures and antibiotic prophylaxis, surgical site infections (SSIs) remain a major hospital-acquired infection in developing countries causing increased morbidity and mortality [4,5].
The global scenario of SSIs ranges from 2.5% to 41.9%. In India, several multicentric studies reported SSI rate ranging from 4.1 to 11 per 100 surgeries [6]. The true data is expected to be much higher as post discharge follow up is a big challenge in SSI surveillance.
The common pathogenic bacteria in SSIs include *Staphylococcus species, Pseudomonas species, Streptococcus species, Enterococcus species, Escherichia coli, Klebsiella species, Enterobacter species, Citrobacter species, Acinetobacter species, Proteus species* etc. [7].
Staphylococcus aureus is implicated as one of the important causes of community acquired and hospital acquired infections worldwide. It is the leading cause of surgical site infections (SSIs), accounting for 20% of the total cases [8].
Staphylococcus species are gram positive cocci arranged in bunch of grapes like clusters. They are frequently found as normal flora of the skin, axilla, and anterior nares of man and animals. They are commonly isolated from infections of the skin and soft tissues, bloodstream infections, respiratory infections and surgical wound infections [8,9].
Methicillin resistant *Staphylococcus aureus* (MRSA) is a significant global threat. It carries the mecA or mecC gene on SCCmec gene which encodes a modified penicillin-binding protein (PBP2a) that reduces the effectiveness of beta-lactam antibiotics [10].
In India, prevalence rate of MRSA varies from 25-85% in different parts and has now become endemic [10].
Vancomycin is used as the drug of choice for treatment of multidrug-resistant MRSA infections. This has led to the development of isolates with reduced susceptibility to Vancomycin as Vancomycin-resistant *Staphylococcus aureus* (VRSA) and Vancomycin-intermediate *Staphylococcus aureus* (VISA). The global prevalence of VRSA, VISA are 1.5% and 1.7% respectively [10,11,12].
Vancomycin resistance in *S. aureus* (VRSA) is acquired by horizontal transfer of a plasmid- borne transposon (Tn1546) carrying van A gene from Vancomycin-resistant Enterococcus (VRE) while VISA is associated with a thickened and poorly cross-linked cell wall. These resistant strains developing thicker cell walls trap the antibiotic in outer layers and prevent their action, leading to resistance [11,12,13,14].
The emergence of VRSA and VISA, though rare, is a major public health concern due to its high virulence and limited treatment options. Therefore, constant monitoring of these isolates is important as *S. aureus* is a very common cause of human infections. However, there are many hurdles, since most of the laboratories perform disc diffusion test for antibiotic susceptibility which is not reliable for testing vancomycin susceptibility [13]. As per latest CLSI guidelines, detection of growth on vancomycin screen agar and confirmation by broth microdilution for determining MIC are reliable methods for testing vancomycin susceptibility in *S. aureus* [13].
As there is limited data on the epidemiology of VISA and VRSA in India, the present study was undertaken to identify MRSA, VISA and VRSA among clinical isolates from surgical site infections and to study the antimicrobial susceptibility pattern of *Staphylococcus aureus*.

MATERIALS AND METHODS

The present study was a hospital based descriptive type of observational study conducted in the Bacteriology Laboratory of Department of Microbiology, SMS Medical College, Jaipur (Rajasthan) from January 2024 to December 2024 after approval from the research review board and the institutional ethical committee. A total of 647 samples received from clinically suspected cases of SSI (as per CDC criteria) were inoculated onto Nutrient agar, Blood agar and MacConkey agar, and incubated aerobically at 37ºC overnight.
Isolation and identification of *Staphylococcus aureus* was done on the basis of colony morphology, gram staining and biochemical reactions as per standard microbiological protocol.
145 non-duplicated *Staphylococcus aureus* isolates were further processed for identification of MRSA, VISA and VRSA.
Antibiotic susceptibility testing (AST)
In vitro antimicrobial susceptibility testing of all the *S. aureus* isolates was done for Cefoxitin (30 µg), Teicoplanin (30µg), Gentamicin (10µg), Doxycycline (30µg), Linezolid (30µg), Ciprofloxacin (15µg), Erythromycin (5µg), Cotrimoxazole (1.25/23.75µg) and Clindamycin (2µg).
Staphylococcus aureus ATCC 25923 was used as control strain to check the potency of every batch of antibiotic sensitivity testing.
Detection of MRSA isolates was done by using commercially available Cefoxitin disc, 30µg (Hi Media, Mumbai) by Kirby Bauer disc diffusion method as per CLSI guidelines 2024.
Interpretation: Strains having the diameter of zone of inhibition of ≤21mm were identified as MRSA.

