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MULTI DRUG RESISTANT *STAPHYLOCOCCUS AUREUS* AND COAGULASE NEGATIVE STAPHYLOCOCCI FROM CLINICAL CASES IN KARACHI, PAKISTAN

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ABSTRACT

Staphylococcus aureus and Coagulase Negative Staphylococci (CoNS) are significant pathogens of humans and emergence of multidrug resistant (MDR) strains is limiting the treatment options against these organisms. The resistance pattern of 60 strains of staphylococci isolated from different clinical specimens was determined using the disc diffusion method. Of the 60 staphylococcus isolates, 43 were identified as *S. aureus* and 17 were CoNS. In the present study, 98% (442/43) of *S. aureus* isolates were resistant to amoxicillin, 88% (38/43) to gentamicin, 81% (35/43) to penicillin and 67% (29/43) to tetracycline. This study showed a high rate of methicillin resistance among *S. aureus* (37%) and CoNS (59%). Emergence of vancomycin resistance was observed not only in *S. aureus* (1/43) but also in CoNS (2/17). The study also revealed prevalence of multidrug resistance among both *S. aureus* (86%) and CoNS (82%) isolates. Only one isolate of CoNS was susceptible to all the six antibiotics used in this study.

Key-words: *S. aureus*, Coagulase negative Staphylococci, MRSA, Vancomycin resistance, multidrug resistance.

INTRODUCTION

Staphylococcus species are wide spread in nature and found on the skin, mucosa and internal nares of human as part of the normal flora (Kluytmans *et al*., 1997). From clinical perspective, the staphylococcal species have been grouped as coagulase positive staphylococci and coagulase negative staphylococci (CoNS). *S. aureus* is coagulase positive, opportunistic pathogen and can infect almost every tissue in the body of humans as well as animals. It has been implicated in various clinical conditions ranging from minor skin infections to severe toxin mediated diseases, bacteremia (Romero-Pastrana *et al*., 2010), meningitis, bacterial arthritis (Coutinho *et al*., 2009), nosocomial pneumonia (Islam *et al*., 2008) and surgical site infections (Fagade *et al*., 2010; Islam *et al*., 2008). Some strains of *S. aureus* have the ability to produce exfoliative toxins which cause staphylococcal scalded skin syndrome and bullous impetigo (Verkaik *et al*., 2010).

Coagulase Negative Staphylococci (CoNS) are also members of the normal flora of skin and mucus membranes in humans and can cause opportunistic infections (Fagade *et al*., 2010). They have the ability to colonize the urethra and periurethra in humans and their incidence in urinary tract infection is around 10-20% (Nandy *et al*., 2009). Coagulase Negative Staphylococci have also been associated with bacteremia (Sloos *et al*., 2000) in those patients dependent on invasive procedures and indwelling medical devices (Sloos *et al.*, 2000; Center *et al*., 2003).

B-lactam antibiotics are commonly prescribed to control *Staphylococcus* infections (Islam *et al*, 2008). However, their extensive and frequent use facilitates the emergence of resistant strains (Coutinho *et al*., 2009; Islam *et al*., 2008). *S. aureus* has acquired resistance against β-lactam antibiotics by production of enzyme β-lactamase.

Methicillin, a semi-synthetic β-lactam antibiotic was introduced in 1950s effective against β-lactamase producing strains, but methicillin resistant *S. aureus* (MRSA) strains emerged rapidly (Hardy *et al*, 2004). Methicillin resistant Staphylococcal strains are also resistant to multiple antibiotics like penicillin, amoxicillin and cephalexin and their treatment is more difficult (Fagade e*t al*., 2010). Oxacillin resistant *Staphylococcus aureus* and multiple drug resistant *S. aureus* are other names of MRSA (Islam *et al*., 2008; Merlino *et al*., 2002). Coagulase negative strains isolated from hospitalized patients are also resistant to methicillin (Center *et al*., 2003).

