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Antimicrobial susceptibility and frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from skin infected patients in District Peshawar, KPK, Pakistan

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Abstract

Staphylococcus aureus (S. *aureus*) is an important pathogen which causes community associated Methicillin-resistant *Staphylococcus aureus* in fection (CA-MRSA) and hospital-associated Methicillin-resistant *Staphylococcus aureus* (HA-MRSA). The objective of the current study was to investigate the frequency and antimicrobial susceptibility of MRSA isolated from clinical settings of Peshawar. Clinical samples including pus and swab were collected, followed by identification of MRSA by microscopic and biochemical based methods. Out of 97 isolates, (27%) were found positive. The frequency of MRSA strains was more in females (57%) than in males (43%). *S. aureus* was more prevalent in the age group 31-45 years with 42% followed by the age groups 46-60, 16-30 and 1-15 years with 26%, 18%,and 14% respectively. *S. aureus* showed high resistance to Oxacillin (27%), followed by Clarithromycin (18%), Amikacin and Doxycycline (16%), vancomycin (15%), Cefoxitin (13%), Amoxicillin (12%), linezolid (8%) and Sulzone (7%). The present study highlighted that linezolid and Sulzone were most effective to treat *S. aureus* infected patients.

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Introduction

S. aureus has heterogeneous groups and consists of 30 species. *S. aureus* is one of the clinically essential species. The *S. aureus* is normally a flora of skin of the human body and generally assail on the skin or in the nosel region of a healthy human being which makes it easy to be transmitted by air or fomites from patients or carriers (Brown *et al.*, 2005). For that reason, the avoidance of staphylococcal infections is currently more essential. Carriage of *S. aureus* in the nose appears to co-operate a key task in the epidemiology and pathogenesis of infection (Williams, 1963).

S. aureus causes a broad variety of disease like abscesses, furuncles, osteomyelitis, infective endocarditis, and bacteremia. It is a major source of causing nosocomial and community-acquired infections (Ghebremedhin *et al.*, 2009). Due to the synthesis of Penicillin-binding protein (PBP) 2A *S. aureus* shows resistance to all β-lactam antimicrobial agents (Von Eiff *et al.*, 2001).

Community-associated MRSA is able to initiate severe diseases like necrotizing pneumonitis and toxic shock syndrome in case if the bacteria carry and express toxin-producing gene (Boucherand Corey, 2008).

Several medical studies have indicated that MRSA strains are more dangerous than Methicillin-susceptible Staphylococcus aureus (MSSA) strains (Hartman and Tomasz, 1986).

The present study was conducted in Peshawar to find out the antibiotic sensitivity pattern and frequency of MRSA in infected individuals.

Materials and methods

Study design and sample collection

The present study was conducted in the department of microbiology Khyber teaching hospital (KTH) Peshawar.

A total of 97 samples were collected from visiting and admitted patients from October 2017 to December 2017. Specimens of patients irrespective of their age and gender were included in the study. Pre-informed consent was taken from patients during sampling.

Isolation and identification of S. aureus from clinical isolates

Samples were inoculated on blood agar, MacConkey's and mannitol salt agar. The inoculation step was followed by identification of the strains by Gram's staining, culture characteristics and biochemical reactions such as catalase, coagulase and DNase.

Antibiogram and screening of MRSA

Antibiotic susceptibility tests were carried out by disc diffusion method. The *S. aureus* isolates were inoculated on Mueller-Hinton agar plates and various antibiotic discs were placed on the plates followed by incubation at 37°C for 24 hours. Post-overnight incubation, antibiotic susceptibility pattern was determined.

The zones of inhibition were measured and compared according to the national committee for clinical laboratory standard guidelines. All confirmed *S. aureus* isolates were screened for Methicillin resistance.

The 5µg Methicillin (Cefoxitin FOX) disc (Oxoid, USA) was aseptically placed on the surface of the inoculated plates aerobically at 37°C for 18-24 hour.

Statistical analysis

All the collected data were entered in Microsoft Excel sheet and analyzed by using (SPSS-18). Results have been presented in the form of figures and tables.

Results

In the current study, a total of 97 *S. aureus* positive clinical samples were collected from KTH Peshawar. Out of 97 isolates, 26 (27%) were detected MRSA positive as shown in Table 1.