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Detection of VISA and VRSA

Detection of VISA and VRSA isolates was done by detection of growth on Vancomycin Screen Agar (VSA) containing 6 µg/ml vancomycin and MIC determination by broth microdilution method (BMD) as per CLSI guidelines, 2024.

Interpretation: Presence of more than one colony of the strain or light film of growth on VSA when examined with transmitted light is interpreted as reduced susceptibility to vancomycin.

On broth microdilution, MIC ≤2 µg/ml of vancomycin are considered as Vancomycin sensitive *Staphylococcus aureus* (VSSA), MIC range from 4 - 8 µg/ml of vancomycin are considered as Vancomycin-intermediate *Staphylococcus aureus* (VISA) and MIC ≥16 µg/ml of vancomycin are considered as Vancomycin-resistant *Staphylococcus aureus* (VRSA).

RESULTS

Out of 647 clinically suspected cases of SSI screened, 550 (85%) were culture positive and 97 (15%) were culture negative. Out of 550 culture positive samples, 145 *Staphylococcus aureus* (26.3%) were isolated followed by 26 *Enterococcus species* (4.7%) and 19 CONS (3.4%) among gram positive bacteria. Among gram negative bacteria, 110 *Escherichia coli* (20%) followed by 99 *Pseudomonas species* (18%), 88 *Klebsiella species* (16%), 33 *Acinetobacter species* (6%), 18 *Proteus species* (3.3%), 7 *Enterobacter species* (1.3%) and 5 *Citrobacter species* (1%) were isolated. The most common isolate was *Staphylococcus aureus* (26.3%) followed by *Escherichia coli* (20%), *Pseudomonas species* (18%), *Klebsiella species* (16%) and so on.

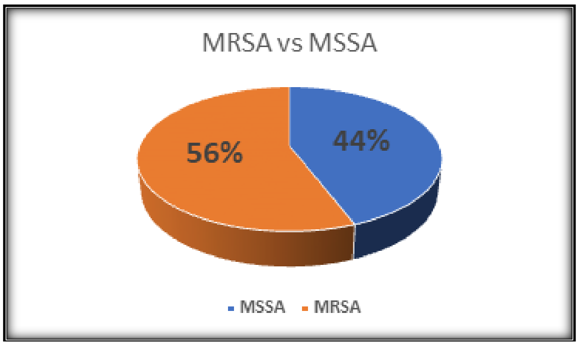

Figure 1: Distribution of MRSA and MSSA in *S. aureus* isolates

Out of 145 *S. aureus* isolates, 81 were detected as MRSA isolates (55.87%) as compared to 64 MSSA isolates (44.13%).

Table 1: Spectrum of Micro-organisms Isolated from Patients of SSI

Sl. No.	Name of Micro-organism	Number (n = 550)	%
1	*Staphylococcus aureus*	145	26.3%
2	*Escherichia coli*	110	20%
3	*Pseudomonas species*	99	18%
4	*Klebsiella species*	88	16%
5	*Acinetobacter species*	33	6%
6	*Enterococcus species*	26	4.7%
7	*Coagulase negative Staphylococci (CONS)*	19	3.4%
8	*Proteus species*	18	3.3%
9	*Enterobacter species*	7	1.3%
10	*Citrobacter species*	5	1%

Table 2: Age-wise distribution of MRSA in SSI

Age (Years)	Total (*S. aureus*) (n=145)	MRSA (n = 81)	MSSA (n=64)
<18	30	17 (21%)	13 (20.3%)
18 – 45	82	44 (54%)	38 (59.4%)
>45	33	20 (25%)	13(20.3%)

Table 3: Gender-wise distribution of MRSA and MSSA in SSI

Gender	Total (*S. aureus*) (n= 145)	MRSA (n= 81)	MSSA (n= 64)
Male	65	37 (46%)	28 (43.7%)
Female	80	44 (54%)	36 (56.3%)

The percentage of MRSA was more among females (54%) as compared to males (46%) with male to female ratio of 1:1.2 and was maximum in the age group 18-45 years (54%). Maximum number of MRSA isolates were from the department of General Surgery (44.5%) followed by Orthopaedics Department (18.5%) and Paediatric surgery (13.6%).

