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MRSA Isolated through Pus Swabs from Patients Visiting Armed Forces Institute of Pathology, Rawalpindi, Pakistan

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Abstract: To study the Antibiotic resistance determination of S. aureus isolates and screening for MRSA. This was in-vitro study on MRSA isolates received from clinical samples in the department of microbiology during three months (January 2015-March 2015). Staphylococci were identified by catalase, coagulase and DNAse tests. The samples were cultured on blood agar and MacConkey's agar plates. Antibiotic susceptibility tests were carried out by disc diffusion method. The disc used was Amikacin (30µg), Doxycycline (30µg), Co-trim0oxazole (25µg), Gentamicin (10µg), Penicilin (10µg), Erythromycin (15µg), Clindamycin (2µg), Vancomycin (30µg), Linezolid (30µg), Cefoxitin (30µg), Fusidic acid (10 g), Chloramphenicol (30µg) and Ciprofloxacin (5µg). Our results indicate that out of 112 positive isolates of S. aureus, 34 (30.35%) were found to be MRSA. These isolates were also resistant to different commonly prescribed other anti-staphylococcal antibiotics. A total of 34 (30.35%) MRSA was isolated from various clinical samples. The susceptibility of MRSA to antibiotics is changing. Upon the isolation of MRSA isolates many of them showed resistance against different antibiotics.

Key words: Methicillin Resistant Staphylococcus aureus, Antimicrobial susceptibility pattern, Pus swab, Rawalpindi, Pakistan

Introduction

Staphylococcus aureus is an important cause of community- and hospital-acquired infections. Infections caused by methicillin- or oxacillin-resistant

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S. aureus (MRSA) are mostly nosocomial and are increasingly reported from many countries worldwide (Lowy). Observation of MRSA provides relevant information on the level of the MRSA epidemic, identifies the importance of infection control and the need for adjustments in antimicrobial drug policy, and guides intervention programs (World Health Organization, 2004). S. aureus is mainly isolated from hospitalized patients and the most common from patients in outpatient settings. S. aureus causes an extensive range of syndromes, from minor skin and soft tissue infection to life-threatening pneumonia and toxinoses such as toxic shock syndrome (Lowy, 1998). In a climate of increasing S. aureus antibiotic resistance, the study of MRSA epidemiology has expected new importance, because any strategies to contain the spread of MRSA at the local (hospital), national or international level require knowledge of how strains are spread and how MRSA epidemics occur (Robinson et al, 2004). Each year, approximately two million hospitalizations result due to nosocomial infections (Haley, 1985). In a study of serious ill patients in a large teaching hospital, illness attributable to nosocomial bacteremia increased intensive care unit stay by 8 days, hospital stay by 14 days, and the death rate by 35% (Pitte et al., 1994). In Pakistan, the prevalence of methicillin resistance in S. aureus has been reported in the range from 42 to 51 % (Akinkunmi et al.,). With the increase of MRSA associated infections, the use of glycopeptides (vancomycin and teicoplanin) is also rising slowly (Wayne et al., 2012).

Transmission of MRSA clones from one city to another, from country to country, and even from continent to continent has been drawn to the transfer of patients infected or colonized with MRSA (Diekema et al., 2000; Deplano et al., 2000). Transmission of MRSA within and between healthcare facilities has been well documented by using pulsed-field gel electrophoresis (PFGE) (Haley et al., 1995; Givney et al., 1998; Vriens et al., 2002) A large molecular epidemiological study of MRSA bloodstream isolates collected from five continents demonstrated numerous clusters of clonal dissemination within individual medical centers (Diekema, et al., 2000).

Rifampin is extremely active against susceptible community-associated MRSA isolates (Strausbaugh et al., 1992). Thus, a combination of trimethoprim–sulfamethoxazole or doxycycline with rifampin is sometimes used for the treatment of skin and soft-tissue infections caused by community-associated MRSA (Iyer et al., 2004). Skin and soft-tissue infections caused by community- associated MRSA should not be treated by fluoroquinolones. Resistance to them develops readily in S. aureus and is already widely prevalent (Moran et al., 2006). Vancomycin is the only drug that can constantly treat MRSA (Vriens, et al., 2000). Increased vancomycin lead to cross-resistance in *S. aureus* (Diekema et al., 2000).

Material and Methods

The current study was conducted over a period of three months (January 2015- March 2015). *Staph aureus* was isolated from pus samples collected from patients suspected of bacterial infection at Armed forces institute of pathology, Rawalpindi, including 112 isolates of S. aureus. Each sample was collected by sterile microbiological swab. The samples were cultured on blood and MacConkey's agar plates. These plates were incubated at 37^{o}C for 24 hours. S. aureus was identified on the basis of Gram's staining, culture characteristics and biochemical reactions such as catalase, coagulase and DNAase reactions following standard microbiological procedures (Brown, et al., 2005). Antibiotic susceptibility tests were carried out by disc diffusion method (Kaleem, et al., 2012) following NCCLS guidelines. S. aureus

isolates were inoculated on Mueller-Hinton agar plates and various antibiotic discs were placed and incubated at 37°C for 24 hrs. Antibiotics used were Amikacin (30µg), Doxycycline (30µg), Co-trim0oxazole (25µg), Gentamicin (10µg), Penicilin (10µg), Erythromycin (15µg), Clindamycin (2 µg), Vancomycin (30µg), Linezolid (30µg), Cefoxitin (30µg), Fusidic acid (10µg), Chloramphenicol (30µg), Ciprofloxacin (5µg).

