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Frequency of Methicillin Resistant *Staphylococcus aureus* Among Isolates of Wound Infections from Hyderabad

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Abstract: *S. aureus* commonly colonizes human skin and mucosa and can cause skin and skin structure infections in human. *S. aureus* resistant to methicillin (MRSA) is an important pathogen associated with hospital and community acquired infections triggering high morbidity and mortality. The aim of this study was to assess the frequency of oxacillin resistant strains of *S. aureus* from wound infection circulating in Hyderabad, for guiding rational use of antibiotics in clinic. *S. aureus* isolates from 153 wound specimens were evaluated for susceptibility to various commonly used antibiotics and oxacillin was particularly used to screen for MRSA using disk diffusion method. The data revealed that out of 52 *S. aureus* isolates, 19 (36.5%) were MRSA. Overall, *S. aureus* was found to be the main organism which contaminates wound infections. Furthermore, all *S. aureus* isolates in this study were shown sensitive to vancomycin and piperacillin antibiotics. In summary, *S. aureus* was found dominant among wound specimen isolates at Hyderabad and prevalence of MRSA was 36.5% and all MRSA were susceptible to vancomyicn.

Keywords: *S. aureus*, MRSA, Antibiotic Resistance, Vancomycin-Resistant, Wound Infections

1. INTRODUCTION

S. aureus is coagulase positive Staphylococcus, which is commonly associated with its colonization on human skin and nasopharynx and causing various suppurative (pus-forming) infections. In addition to skin and skin structure infections, *S. aureus* can cause more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as Osteomylitis and endocarditis (Lowy, 1998).

Methicillin antibiotic was introduced in clinical settings to combat infections caused by penicillin resistant Staphylococcus species. Shortly, a proportion of organisms acquired resistant to methicillin (Barber, 1961), and the resistant strains are termed as MRSA and often require different types of antibiotic to treat them. Since the first description in 1961 (Barber, 1961) the incidences of MRSA have gradually increased and now hospital and community associated MRSA infections are a growing public health concern around the world (Ippolito *et al.*, 2010; Rajaduraipandi *et al.*, 2006).

MRSA has become major cause of nosocomial infections with a potentially deadly strain of Healthcare-acquired methicillin-resistant *S. aureus*, or HA-MRSA that has developed resistance to several antibiotics (Bishara *et al.*, 2003; Hafiz *et al.*, 2002). HA-MRSA infections may include surgical wound infections, urinary tract infections, bloodstream infections, and pneumonia. Antibiotics other than methicillin such as clindamycin, linezolid, tetracycline, trimethoprim-sulfamethoxazole, or vancomycin, are usually the first choice of treatment for HA-MRSA infections (Boucher *et al.*, 2010).

MRSA, previously confined to healthcare exposure, has now been shown to be responsible for a growing number of infections among athletes, students, and the military having no required previous history of healthcare exposure (*Centers for Disease Control and Prevention*,CDC, 2001, 2003; Kazakova S.V, 2005). Such strains of MRSA are known as Community Acquired MRSA (CA-MRSA). Since late 1990s, a rapid emergence of the CA-MRSA brought a major change in epidemiology of Staphylococcal infections (Carleton *et al.*, 2004; Chambers, 2001). The majority of CA-MRSA clinical isolates are also recovered from skin or soft tissues (Maltezou HC and Giamarellou H, 2006). Recently, an increase in the incidence of CA-MRSA skin and soft tissue infections have been observed (Siddiqui and Bernstein, 2010).

Bacterial colonization is the initial manifest of a wound infection. If the colonization, associates with other factors such as decreased vascular supply, intrinsic virulence of specific bacterium (eg. *S. aureus*) and host immune factors, proper infection will appear (Siddiqui and Bernstein, 2010). The main causes of the contamination of all wounds include various factors such as surrounding skin, the local environment, and endogenous patient (Siddiqui and Bernstein, 2010).

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Moreover, the hospitalization, surgical procedures, and prolonged or broad-spectrum antibiotic therapy of patient may influence bacterial colonization of wound or infection, or both, with resistant organisms, including MRSA (Baveja *et al.*, 2010; Hartemann-Heurtier *et al.*, 2004). The antibiotic-resistant bacteria pose a major concern to wound care because of their ability to resist many of the antibiotics used today to treat infections (Percival *et al.*, 2011).