Table 4: Distribution of MRSA in SSI in relation to type of surgery

Type of Surgery	Total (*S. aureus*)	MRSA	MSSA
Emergency	86	50 (61.7%)	36 (56.3%)
Elective	59	31(38.3%)	28 (43.7%)
Total	145	81	64

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The isolation rate of MRSA was more in case of emergency surgeries (61.7%) as compared to elective surgeries (38.3%). Prolonged post-operative stay of >2weeks was associated with higher rate of MRSA related SSI (43.2%).

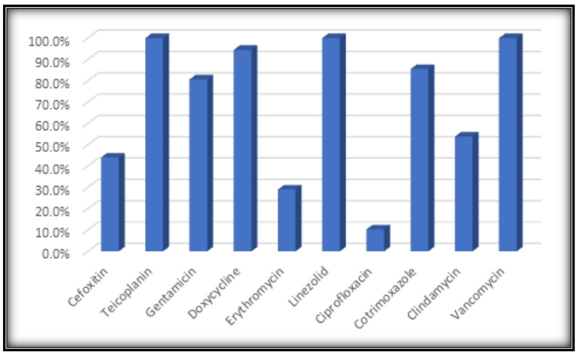

Figure 2: Antimicrobial sensitivity pattern of *Staphylococcus aureus* isolates (%)

All the 145 *S. aureus* isolates were 100% susceptible to vancomycin, teicoplanin and linezolid while the isolates were least susceptible to ciprofloxacin (10.3%) followed by erythromycin (29%). All the 81 MRSA isolates were 100% susceptible to vancomycin, teicoplanin and linezolid and least susceptible to ciprofloxacin (6%) and erythromycin (22%). Multidrug resistant MRSA were predominant (63%) in the study as compared to non-MDR isolates (37%). All the 145 *S. aureus* isolates were found to be susceptible to vancomycin on screening by VSA and confirmed by broth microdilution. All the isolates showed MIC ≤ 2µg/ml of Vancomycin by BMD. No VISA and VRSA were detected in our study.

DISCUSSION

Surgical site infections (SSIs) remain a significant and preventable challenge in healthcare settings, even in hospitals equipped with advanced infrastructure and standardized protocols for pre-operative care and antibiotic use [6,7]. The risk of developing an SSI depends on multiple factors, including the type and nature of the surgery, duration of hospital stay and patient-related conditions such as age, gender, and underlying health issues. *Staphylococcus aureus* is one of the most commonly isolated organisms in SSIs. It is found in the skin and nasal passage of over 80% of healthy individuals, and any surgical breach in skin integrity can facilitate infections. Methicillin-resistant *S. aureus* (MRSA) is particularly concerning, as it is a leading cause of hospital-acquired infections globally [19].

In the present study, out of 647 clinically diagnosed cases of SSI screened, 550 (85%) were culture positive and 97(15%) samples yielded no growth. This finding is similar to a study conducted by Kaur G et al [6] who reported 85.57% culture positivity and 14.4% culture negativity. It is also comparable to studies conducted by Geetanjali A et al [7], Dessie W et al [20], Shankar N et al [22], Sridhar P et al [23] and Negi V et al [24] who reported culture positivity rates of 76%, 84%, 79.5%, 93% and 96% respectively. Higher culture positivity rate may be due to contamination during or after surgery, patient related risk factors like diabetes, obesity, low immunity etc. or inadequate infection control measures. In contrast, Bhatta DR et al [2], reported lower culture positivity of 49.7% in their study. Culture negativity may be due to prior antibiotic therapy, presence of fastidious or atypical organisms that do not grow on standard culture media or better adherence to infection control measures.

