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The emergence of vancomycin resistance among methicillin-resistant Staphylococcus aureus isolates from post-operative surgical site infections at District Jacobabad

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ABSTRACT

Surgical site infections (SSIs) are the most common post-operative infections and *Staphylococcus aureus* is the leading microbial cause. SSIs contribute significantly to morbidity and healthcare costs. The epidemiology and susceptibility pattern of *S. aureus* is fluctuating over time with the propagation of newer clones and the emergence of highly resistant strains. This cross-sectional study was conducted at Jacobabad Institute of Medical Sciences (JIMS) to determine the prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients suffering from SSIs from January to October 2021. A total of 180 pus aspirate and pus swabs were collected from patients who had undergone surgical procedures. Of these, 146 (81%) specimens were bacterial culture positive. Mono-bacterial growth was isolated from 111 (76%) patients and the remaining 35 (24%) patients revealed growth of either two organisms or mixed bacterial growth. Diabetes mellitus and chronic liver disease were major underlying medical conditions. The most common bacterial isolate was *S. aureus* 88 (79%) of which 38 (43%) were MRSA. The highest sensitivity of MRSA was revealed against vancomycin (87%), followed by Amikacin and Doxycyclin 84% and 82% respectively. Minimum inhibitory concentration (MIC) of vancomycin revealed, 5 (13%) of MRSA isolates were resistant to vancomycin exhibiting three isolates MIC 32 µg/ mL and two isolates MIC 64 µg/ mL. None of the methicillin-sensitive *Staphylococcus aureus* (MSSA) was resistant against vancomycin. Among MRSA and MSSA, the highest rate of resistance was observed against sulfamethoxazole+trimethoprime and ciprofloxacin. This report highlight the emergence of a significant fraction of VRSA among MRSA isolates amongst post-operative SSIs. The findings of this study call for regular monitoring of sensitivity patterns and the necessity of new and effective antibiotics.

Keywords: Emergence, Prevalence, Vancomycin, Surgical site infections, Methicillin resistance, Staphylococcus aureus

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INTRODUCTION

Surgical site infections (SSIs) have been estimated the third most common nosocomial infections worldwide and remain a great source of morbidity, a challenge for surgeons, delay wound healing, prolonged hospital stay, pain, increase in medical cost, and long-lasting disability among post-operative patients despite the antimicrobial evolution and advancement in modern aseptic techniques (1,2,3). SSIs frequently occur within 30 days following surgery (4). It has been estimated that there are about 234 million major surgeries worldwide per year. SSIs complicate 2-15% of all patients undergoing inpatient surgical procedures (5). According to the Center for Disease Control and Prevention (CDC), SSIs are of three types, superficial, deep incisional, and organ-space SSIs and among these, superficial incisional SSIs are more