Glycopeptides like vancomycin or teicoplanin are the drugs of choice for treatment of methicillin resistant Staphylococci (Sloos *et al*., 2000). However, their susceptibility has decreased in recent years (Center *et al*., 2003). *S. aureus* strains express two types of Vancomycin resistance; complete resistance known as vancomycin resistant *S. aureus* (VRSA), due to the acquisition of plasmid associated *VanA* resistance gene from *Enterococcus faecalis* (Nandy *et al*., 2009) and intermediate resistance known as vancomycin intermediate resistant *Staphylococcus aureus* (VISA), due to a genetic mutation resulting in thicker cell wall which reduces the uptake of vancomycin by the cell (Romero-Pastrana *et al*., 2010).

The aim of this study was to determine the current status of antibiotic resistance among clinical isolates of *Staphylococcus* species prevailing in the metropolis of Karachi, Pakistan.

MATERIALS AND METHODS

Bacterial isolates

Staphylococcus strains isolated from different clinical specimens such as blood, cerebrospinal fluid, pus and wound were collected from various clinical microbiology laboratories in Karachi.

Antibiotic susceptibility test

The antibiotic susceptibility pattern of the isolates was determined by the disc diffusion method on Mueller Hinton agar (Oxoid, UK) according to the Clinical Laboratory Standards Institute (CLSI 2010) guidelines. Briefly, a lawn of bacterial culture was prepared on MH agar plates and antibiotic discs (Oxoid, UK) were placed on the inoculated plates, incubated at 37°C for 24 hours. The results were interpreted according to standard specifications of CLSI (CLSI, 2010; Cheesbrough, 2006; Margaret, 1997). The following antibiotics were used: Penicillin (10U), Ampicillin (10µg), Gentamicin (10µg), Methicillin (1µg), Tetracycline (30µg) and Vancomycin (30µg).

RESULTS AND DISCUSSION

Members of the genus *Staphylococcus* exist as part of the normal flora in humans, and can sometimes cause serious life-threatening infections. These organisms have acquired resistance to a number of antibiotics making the treatment and control of such infections very challenging. Emergence of multidrug resistance in Staphylococci is a cause of major concern among the medical community, owing to the fact that such infections result in increased costs, treatment failures and lead to high mortality rates.

A total of 60 isolates of Staphylococcus were cultured from different clinical specimen. Forty (40) isolates were from wound, 11 from blood, 5 from cerebrospinal fluid (CSF) and 4 isolates were from pus specimens. Characterization of these isolates identified four different species of the genus Staphylococcus. *Staphylococcus aureus* was isolated in highest number i.e. 71% (43/60) while the incidence of Coagulase Negative Staphylococcus (CoNS) speicess was noted as; 25% (15/60) isolates of *Staphylococcus haemolyticus* and 1 isolate each of *Staphylococcus capitis* and *Staphylococcus hominis*. Perveen *et al.* (2013) have also reported isolation of *S. aureus* (70%) and CoNS (30%) from different clinical samples in similar proportions as observed in the present study. *Staphylococcus* species were isolated in varying numbers from different clinical specimens. *Staphylococcus aureus* was isolated from 28 (70%) of the 40 wound specimens, 7 (64%) of the 11 blood, 4 (80%) of the 5 CSF specimens and all (4) of the pus specimens. Prevalence of *S. haemolyticus* was observed as 10/40 (25%) wound specimens, 4/11 (36%) of the blood, and 1/5 (20%) from CSF specimens. Whereas, a single isolate each of S. *capitis* and *S. hominis* was recovered from wound specimens (Table 1).