Results

The S. aureus isolates were identified as coagulase positive by the test. Table 1 shows that out of 112 S. aureus clinical isolates, 34 (30.35%) isolates were identified to be MRSA. The samples of pus were collected from inpatient and outpatient referred to department of Microbiology, Armed forces institute of pathology, Rawalpindi, Pakistan.

Table 1. Percentage of MRSA

Source	Total Staphylococcus aureus isolates	MRSA
Pus samples	112	34(30.35 %)

Table 2 shows the gender, wise distribution of Methicillin Resistant S. Aureus. Out of 30.35% MRSA, 8.41% were females while 21.4% were males.

Table 2. Gender wise distribution of MRSA served during current study.

MRSA in Males (%)	MRSA in Females (%)
24(21.4%)	10(8.41%)

Table 3 shows that the antibiotic sensitivity patterns of MRSA isolated from pus samples were found to be highly variable. All the 112 MRSA isolates were isolated from hospital of Rawalpindi were resistant to methicillin (used for screening), followed by penicillin (58.03%), Erythromycin (45.53%), Cefoxitin (29.46%), Clindamycin & Chloramphenicol (25.00%), Ciprofloxacin (20.53%) and Gentamicin (20.53%), Doxycomycin (16.96), Vancomycin (16.96), while Amikacin, Co-trimoxazole, Fusidic acid and Linezolid. The antimicrobial susceptibility pattern of MRSA isolates against different classes of antibiotics is shown in Table 3.

Table 3. Antibiotic sensitivity pattern of Methicillin Resistant *Staphylococcus aureus*.

Antibiotics	Resistant(n)	%
Penicillin	65	58.03
Erythromycin	51	45.53
Cefoxitin	33	29.46
Clindamycin	28	25.00
Chloramphenicol	28	25.00
Ciprofloxacin	23	20.53
Gentamicin	23	20.53
Doxycycline	19	16.96
Vancomycin	09	8.03
Amikacin	0	0.00
Co-trimoxazole	0	0.00
Fusidic acid	0	0.00
Linezolid	0	0.00

Discussion

The results of the present study reported a prevalence rate for MRSA in pus samples to be 30 % for Rawalpindi. Previous reports have displayed variable prevalence of MRSA isolates between various cities of Pakistan such as 61% in Lahore, 57% in Karachi, 46% in Rawalpindi and 54 % in Peshawar (Ahmad et al., 2000; Hafiz et al., 2002; Qureshi et al., 2004; Shafiq et al., 2011). This high isolation of MRSA with the

passage of time may be credited to the transfer of resistance genes between bacterial cells and persistence of bacteria in hospital environment due to antibiotic resistance (Saima et al., 2007).

The results of the present study showed multiple drug resistance of MRSA isolates, isolated from hospital in Rawalpindi. The current study also reported that 45.53% MRSA showed resistance against Erythromycin that is less than the 59.10% of MRSA resistance toward Erythromycin as described by Basit et al., (2013). During the current study, 25.00% MRSA showed resistance against Clindamycin that is less than the 94.3% of MRSA resistance toward Clindamycin as described by Bradley et al. (2005). During the current study, In addition, 25.00% MRSA showed resistance against Chloramphenicol that is lower than the 38.3% of MRSA resistance toward Chloramphenicol as described by Qureshi et al. (2004). The current study also reported that 20.53% MRSA showed resistance against Ciprofloxacin that is lower than the 59.16% of MRSA resistance towards Ciprofloxacin as described by Basit et al. (2013). During the current study, 20.53% MRSA showed resistance against gentamicin that is lower than the 76.35% of MRSA resistance towards gentamicin as described by Perveen et al. (2013). Gentamicin is an aminoglycoside and is most often prescribed because of its low cost and synergistic activity with ß-lactum antibiotics described by Hafiz et al. (2002). During the current study, we examined that 16.96% MRSA showed resistance against Doxycycline that is lower than the 63.35% of MRSA resistance towards Doxycycline as described by Basit et al. (2013). During the current study, we examined that 8.03% MRSA showed resistance against Vancomycin that is higher than the 0.46% of MRSA resistance toward Vancomycin as described by Basit et al. (2013).

During the current study, we examined that 0.00% MRSA showed resistance against Amikacin that is lower than the 54% of MRSA resistance toward Amikacin as described by Perwaiz et al. (2007). During the current study, we examined that 0.00% MRSA showed resistance against Fusidic acid that is lower than the 2 % of MRSA resistance toward Fusidic acid as described by Perwaiz et al. (2007). It is well known that occurrence of bacterial resistance is promoted by excessive use of antibiotics. In livestock products like milk and meat, antibiotic residues present, which is responsible for maintaining resistant strains in environment (Persoon et al. 2009). The healthcare workers in this epidemic chain have great importance in the increasing resistance of contaminants, serving as a source of transmission and information for empirical prescription of antibiotics.

Conclusion

The current study reported that resistance of S. aureus towards methicillin is increasing and its susceptibility toward antibiotics is changing. Amongst the clinical isolates, mostly pus samples were reported with higher frequency of Methicillin Resistant Staphylococcus aureus (MRSA) which show high risk to chronic kidney patients and immune comprised persons. Upon isolation, selected MRSA isolates showed resistance against different commonly prescribed antibiotics.

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