Percentage of different antibiotics against S. aureus

The data regarding antibiogram of different antibiotics against *S. aureus* is presented in Fig. 1. In

the results Oxacillin was noted with higher resistance (26.8%) against *S. aureus* followed by Clarithromycin (17.5%), Doxycycline (16.4%), Amikacin (16.4%), Vancomycin (14.9%), Cefoxitin (13.4%), Amoxicillin (12.3%), Linezolid (8.2%) and Sulzone (7.2%). Similarly *S. aureus* was found more sensitive to Linezolid (92%) Sulzone (93%), followed by Amoxicillin (87%), Cefoxitin (86.5%), Vancomycin (84%) Amikacin (83%) Doxycycline (82%), Oxacillin (73%) and Clarithromycin (72%).

Table 1. Number of MRSA and MSSA among the isolates.

S. aureus strains	Number of samples	Percentage %
Methicillin resistance S. aureus (MRSA)	26	27%
Methicillin susceptible S. aureus(MSSA)	71	73%
Total	97	100%

Table 2. data represents the presence of S. aureus in the pus samples of male and females. In the total studied population 42 (43%) male patients were positive and 55 (57%) female patients showed the presence of S. aureus.

Discussion

The current study was conducted to determine the frequency of MRSA and pattern of antimicrobial susceptibility to MRSA. The MRSA was identified as a nosocomial pathogen. In the current study, the presence of S. aureus was investigated in hospitalized patients which may cause nosocomial infections. In a similar study 35%, MRSA was isolated of which 82% were found in hospitalized patients and 17% from outpatients. The present study shows the presence of MRSA at a rate of 27%. Our colonization rate of 27% for MRSA was low as compared to the study results (Aghazadeh *et al.*, 2009).

Table 2. Gender wise distribution of *S. aureus* isolates.

Gender	Number of isolates	Percentage%
Male	42	43%
Female	55	57%
Total	97	100%

Table 3. shows the age-based analysis of the presence of S. aureus. The samples were divided into age groups. The high rate of the *S. aureus* was observed in the age group 31-45 years with 42%, followed by the age group 46-60 with 25%, 16-25 with 17% and 1-15 years with 14%.

The resistance showed by *S. aureus* may be due to excess used of antibiotics, either because of mutation in the genetic material or because of climate change. In another study 56% MRSA was isolated of which 70% of MRSA was found in hospitalized patients (Chandrashekhar *et al.*, 2012). Another study reported that the *S. aureus* was more prominent in male with 58% as compared to female with 41% (Mahmood *et al.*, 2010).

In our study, the S. aureus was more prevalent in female with 56% as compared to male with 43%. The significant cause for the greater frequency in the female population could be the main occupation of the female as a housewife. While the male are not involved in the house chores more commonly (Bhatt *et al.*, 2014). The results werenot in accordance with the results of our study that *S. aureus* was more prominent in female than male.

In the present study, the patients were divided into various age groups. The high rate of S. aureus was observed in the age group of 31-45 years with 42% followed by the age group of 46-60 years with 25%

and 17% in the age group of 16-30 years. A high rate of *S. aureus* in the age group 31-45 was observed with 42%. This may be due to excess time period spent by an adult of age group 31-45 years outside the home. The *S. aureus* in age group 45-60 years with 25% prevalence might be because of weak immunity. *S. aureus* with 17% in age group 16-31 years may be because of the fact that most of them were students and also in the age group 1-15 years with a frequency of 14%.

Table 3. Age wise prevalence of MRSA and MSSA.

Age Groups (Years)	*S. aureus* (N)	MRSA N (%)	MSSA N (%)
1-15	14	5(35.7%)	9(64.3%)
16-30	17	6(35.3%)	11(64.7%)
31-45	41	12(29.3%)	29(70.7%)
45-60	25	3(12.0%)	22(88.0%)

A study presented the age wise prevalence of *S. aureus* in the patients. High rates of *S. aureus* were observed in the age group of 30-50 years with 88%, followed by the age group than 50 years and above with 78% and 57% 30 years and below age group (Khanal *et al.*, 2018).

In a relevant study, infected individuals were divided into different age groups. The high rate of S. aureus was observed in the age group of 1- 10 years with 18% isolates followed by the age group 21-30 years with 17% isolates (Chandrashekhar *et al.*, 2012).Previously a study showed the percentage of different age groups. A high percentage of S. aureus was observed in the age group of 0-10 years with 24% followed by the age group of 51-60 years with 19% and 18% in the age group of 21-30 years (Bhatt *et al.*, 2014). A relevant study described various age groups of *S. aureus* infected persons.

