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FREQUENCY AND RESISTANCE PATTERNS OF METHICILLIN- SENSITIVE AND METHICILLIN- RESISTANT *STAPHYLOCOCCUS AUREUS* IN PNS SHIFA HOSPITAL KARACHI, PAKISTAN

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ABSTRACT

An increase in resistance of *Staphylococcus aureus* to antibiotics; especially methicillin, vancomycin and linezolid; is a growing concern limiting the treatment modalities. The current study was conducted to find out the frequency and antibiotic resistant pattern of methicillin-sensitive and –resistant *S. aureus*. All the clinical samples received at Diagnostic Microbiological Laboratory PNS Shifa Hospital Karachi were processed. Staphylococci were identified by standard procedures. Methicillin-resistant *S. aureus* (MRSA) isolated were identified by a slide latex agglutination kit for the detection of penicillin binding protein 2 (PBP 2). Two hundred and fifty six *Staphylococcus* isolates were recovered from different samples of blood, pus, urine, ear swab, sputum, ascetic fluid, throat swab and pleural fluid. One hundred and forty eight (60.2%) were coagulase positive. Of these, 32 (21.8%) were found to be MRSA. Antibiogram of methicillin-sensitive *S. aureus* (MSSA) and MRSA was determined by standard Kirby-Bauer disc diffusion method. Co-existence of resistance to other antibiotics with methicillin was also noted in the present study. Vancomycin, linezolid and rifampicin were found to be the most effective drugs against both MRSA and MSSA. Highest resistance was found against penicillin i.e. 81.25% in MRSA and 75% in MSSA. It is concluded that due to the high and changing pattern of resistance of *S. aureus*, antimicrobial susceptibility testing is mandatory.

Key words: MRSA, MSSA, methicillin, vancomycin, linezolid, Penicillin binding protein (PBP 2)

INTRODUCTION

Staphylococcus aureus, a Gram positive coccus is a common pathogen causing a variety of infectious diseases such as food poisoning, pneumonia, Osteomyelitis, endocarditis, skin infections, post operative infections, toxic shock syndrome and bacterimia (Ali *et al*., 2007; Hannan *et al*., 2009). Penicillin-resistant strains were reported after few years of discover of penicillin in 1941. Chloroamphenicol, aminoglycosides and erythromycin resistance was reported during 1950s. After that, B-lectamase-resistant strains of *S. aureus* were also reported during 1960s. In early 1960s, the B-lectamase-resistant semi-synthetic penicillin were introduced which provided a temporary relief. But soon after the discovery of methicillin, methicillin-resistant *S. aureus* (MRSA) strains emerged rapidly (Hafeez *et al.,* 2004).

Methicillin resistance in *S. aureus* was first time noted in UK in 1961 (Jevons, 1961). However, MRSA is now recognized as an important pathogen causing community and hospital acquired infections (Ajmal *et al*., 2009). MRSA is also called as oxicillin-resistant *S. aureus* (ORSA) (Siddiqi *et al*., 2009). During recent years, infections caused by MRSA have increased rapidly though out the world (Akhtar *et al*., 2009). This increasing resistant has become a cause of treatment failure (Mehdinejad *et al*., 2008). Certain risk factors are associated with colonization and infections caused by MRSA include prolong hospitalization particularly in intensive care unit (ICUs), intravascular catheterization, excessive and unnecessary use of antibiotics and immuno-compromised health status of individual (Ajmal *et al*., 2009). Carriers and infected individuals serve as reservoirs of MRSA. Another important mode of transmission is through contaminated hands of health care personals (Brown *et al*., 2005).

In case *S. aureus* the mechanism of methicillin resistance is the production of an additional penicillin binding protein 2 (PBP 2), a product of 'mec A' gene. It is an additional gene present in MRSA and absent in MSSA. Several additional genes for methicillin resistance are also present in *S. aureus* but these are also present in methicillin sensitive strain of *S. aureus* (Rahbar *et al.,* 2006). MRSA is widely distributed but the frequency varies with respect to time and place (Shabir *et al.,* 2010). A small amount of data is available about the prevalence of MRSA in different cities of Pakistan. Research papers claimed around 35% MRSA in Pakistan (Hafiz *et al.,* 2002). The current study was performed to find out the frequency of MRSA in Karachi, Pakistan. In addition, the

comparison of antibiotics resistance pattern were also determined among methicillin-sensitive and methicillin-resistant *S. aureus*.

MATERIALS AND METHODS

The study was carried out in PNS Shifa Hospital Karachi, Pakistan from July, 2011 to October 2011. All samples of blood, pus, urine, ear swab, sputum, ascetic fluids, throat swab and pleural fluid, received at diagnostic Microbiological Laboratory, PNS Shifa Hospital, were processed. Identification of staphylococci was performed by colonial and morphological characteristics, Gram staining, catalase test, coagulase test and mannitol fermentation.

Screening for MRSA was performed, the detection of PBP 2, by slide latex agglutination kit. Antibiotic resistance was carried out by standard Kirby Bauer disc diffusion method against vancomycin, rifampicin, gentamicin, clindamycin, cefoxitin, ciprofloxacin, penicillin, erythromycin, doxycyclin, trimethoprim-sulfamethoxazole, amikacin, tigecycline, linezolid, ofloxacin and fusidic acid. Mueller-Hinton agar (Oxoid) was used as base medium. The inoculated plates were incubated at 37 ^{o}C for 18-24 hours. The inhibition zone diameters were measured in millimeter and were interpreted on the basis of guidelines published by the NCCLS.