common (6). Risk factors include emergency procedures, patients' age, level of care, and duration of surgery. Appropriate surgical antibiotics prophylaxis (SAP) may reduce the risk of SSIs (5).
Gram-positive cocci are generally considered the most common etiological agents over gram-negative bacteria for SSIs (7). The most common gram-positive cocci causing SSIs is *S. aureus* (7,8). *S. aureus* is usually found in the hospital environment and gains access to the surgical sites by direct contact with contaminated objects. They may also be transmitted through air droplets and aerosols produced by the health care personnel infected with *S. aureus* (9). A variant of *S. aureus* which is more virulent and causes life-threatening invasive infections among post-operative surgical patients is methicillin-resistant *S. aureus* (MRSA) (10). MRSA infections are associated with significant morbidity in both community and hospital environments (11). Since its emergence in 1961, the incidence of MRSA infections are increasing worldwide and a high surge in MRSA especially in the last decade has gotten attention from the medical community and scientists to find out the root causes and to overcome the mishaps (12).
The first choice of treatment for *S. aureus* infections is beta-lactam antibiotics. Resistance against methicillin is the indicator of resistance against most of the members of the beta-lactam group such as penicillin and cephalosporin (13) . The prevalence estimation of MRSA is important to combat the menace of this microorganism (14). The frequency of SSIs due to MRSA varies geographically and from hospital to hospital. Compared to northern Europe, the prevalence of MRSA is higher in Pakistan and India. The prevalence of MRSA in Pakistan has been estimated at 42-51% and in India at 41% (15). Glycopeptides such as vancomycin are frequently the treatment of choice for complicated MRSA infections (16). Infections due to Vancomycin-resistant *S. aureus* (VRSA) have also been reported from various parts of the world. The present study attempts to find the prevalence of MRSA and VRSA and their antimicrobial susceptibility pattern among patients suffering from post-operative SSIs.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the surgery and pathology department of Jacobabad Institute of Medical Sciences (JIMS), Jacobabad, Pakistan from January to October 2021. A total of 180 patients were recruited for this study and monitored for 30 days after surgery. Pus swabs and aspirates were collected from the surgery site of post-operative patients and inoculated on mannitol salt agar, chocolate agar, and McConkey agar. Organisms were identified using conventional methods including colony morphology, gram staining, catalase, coagulase, DNase, pigmentation, and fermentation of different sugars (17). We confined our study to gram-positive cocci and the antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method against amikacin, ciprofloxacin erythromycin, sulphamethoxazole/trimethoprim, clindamycin, vancomycin, tetracycline, fusidic Acid, and doxycycline. Resistance against methicillin was determined using cefoxitin 30 µg disc. *S. aureus* isolates showing resistance against vancomycin disc were further confirmed by determining their level of resistance by minimum inhibitory concentration using the broth dilution method. The interpretation was done according to the CLSI-2018 guidelines. Statistical analysis was done using SPSS version 20.

RESULTS

In this study, a total of 180 samples of pus swabs (n=147) and pus aspirates (n=33) were collected from the surgical site of post-operative patients ranging in age from 11-60 years with a mean age of 12.76 $\pm$ 08.27 years. A total of 146 (81%) specimens were bacterial culture positive. Mono-bacterial growth was isolated from 111 (76%) patients, no growth from 34 (23%) patients, and the remaining 35 (24%) patients revealed growth of either two organisms or poly-microbial growth. The most common isolated organism was *S. aureus* 88 (60.2%), followed by *Escherichia. coli* 44 (30.1%), *Pseudomonas aeruginosa* 10 (6.8%) and *Klebsiella spp*. 4 (2.7%).
Out of 88 *S. aureus*, 42 (48%) were isolated from males and 46 (52%) from females. A large number of *S. aureus* were isolated from age group 21-30 years 25 (28%), followed by 31-40 years 20 (23%), 11-20 and 51-60 years 15 (17% each) and from 41-50 years 13 (15%). The common sites for SSIs were legs 26 (30%), feet 22 (25%), and abdomen 18 (20%) and the least common sites were head 12 (14%), hands 8 (9%), and back 2 (2%). The most common underlying medical condition was diabetes mellitus 28 (32%), followed by chronic liver disease 24 (27%), malignancy 18 (20%), immune-suppression 10 (11%), chronic kidney disease 6 (7%), and heart failure 2 (2%) **(Table: 01).** *S. aureus* showed the highest resistance against sulfamethoxazole-trimethoprim 37 (74%) and ciprofloxacin 25 (50%) followed by clindamycin 16 (32%), fusidic acid 14 (28%), tetracycline 11 (22%), erythromycin 10 (20%), doxycycline 8 (16%), and amikacin 4 (8%) None of the methicillin-sensitive *S. aureus* (MSSA)

Table: 01. Demographic characteristics and distribution of *Staphylococcus aureus* among patients suffering from Surgical site infections (SSIs)

Characteristics	***Staphylococcus aureus* (n=88)**	
	Frequency	%
Gender		
Male	42	48
Female	46	52
Age in (years)		
11-20	15	17
21-30	25	28
31-40	20	23
41-50	13	15
51-60	15	17
Site of infection		
Legs	26	30
Feet	22	25
Abdomen	18	20
Head	12	14
Hands	8	9
Back	2	2
Underlying medical condition		
Diabetes mellitus	28	32
Chronic liver disease	24	27
Malignancy	18	20
Immune-suppression	10	11
Chronic kidney disease	6	7
Heart failure	2	2