The high rate of *S. aureus* shown in the age group of 41-80years with 64% followed by age group of 21-40 years with 19% and 11.32%in age group of 1-20 years (Mahmood *et al.*, 2010).

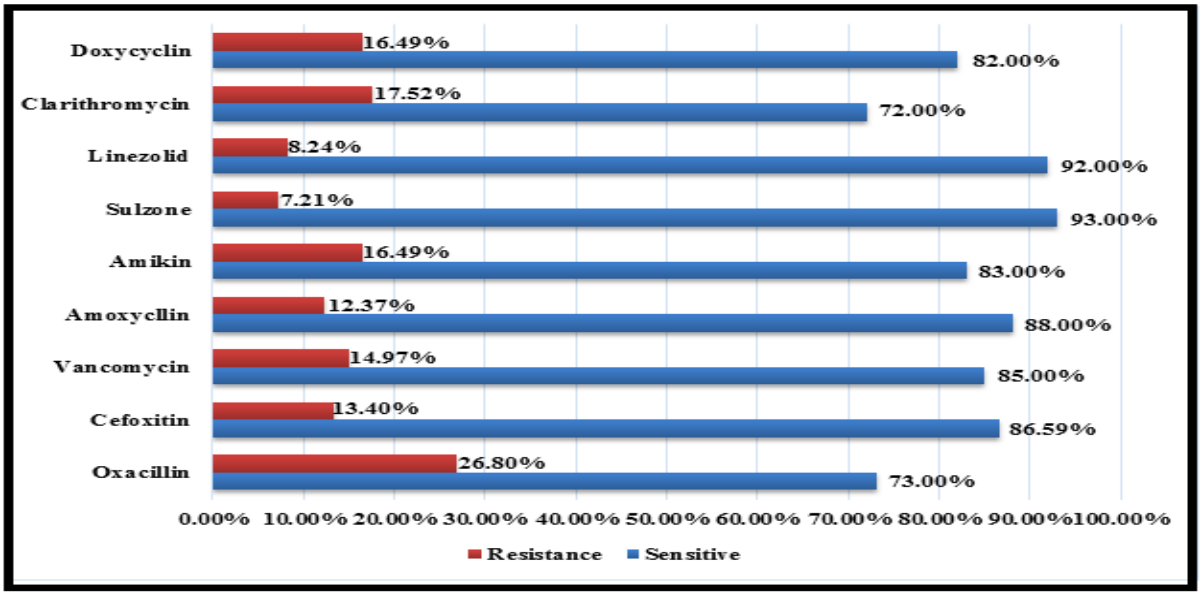

Fig. 1. Percentage of different antibiotics against *S. aureus*.

In the present study S. aureus show 26% resistance to Oxacillin followed by Clarithromycin 17%, 16% by Amikin and Doxycycline, Vancomycin 14%, Cefoxitin 13%, Amoxicillin 12%, Linezolid 8% and Sulzone 7%. (Bhatt *et al.*, 2014) presented the antimicrobial susceptibility of MRSA in different patients, and reported that 100% sensitivity of MRSA to Vancomycin, 84% to Amikacin, 63% to Tetracycline,

42% to Ciprofloxacin, 36% to Gentamycin and in urine isolates Nitrofurantoin with87%, followed by Norfloxacin with 75%sensitivity. The antimicrobial susceptibility of MRSA in different isolates was presented in another study, in which it was reported that 100% of MRSA isolates were resistant to Penicillin, 91% to Erythromycin, 90% to Cefepime, Ampicillin and sulbactam, 83% to Amoxicillin, 81% to Norfloxacin, 78% Cefuroxime and 25% to Amikacin (Chandrashekhar *et al.*, 2012).

Conclusion

It was concluded from the study that the environment is contaminated with different types of microorganisms including S. aureus. Its pathogenicity increases when it turns out to be resistant to the different antibiotics.

The main reservoirs for the spreading of *S. aureus* are the environment, primary health care centers, and hospitals. With conducting special activities for the cleanliness of the environment and hospitals the rate of *S. aureus* infection could be reduced. The recommendations which were observed to be essential includes, proper ventilation program in the hospitals.

The environment should be kept clean. The government should construct laboratories for checking the rate of such type of infections. The public should care about their health and with the clean environment, they must also conduct routine checkup for the detection of such type of infections. Further study would be valuable to know the exact occurrence of the MRSA in hospitalized patients as well as in the general population of Pakistan, particularly in KPK.

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