In the present study, *Staphylococcus aureus* (26.3%) was the most common isolate followed by E. coli (20%), *Pseudomonas species* (18%) and *Klebsiella species* (16%). This is in accordance with various previous studies conducted by Kaur G et al [6], Geetanjali A et al [7], Ali AM et al [10], and Bhattacharya S et al [18]. The predominance of *Staphylococcus aureus* in SSI in the present study and many other studies may be due to colonization in the skin or nasal flora in more than 70- 80% of healthy individuals, contamination from environment, surgical tools, presence of silent carriers among hospital staffs, strong virulence factors of *Staphylococcus species* or emergence of multidrug resistant strains like MRSA.

In the present study, among 145 isolates of *S. aureus*, 81(56%) were detected as MRSA which is similar to studies conducted by Tiwari HK et al [25] who reported 40.61% MRSA, Kulshrestha A et al [8] who reported 51% MRSA and Bhatta DR et al [2] who reported 54.4% MRSA, while Nashra A et al [15] reported only 28.5% MRSA.

Variation in incidence of MRSA may be due to difference in the location and time period of the study, pre- & post-operative antibiotic policy and infection control surveillance program prevailing in different set up. Tertiary care hospitals have higher incidence as most of their patients were previously admitted in other facilities and received previous antimicrobial therapy, which increases the risk of acquiring resistant strains. In our study, MRSA was more among females (54%) as compared to males (46%) and was maximum in the age group 18-45 years (54%).). This is in accordance with Tafera S et al [11] who reported maximum MRSA cases (38.4%) in (15-30 years) age group and DK Turbawaty et al [19] who reported 93% of MRSA cases in adult age group. Kulshrestha A et al [8] and Ranjan KP et al [26] also reported similar findings of MRSA predominance (26%) in the age group of 21-30 years and 52% MRSA in 21-40 years age group respectively. This may be due to the reason that this age group might be more prone to accidents and trauma requiring emergency procedures which carry a higher risk of SSI, other contributing factors may include life style factors like smoking, alcohol consumption etc. In contrast, Negi V et al [24] and Bhatta DR et al [2] reported predominance in patients aged >50 years (51.8%) and 61-80 years (30%) respectively. The reasons may include age-related immune decline, chronic co-morbidities and longer recovery period. Similar female predominance was reported by Dessie W et al [20] (52.3%) and Snehaa K et al [27] (68%). Possible contributing factors in females may be more frequent exposure to surgical interventions (e.g., caesarean section and other gynaecological surgeries). In contrast, Bhatta DR et al [2], Geetanjali A et al [7], Kulshrestha A et al [8] and many other studies reported higher MRSA in SSI as compared to females. However, gender is usually not considered a definitive risk factor for surgical site infections (SSI). While some studies report a higher number of cases in males, the evidence remains inconclusive.

In our study, out of 81 MRSA isolates, highest number of MRSA were isolated from General surgery (44%) followed by Ortho Department (19%) and Paediatric Surgery (14%). This finding is in accordance with studies conducted by Kaur G et al [6] who reported maximum MRSA cases from General surgery (51%) and Ortho (22%). Similar findings were also observed in studies by Bhattacharya S et al [18], DK Turbawaty et al [19], Nishanthy M et al [28] and Naik G et al [29]. Incidence of MRSA among SSI was significantly more common in emergency surgeries (61.7%). Similar findings were observed in studies done by Dessie W et al [20] (61.7%%), Sridhar P et al [23] (54%), Chadhary SM et al [30] (63%) and Pathak A et al [31] (56%). Emergency surgeries accounted for a higher proportion of MRSA cases, suggesting increased vulnerability due to limited pre-operative optimization, contaminated wound environments, and urgent procedural conditions. Elective surgeries, while more controlled, still showed a substantial MRSA rate (38.27%), indicating that even planned procedures is not immune to resistant infections.

The present study demonstrates a statistically significant association (P = 0.01) between the duration of post-operative hospital stay and the incidence of MRSA in SSI cases. MRSA was most common in patients who stayed for >2 weeks post operatively (43.2%) as compared to <1 week stay (27%). This finding is in accordance with the studies conducted by Geetanjali A et al [7], Sridhar P et al [23], Mujagic E et al [32], Nage AR [33], Hou Y et al [34], and Yang L et al [35]. Longer hospital stays (>2 weeks) are usually associated with resistant strains, likely due to extended exposure to hospital flora, invasive procedures, and prolonged antibiotic use.