MRSA infections are not only limited to developed countries, but they also have been seen in developing countries and an alarming increase has also been reported in Pakistani hospitals (Hafiz *et al.*, 2002). MRSA has been found in health care settings and emerging as a major nosocomial pathogen in Pakistan (Ashiq & Tareen, 1989; Hafiz *et al.*, 2002; Zafar *et al.*, 2011). It has also been shown to prevail in local population with frequencies varying between 2-61% highest incidence in the major cities of the country, however, no vancomycin resistant *S. aureus* (VRSA) has yet been reported in Pakistan (Butt *et al.*, 2004; Hafiz *et al.*, 2002; Hakim *et al.*, 2007).

2. MATERIALS AND METHODS

2.1 Bacterial isolates

A total of 107 bacterial strains were isolated from 153 wound specimens. The specimens were obtained by either swabs or pus samples from inpatients and outpatients from various medical centers of Hyderabad from July 2011 to December 2011.

2.2 Antibiotic Susceptibility testing

The antibiotic susceptibility was determined by Kirby-Bauer disc diffusion to investigate the antibiotic susceptibility patterns of the *S. aureus* isolates of wound infections using various commercially available antibiotics **(Table.1)** to record

Table 1: List of antibiotics used in this study

S.No	Name of Antibiotic	Class of antibiotic
1	Amikacin (30µg)	Aminoglycoside
2	Clindamycin (2µg)	Lincosamide
3	Ciprofloxacin (5µg)	Quinolone
4	Erythromycin (15µg)	Macrolide
5	Fosfomycin (50µg)	Cell wall inhibitor
6	Ofloxacin (5µg)	Fluoroquinolone
7	Oxacillin (1µg)	β-lactam
8	Piperacillin (75µg)	β-lactam
9	Tetracycline (30µg)	Protein inhibitor
10	Vancomycin (30µg)	Cell wall inhibitor

the current status of *S. aureus* response to commonly used antibiotics and particularly to observe frequency of MRSA among wound isolates in Hyderabad, Sindh. Statistical analysis to obtain ratio of bacterial contamination in wound infections and antibiotic susceptibility pattern of *S. aureus* isolates of wound infections was carried out using MS-Excel 2007.

3. RESULTS AND DISCUSSION

3.1 Ratio of bacterial colonization in wound infections

The consequences of presence of bacteria in wounds include contamination, colonization to critical colonization thus leading to infection (Frank *et al.*, 2005). A wound infection may be accompanied by drainage of pus or a cloudy fluid from the wound and a yellow crust can develop (Butt *et al.*, 2004). In this study, a total of 153 pus swabs and pus specimens were processed for culture on appropriate diagnostic media. The statistical analysis of the result obtained revealed that 69.93% (n=107) wound infections were contaminated with different bacterial pathogens **(Fig1)**, and 30.07% (n=46) samples did not show presence of any bacterial growth **(Fig 1)**. Furthermore, the positive samples were equally distributed among male and female samples. There was no discrimination found between the presence of bacteria and gender and age as well. In addition, no particular age was favorable for the bacterial growth, since positive cultures were found in samples obtained from all age groups.

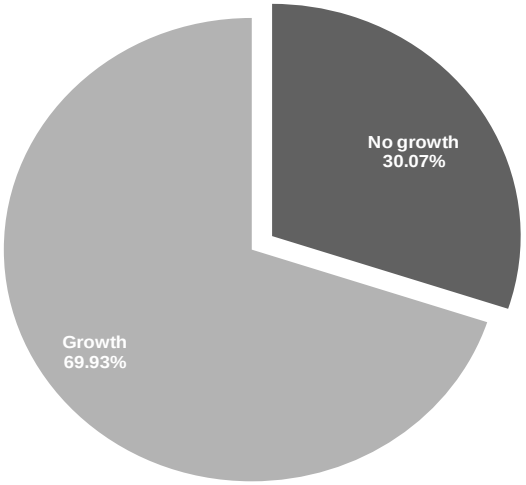


Fig 1. Graph showing percentage of bacterial contamination of wound infections

3.2 Distribution of Bacterial isolates among wound specimens

Isolation and characterization of 107 positive cultures was performed (Hafiz *et al.*, 2002; Rajaduraipandi *et al.*, 2006). The results showed that *Staphylococcus spp* (76.64%, n= 82) were predominant

among all wound isolates, followed by *Klebsiella spp*, 12 (11.21%), *E.coli* 7 (6.54%) and *Proteus spp* 3 (2.80%) **(Fig 2).** Further characterization of Staphylococci species revealed that 52, (48.60%) were *S. aureus*. Of these 19 (36.5%) were MRSA identified by using oxacillin disc diffusion method.