was resistant to vancomycin in this study (**Figure: 01).**

We found a very high prevalence of MRSA in our study patients as out of 88 *S. aureus*, 38 (43%) were methicillin-resistant (MRSA). Vancomycin, amikacin, and doxycyclin were found the most effective antibiotic treatments for MRSA as 87%, 84% and 82% of MRSA isolates were sensitive to these antibiotics respectively. MRSA exhibited the highest rate of resistance against sulfamethoxazole+trimethoprime (79%) and ciprofloxacin (66%). MRSA resistance against other antibiotics was fusidic acid (42%), tetracycline (39%), a macrolide (34%), and lincosamide (32%). Notably, in this study we found 5 (13%) of MRSA isolates were resistant to vancomycin, three isolates with MIC 32 µg/ml and the two strains with MIC 64 µg/ml (Figure: 02)

DISCUSSION

Despite the modern surgical techniques and antibiotic prophylaxis, SSIs contribute to a large burden of disease and remains a global challenge for the medical community. Compared to developed countries the rate of SSIs is higher in developing countries (5). The present study reveals a very high SSI rate 146/180 (81%) among post-surgical patients in our hospital. Comparable findings were reported from Mardan, Pakistan where the rate of infection was reported 102/136 (75%) (18). Recently, a study from Islamabad reported the SSIs prevalence (29.85%) (5). In contrast, a very low frequency of SSIs 82/1120 (7.7%) among post-surgical patients was reported from Karachi and Peshawar 25/269 (9.3%) (19) . These variations in the prevalence of SSIs have been attributed to differences in hospital environments, overcrowding, poor sanitation, contaminated water, and lack of infection control policies (20).

S. *aureus* is normally found on the skin or anterior nares of 80% of healthy individuals which makes the *S. aureus* the most common etiological agent of SSIs (21). In this study, the most common isolated organism from SSIs was *S. aureus* (60.2%), followed by *E. coli* (30.1%), *P. aeruginosa* (6.8%), and *Klebsiella spp*. (2.7%). Several other studies have reported similar findings. A study from Pakistan reports the main causative agent of these infections is *S. aureus* (23.80%), followed by *E. coli* (16.66%), *P. aeroginosa*, and *Enterobacter spp.* (14.28% each). In this study, we found a very high prevalence (60.2%) of *S. aureus* among post-surgical infections compared to other studies conducted at Peshawar, Mardan, Attock and Gilgit where the prevalence of *S. aureus*