Of the 60 *Staphylococcus* isolates, 26 (43%) were resistant to methicillin. Among the 43 *S. aureus* strains, 16 (37%) were resistant to methicillin (MRSA) and of the 17 CoNS, 10 (59%) were methicillin resistant (Table 2). The incidence of MRSA observed in this study is comparable with those reported in similar studies by Taj *et al.* (2010) and Ansari *et al*. (2014) where resistance was noted as 38.6% and 39.2%, respectively however, Perveen *et al*. (2013) reported a higher (60%) number of MRSA. The incidence of MRSA tends to vary from one geographical region to other and with time. In the present study, incidence of methicillin resistant CoNS was found to be higher (59%) than that reported by Perveen *et al*. (2013), who reported resistance in 43.8% isolates. A total of 3 (5%) vancomycin resistant strains were obtained in this study, of which one (2.3%) was *S. aureus* (VRSA) and 2 (11.7%) were CoNS. Emergence of VRSA and VISA in Pakistan has been reported in earlier studies (Taj *et al*., 2010; Hakim *et al*., 2007), however, the present study accounts for vancomycin resistant CoNS for the first time in this region. VRSA have also been reported from other parts of the world (Hanaki *et al*., 2007) and is considered an alarming situation as the antibiotic is used as the drug of last resort against MDR strains of *S. aureus*. The resistance pattern against other commonly used antibiotics was also noted. Almost all of the *S. aureus* isolates (98%) were resistant to amoxicillin, followed by gentamicin (88%), penicillin (81%) and tetracycline (67%). Coagulase Negative Staphylococci showed similar resistance rates against penicillin, gentamicin and amoxicillin (82%) whereas resistance to tetracycline was observed in 53% isolates. In general, the emergence of resistance to different antibiotics was higher in case of *S. aureus* as compared to CoNS. The least number of resistant isolates of *S. aureus* was observed for oxacillin while least resistant isolates of CoNS was observed for tetracycline.

The incidence of multidrug resistant strains of *S. aureus* and CoNS is presented in Table 3. It was observed that 37 (86%) isolates of *S. aureus* were resistant to multiple antibiotics, 4 (9%) isolates were resistant to two antibiotics and 2 (5%) to one antibiotic. None of the *S. aureus* isolated included in this study was susceptible to all of the antibiotics used. In case of CoNS, out of 17 isolates 14 (82%) were resistant to multiple antibiotics, 1 (6%) to two antibiotics and 1 (6%) isolate was resistant to one antibiotic. In case of CoNS, only 1 (6%) isolate was completely susceptible to all antibiotics used in this study.

Table 1. Distribution of *Staphylococcus* species from different clinical specimens.

Clinical Specimens	Bacterial Isolates (%)				Total
		CoNS			
	S. aureus	*S. haemolyticus*	*S. capitis*	*S. hominis*	
Wound	28 (47%)	10 (17)	1 (2)	1 (2)	40 (66.7)
Blood	7 (12%)	4 (6)	0	0	11 (18.3)
CSF	4 (6)	1 (2)	0	0	5 (8.3)
Pus	4 (6)	0	0	0	4 (6.7)
Total	43 (71.6)	15 (25)	1 (1.7)	1 (1.7)	60

Table 2. Antibiotic resistance pattern of *Staphylococci*.

Antibiotics	Resistant isolates		
	S. aureus (n=43)	CoNS (n=17)	Total (n=60)
	Number (%)	Number (%)	Number (%)
Methicillin	16(37)	10 (59)	26 (43.3)
Gentamicin	38(88)	14 (82)	52 (86.7)
Tetracycline	29(67)	9 (53)	38 (63.3)
Penicillin	35 (81)	14 (82)	49 (81.7)
Amoxicillin	42 (98)	14 (82)	56 (93.3)
Vancomycin	1 (2.3)	2 (5)	3 (5)

Table 3. Multidrug resistance of isolates.

Organism	No. of isolates	No. of antibiotics to which isolate was resistant			
		0	1	2	3 or more
		No. (%)	No. (%)	No. (%)	No. (%)
S.aureus	43	0	2 (5)	4 (9)	37 (86)
CoNS	17	1 (6)	1 (6)	1 (6)	14 (82)
Total	60	1 (2)	3 (5)	5 (8)	51 (85)

In the current study, we observed a very high number (85%) of MDR *Staphylococcus* strains. Over the counter availability, self medication practices, unqualified medical personnel, poor quality of medicines and non compliance of the patients are the significant factors contributing to increasing antibiotic resistance in our region. The infections caused by highly resistant bacteria are difficult to manage especially in developing countries like Pakistan where basic health facilities are not easily available. Therefore, the knowledge about the antibiotic susceptibility pattern of pathogenic bacteria in the community is helpful in determining the treatment options for various infections and controlling further spread of resistant strains.

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