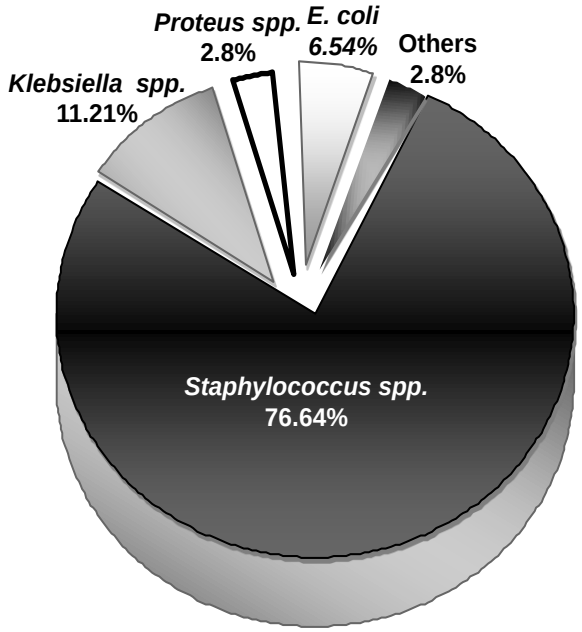


Fig 2. Graph demonstrating distribution of bacterial isolates of wound infections.

3.3 Antibiotic susceptibility patterns of *S. aureus* isolates of wound infections

Although various studies have been conducted on prevalence of MRSA in different regions of Pakistan, individual data presenting frequency of MRSA in wound infections in Hyderabad is lacking. Therefore, in present study, antibiotic susceptibility of only *S. aureus* isolates of wound infections was carried out using disc diffusion method. The data obtained from the antibiotic susceptibility test of *S. aureus* isolates **(Fig. 3)**, demonstrated that 36.5% of total *S. aureus* isolates (48.60%) were oxacillin resistant. Oxacillin is a penicillinase-resistant β-lactam which is similar to methicillin, and has replaced methicillin in clinical use. No vancomycin (which acts by inhibiting proper cell wall synthesis in Gram-positive bacteria) resistant was detected in the present study **(Fig. 3).** In a previous study by Hakim *et al.*, none of the *S. aureus* was reported as vancomycin-resistant (Hakim *et al.*, 2007). In our study, however, one *S. aureus* isolate exhibited intermediate level of resistance to vancomycin.

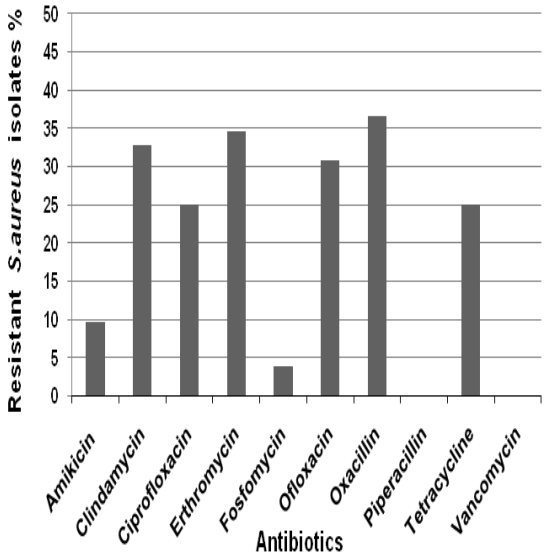


Fig 3. Graphical demonstration of antibiotic resistance patterns of *S. aureus* isolates of wound infections.

Furthermore, *S. aureus* isolates, which showed resistance to oxacillin were sensitive to ofloxacin antibiotic. The antibiotic susceptibility data also demonstrated that vancomycin remains one of the effective antibiotics against *S. aureus* isolates of wound infection since only 01 isolate showed intermediate resistance against the antibiotic. Furthermore, resistance to Amikacin, Clindamycin, Ciprofloxacin, Erythromycin, Fosfomycin, Ofloxacin, and Tetracycline was found 9.61 %, 32.7%, 25.0%, 34.6%, 3.84%, 30.7%, 36.5%, and 25%, respectively **(Fig. 3)**.

4. CONCLUSION

The present study demonstrates the trends of antibiotic resistance among *S. aureus* of wound isolates and frequency of MRSA from wound infections. The data showed that *S. aureus* was most prevalent among isolates of wound infections. The antibiotic susceptibility data showed that 36.5% (n=19) of the total *S. aureus* isolates were resistant to oxacillin antibiotic and thus were MRSA, in wound infections. However, all the isolates (oxacillin resistant and sensitive) were susceptible to vancomycin and piperacillin. Moreover, based on the pattern of antibiotic susceptibility, it is described that the fosfomycin is still one of the effective antibiotics against *S. aureus.* In summary, prevalence of MRSA among wound infection appears to be of grave concern and, thus, active surveillance of MRSA infections is essential to analyze the infection and transmission rates and implement effective control measures.

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