RESULTS AND DISCUSSION

A total of 246 *Staphylococcus* species were isolated, out of which 148 (60.2%) were *S. aureus*. Out of these 148 *S. aureus* 32 (21.8%) were found methicillin-resistant (Table 1). Coexisting resistance to different antibiotics used in the present study with methicillin was found to be significantly higher as compare to MSSA (Table 2).

In Pakistan, the problem of emergence of antibiotic resistance has increased due to the injudicious use of antibiotics in hospital settings, easy availability of antibiotics without prescriptions as well as lack of public awareness (Mulla *et al.,* 2007). The current study highlights the problem of MRSA in Pakistan. In present study the frequency of MRSA noted as 21.8%. These findings are in fair correlation with a study who reported 22.9% frequency of MRSA in Karachi (Akhtar *et al.,* 2009). Many investigators have also reported the frequency of MRSA in Karachi during last ten years. However, our findings are less than that reported in other studies from Karachi 57% (Hafiz *et al.,* 2002), 24.39% (Naqvi *et al.,* 2007), 43% (Perwaiz *et al.,* 2007), 43% (Saima *et al.,* 2007), 48.24% (Ansari *et al.,* 2011). Many studies have also been carried out on growing interest over methicillin resistance in *S. aureus* in other cities of Pakistan i.e. In Lahore 61% (Hafiz *et al.,* 2002), 63.64% (Iffat *et al.,* 2002), 38.5 % (Khatoon *et al.,* 2002), 38.5% (Bukhari *et al.,* 2004), 27.77% (Ajmal *et al.,* 2009), 34.76% (Siddiqi *et al.,* 2009), in Islamabad 46%, Peshawar 36%, Sukkhar 26% and Azad Kashmir 32% (Hafiz *et al.,* 2002), in Sargodha 22.3% (Siddiqi *et al.,* 1999), in Rawalpindi 42.01% (Ali *et al.,* 2007), in Gujranwala 68% (Ahmed *et al.,* 2007) and in Quetta 5.01% (Qureshi *et al.,* 2000) and 26% (Hafiz *et al.,* 2002).

Table 1. Distribution of *Staphylococcus* species with respect to different clinical samples.

Clinical Sample	Coagulase negative Staphylococci	Coagulase positive *S. aureus*			Total
		MSSA	MRSA	Total	
Blood	77	27	05	32	109
Pus	16	84	21	105	121
Urine	02	02	01	03	05
Ear swab	01	0	02	02	03
Sputum	01	0	0	0	01
Ascitic fluid	01	01	02	03	04
Throat swab	0	01	01	02	02
Pleural fluid	0	01	0	01	01
Total	98/246 (39.8%)	116/148 (78.4%)	32/148 (21.8%)	148/246 (60.2%)	246

Table 2. A comparison of antibiotic resistance pattern of methicillin sensitive and resistant *S. aureus.*

Antibiotics	Number (%) of isolates resistant to	
	MSSA	MRSA
Vancomycin	0 (0)	2 (6.25)
Rifampicin	0 (0)	2 (6.25)
Gentamicin	9 (7.76)	8 (25.0)
Clindamycin	6 (5.17)	6 (18.75)
Cefoxitin	0 (0)	20 (62.5)
Ciprofloxacin	11 (9.48)	19 (59.38)
Penicillin	87 (75.0)	26 (81.25)
Erythromycin	29 (25.0)	20 (62.5)
Doxycyclin	34 (29.31)	9 (28.13)
Trimethoprim-sulfamethoxazole	39 (33.62)	13 (40.63)
Amikacin	5 (4.31)	9 (28.13)
Tigecycline	1 (0.86)	2 (6.25)
Linezolid	0 (0)	2 (6.25)
Ofloxacin	15 (12.93)	6 (18.75)
Fusidic acid	4 (3.45)	7 (21.87)

In present study, low rate of resistance was noted in MSSA as compared to MRSA. The highest resistance was seen against penicillin i.e. 81.25% in MRSA and 75% in MSSA. Our findings are in correlation with other studies who reported 100% resistance in MRSA and 81% in MSSA (Perwaiz *et al.*, 2007), while 100% in MRSA and 73.13% in MSSA (Akhtar *et al.*, 2009). Low level of resistance to vancomycin, rifampicin and linezolid (6.25% in MRSA and 0% in MSSA for each of these antibiotics) was reported. The result are not in agreement with other studies conducted in Pakistan in which MRSA and MSSA both were found 100% sensitive to vancomycin (Latif *et al.*, 2000; Naqvi *et al.*, 2007; Perwaiz *et al.*, 2007; Akhtar *et al.*, 2009; Kaleem *et al.*, 2010). However, the data about the resistance pattern of *S. aureus* against rifampicin is lacking the literature.

It is apparent that MRSA is emerging as a potential threat to the hospitals. Current study showed varying degree of resistance to all antibiotics tested both MRSA and MSSA. The study also revealed coexisting resistance to other antibiotics with methicillin. Timely detection of strains resistant to multiple antibiotics will help in the prevention of infections caused by multi-drug-resistant strains. Therefore, it is recommended that careful and continuous monitoring if resistance pattern of pathogenic microorganisms is necessary.

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