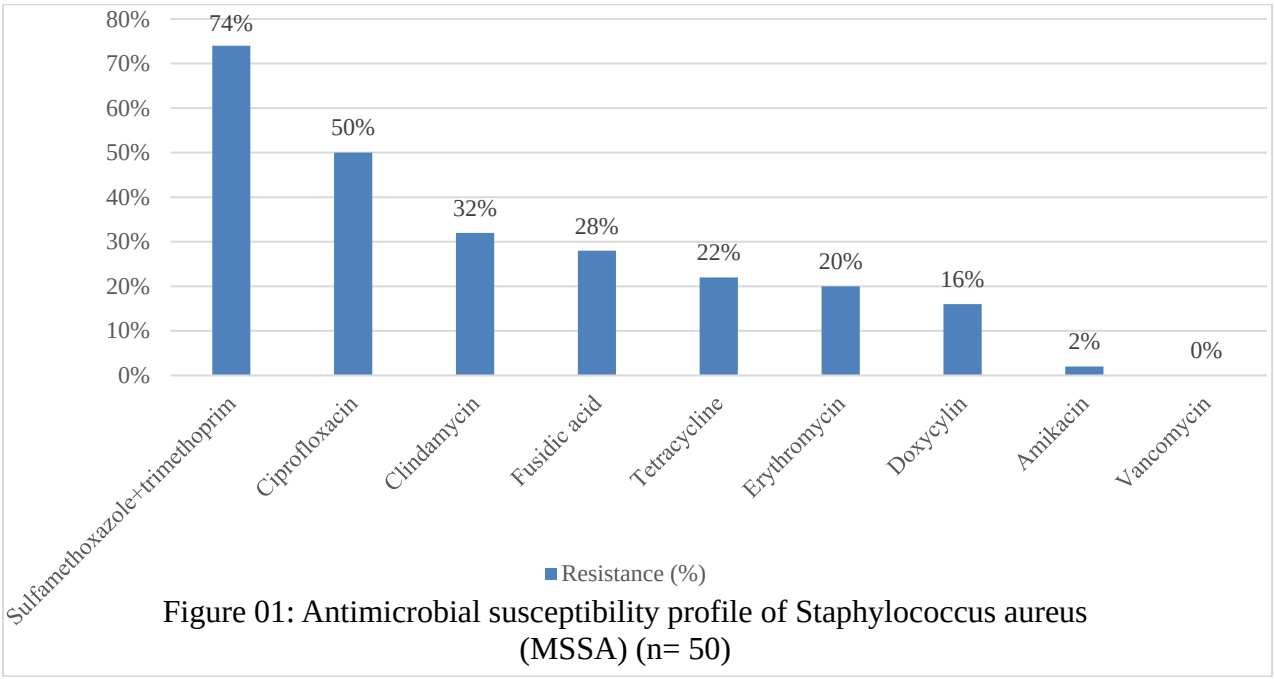

Figure 01: Antimicrobial susceptibility profile of Staphylococcus aureus (MSSA) (n= 50)

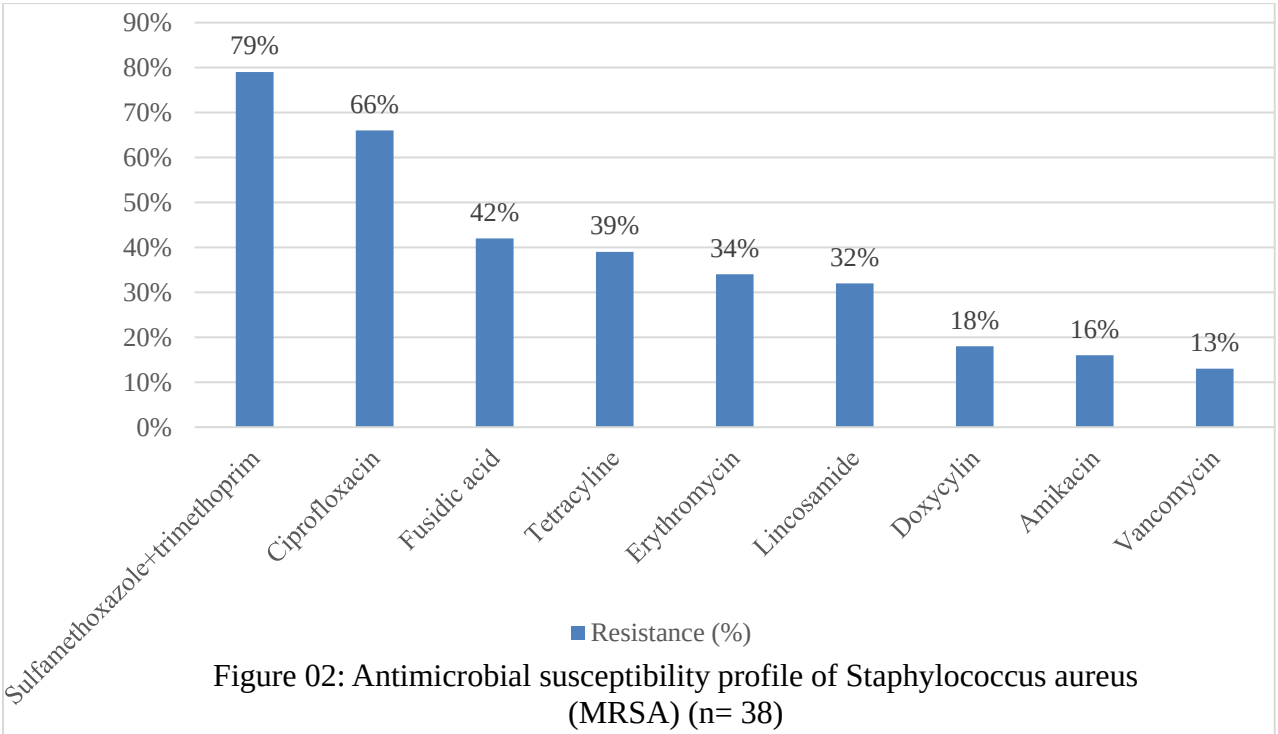

Figure 02: Antimicrobial susceptibility profile of Staphylococcus aureus (MRSA) (n= 38)

among SSIs has been reported ranging from 34-39.3% (22,23) . Our study is comparable to a study from Lahore where the prevalence of *S. aureus* among SSIs has been reported 55% (24). Another study reported the frequency of *Staphylococcus aureus* (43%) (25). Infections due to MRSA complicate the management and treatment of SSIs and beta-lactam antibiotics become no further option for treatment. MRSA has become a global threat due to its resistance to multiple antibiotics (26). In