In the present study, all the 145 *S. aureus* isolates were 100% susceptible to Teicoplanin and Linezolid while the isolates were least susceptible to ciprofloxacin (10.3%) followed by erythromycin (29%). This is in accordance with the study done by Kaur G et al [6] who also reported 100% susceptibility to Teicoplanin and Linezolid, and least susceptibility to Ciprofloxacin (40%) and Erythromycin (59%). Bhatta DR et al [2] also reported least susceptibility to Ciprofloxacin (33%) and Erythromycin (31%), and 100% sensitivity to Teicoplanin. Similar findings were reported by Ansari SK et al [36], Shankar N et al [22], Kulshrestha A et al [8], Kulkarni et al [37] and Krishna S et al [38]. These findings suggest emergence of widespread multi drug resistance. So, treatment should be started according to the antimicrobial susceptibility pattern of the antimicrobial agents. Doxycycline and Cotrimoxazole also showed good sensitivity of 94.5% and 85.5% in the present study. This is in accordance to studies conducted by Bhattacharya et al [18] and Kaur G et al [6]. In the present study, D zone was observed in 30 *S. aureus* isolates (20.6%). This is consistent to studies conducted by Gupta et al [39] (20%), Sah et al [40] (12.1%) and Govindan et al [41] (11.6%). 41 Out of 81 MRSA isolates (63%) were multidrug-resistant (MDR) and 37% were non-MDR. This finding is similar with the studies conducted by Bhatta DR et al [2] who reported 81.2% of MDR cases. Maksoud et al [42] and Kateete et al [43] also reported higher MDR cases of 82% and 95% respectively. High MDR prevalence necessitates careful selection of antibiotics, based on antimicrobial susceptibility pattern, favouring agents like linezolid and teicoplanin which retain efficacy.

No VISA and VRSA were detected in our study by broth microdilution. This is in accordance with the studies done by Shankar et al, Nazeer et al [44] and Rana Khara et al in which no VISA and VRSA were detected. Kaur G et al [6] in their study also reported no VRSA but 3 isolates were confirmed as VISA by BMD. Six VISA (0.76%) and two VRSA (0.25%) were reported from a study in Northern India (Khan AKA et al [45]. In a study conducted by Bhattacharya et al [18], no VRSA were detected but 3 (1.12%) VISA were detected by E-test. 19% resistance was seen against vancomycin in a study from Congo, Africa. Zero prevalence of VISA and VRSA in our study is an important finding when some of the studies have reported resistance to vancomycin from our country. So, it proves that vancomycin can be used as drug of choice for MRSA infections.

CONCLUSION

Staphylococcus aureus is a major pathogen responsible for surgical site infections (SSIs). According to the CDC data, individuals at higher risk of methicillin-resistant and vancomycin-resistant *S. aureus* (MRSA/VRSA) infections include those with hospitalization or surgeries, residents of long-term care facilities, and patients with implanted medical devices. The MRSA

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prevalence of 56% in our study is quite high. No VISA and VRSA were detected in our study.

Despite advancements in antimicrobial development, resistance to antibiotics remains a concern. This is largely attributed to the inappropriate and excessive use of antibiotics, which not only reduces their effectiveness but also increases toxicity. Therefore, initiating therapy based on Antimicrobial Susceptibility Testing (AST) is essential to ensure appropriate and targeted treatment [6]. Regular antibiogram updates are essential to guide local prescribing practices.

Effective prevention of SSIs requires strict adherence to pre- and post- operative care protocols. This includes regular wound monitoring for signs of infection - such as redness, swelling, warmth and discharge, and enforcing standard infection control measures like hand hygiene, environmental sanitation, sterilization of surgical instruments, and ongoing education and surveillance for both patients and healthcare workers. There should be a policy in every hospital for infection control practices and regular surveillance to reduce SSIs. Also, there is a need to emphasize the rational use of antibiotics and strict adherence to the concept of reserve drugs to minimize the misuse of avoidable antimicrobials.

As the current study only indicates the tip of iceberg, more and more studies should be undertaken in future to monitor the emergence of these multidrug resistant strains.

Limitations

Genotypic detection of MRSA, VISA and VRSA were not included in the study.

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