Karachi, the first case of MRSA was reported in 1989 with a prevalence rate 5% which gradually increased up to 57% in 2002 and then gradually decreased to 38.6% in 2009 (26)**.** In this study, we report 43% of *S. aureus* were resistant to methicillin which is in concordance with several studies from other parts of Pakistan (27). Compared to our study, low prevalence of MRSA has been reported from Rawalpindi (20.3%)(28,29) and high prevalence from Mardan (66%) and Peshawar (62.9%) (30, 18). A very high prevalence of MRSA (78.9%) has also been reported from Iran (25). This variation in MRSA prevalence might be due to differences in quality of post-operative management, types of surgeries, or hospital infection control policies.

Patient's age is frequently recognized as a statistically significant factor for SSIs as decrease in immunity, increase in catabolic processes, low healing power, and co-morbid illnesses make the older age group more susceptible to SSIs (19). In this study, we report 51% of SSIs were among patients ranging in age 21-40 years which is in contrary to several other studies which report a high prevalence of SSIs among older age groups >50 years (31). In our study, we found only 17% of SSIs were among age group >51 years whereas a similar study from Pakistan has reported the highest prevalence (45.23%) in age group ranging from 65-80 years (33). Similar to our finding, few studies also report the maximum number of SSIs in the age group of 21-40 years (33). In this study, we do not find any significant statistical difference in gender prevalence of SSIs as we report 52% of SSIs were among females and 48% were among males. In our study, the rate of SSIs was slightly higher among female patients, in contrast, other studies from Pakistan report higher prevalence among male patients (25,33). In the present study, we report the diabetes mellitus and chronic liver disease major underlying medical conditions (59%) for SSIs whereas a comparable report from Pakistan reports the diabetes mellitus 30.95% and obesity 21.42% the major threat related with these infections (33).

In practice, when beta-lactam antibiotics remain no more effective treatment due to MRSA infections, glycopeptides (vancomycin) are preferably used to treat these complicated MRSA infections (16). Several studies from Pakistan and other countries claim that vancomycin most effective antibiotic treatment for MRSA infections without any resistance (34). Recent reports from several countries and Pakistan regarding the emergence of vancomycin-resistant isolates is another challenge and have further limited the treatment options (22). This alarming situation calls for the necessity to discover new antibiotics for curing MRSA and VRSA infections. Though rate of VRSA is low it has been reported from several parts of the world including Pakistan (22,24). In the present study, we report non of the MSSA isolate was resistant to vancomycin whereas 13% of MRSA isolates were resistant to vancomycin. Recently, similar resistance rate of 14% has been reported from Lahore (35). Vancomycin resistance from other parts of Pakistan has also been reported (36, 18). Recently, cumulative VRSA prevalence among SSIs in various hospitals of Peshawar has been reported 8.33% and from Lahore 2.5 to 9.8% (22, 24, 37). High rate of vancomycin resistance in our patients may be attributed to frequent and misuse of this antibiotic and improper dosage taken by the patient because literacy rate in Jacobabad district is very low.

CONCLUSION

SSIs are the common bacterial infections among post-operative patients. Gram-positive bacteria are the common cause of SSIs. *S. aureus* is the most common culprit responsible for post-operative SSIs. The high prevalence of MRSA has limited the treatment choices. The emergence of vancomycin-resistant *S. aureus* has further complicated the treatment options. Good infection control practices, strict aseptic techniques, and appropriate use of antibiotics may reduce the spread of MRSA in our hospitals. Further multi-center studies are required to determine the actual emergence of vancomycin-resistant *S. aureus* in Pakistan.

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