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Antibiogram Sensitivity Pattern of Methicillin-resistant *Staphylococcus aureus* Isolates from Pus Samples

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Abstract: A total of 75 pus samples (21 ear pus samples and 54 pus samples from other parts of the body – surgical wounds, acne pus samples etc.) were tested to confirm the presence of *Staphylococcus aureus*. Consequently, *Staphylococcus aureus* was isolated from 33 (44%) cases, 25 (46%) from wound pus and 8 (38%) from ear pus. Of these isolates, 3 (12%) from wound and 5 (63%) from ear pus were determined to be MRSA (based on their antibiogram sensitivity tests). All these isolates were sensitive to vancomycin, however, amongst the wound pus samples, 2 MRSA were found resistant to gentamicin and 1 MRSA resistant to amikacin. As for 5 MRSA strains from ear pus, all were sensitive to vancomycin and amikacin, while 2 were found to be gentamicin resistant. The prevalence of gentamicin-resistant MRSA (GR-MRSA) is of great concern and may be attributed to indiscriminate use of antibiotics, non-compliance of patients, over-the-counter availability of drugs, improper health facilities, care and guidance.

Key words: MRSA, pus samples, gentamicin resistant

Introduction

Staphylococcus aureus is one of the most common causative agents of nosocomial infections worldwide, which also causes abscesses, boils, sties, impetigo, folliculitis, scalded skin syndrome, toxic shock syndrome, cellulitis and more serious infection in persons debilitated by chronic illness, traumatic injury, burns or immuno-suppression. These infections include, Staphylococcal pneumonia, osteomyelitis, and also secondary infections resulting from insect bites, ulcers, burns, wounds and endocarditis (Mohan and Larsen, 1995).

Various reports have confirmed that nosocomial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are an important cause of mortality among hospitalized patients all over the world, mostly among those who use needles frequently and are hospitalized for surgery. It can easily be transferred from person to person in hospitals by contaminated hands of physicians, nurses by contaminated equipments, solutions, sinks and clothing (Lameitre *et al*., 1998; Cuoto *et al*., 1995). Simultaneously, there is growing evidence of community-acquired methicillin-resistant *Staphylococcus aureus* susceptible to gentamicin has been reported in numerous countries (Nimmo *et al*., 2000). Factors such as contact with health care institutions, or workers, are the most common risk factors associated with MRSA transmission (Palmer *et al*., 1994; Sumrall and Nolan, 1996).

Staphylococcal infections are of particular concern because of the causative bacteria offering resistance to a wide range of commonly used antibiotics. Half of all *Staph.* species that circulate in hospitals are resistant to penicillin, due to over production of β-lactamase, as a result methicillin and oxacillin are used as alternative antibiotics. In 1961, shortly after methicillin became available for clinical use, methicillin-resistant *Staph. aureus* (MRSA) emerged in England. As a result vancomycin and gentamicin constitute the uniformly effective drug presently available against MRSA strains (Boyce, 1998).

Furthermore, some strains of *Staph. aureus* have shown intermediate level resistance to vancomycin and complete resistance to gentamicin in developed and developing nations At the moment fortunately such strains are encountered rarely, but are increasing. As a safeguard, clinicians are trying to tackle this problem by using a combination of antibiotics, and counseling patients regarding the deleterious effects of the over and misuse of antibiotics (Martin, 1997; Trakulsomboon *et al*., 2001).

The injudicious use of antibiotics in Pakistani hospitals, communities and because of the easy availability of antibiotics without prescription, the chances of the emergence of resistant strains are enhanced. Lack of public awareness has further deteriorated the situation (Haneef and Khan, 1990).

This study was designed to investigate the prevalence-identification of *Staphylococcus aureus* in different pus samples, their antibiotic resistance pattern (including MRSA, GS-MRSA and GR-MRSA).

Materials and Methods

The study was carried out in the Bacteriology Laboratory of the National Institute of Health (NIH), Islamabad. A total of 75 pus samples, wound pus (54) from all types of wounds, cuts, abscesses, acne pus and pus drained from any organ i.e. gall bladder, spleen, bones, breast and of ear pus (21), both from inner ear and outer pinna, were collected from patients of Rawalpindi/Islamabad area visiting the NIH hospital.

Pus samples were immediately streaked onto Blood and MacConkey agar (DIFCO), with the help of sterilized cotton swab. Plates were then incubated at 37° C for 24 hours and examined for *Staphylococcus aureus* colonies, which grew only on blood agar. The colonies were identified on the basis of colony morphology (β-hemolytic – clear zone around colonies), microscopy and confirmatory biochemical tests, such as catalase and coagulase tests.

Antibiotic sensitivity test: After the confirmation of *Staph. aureus,* antibiotic sensitivity profile was determined. Two separate colonies were mixed in peptone water in test tube. After 15 minutes this suspension was streaked with the help of sterilized cotton swab on Mueller Hinton agar (Oxoid) plate. Antibiotic disks (Oxoid) were applied on plate and incubated at 37° C for 24 hours. The diameter of the zone of inhibition was compared according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) to determine whether *Staph. aureus* is susceptible, intermediate or resistant to antibiotics (Monica, 1991).

Results and Discussion

Of the 54 wound pus samples examined for the incidence of *Staphylococcus aureus*, 25 (46%) were positive, whereas of the 21 ear pus samples, 8 (38%) were found positive for *Staph. aureus*. Among these isolates, 3 from wound pus and 5 from ear pus were found to be MRSA, based on their culture sensitivity patterns. According to sex-wise distribution pattern of *Staph. aureus* among the positive wound pus samples, 18 (72%) were

from males and 7 (28%) from females. However, with regards to 8 ear pus samples, positive for *Staph. aureus*, the opposite was seen, as this study reported 6 (75%) cases in females and only 2 (25%) cases in males (Table 1).

With regards to the antimicrobial sensitivity pattern of the 3 MRSA isolates from wound pus, the results showed that all the strains were sensitive to vancomycin, however, one strain showed resistance to amikacin, while 2 isolates offered resistance to gentamicin (Table 2).

As for the antibiogram pattern of 5 MRSA isolates from ear pus, it was found that all the strains were sensitive to vancomycin and amikacin, while 2 strains were simultaneously resistant to gentamicin (Table 3).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has proved to be one of the most widespread and persistent nosocomial pathogen of the late 20th Century (Ayliffe, 1997; Voss and Doebbeling, 1995) and now community-acquired MRSA is a worldwide phenomenon, appearing in France, West Indies and in children, with no identifiable predisposing risk, in the United States (Lelievre *et al.*, 1999; Herold *et al.*, 1998). Most of these cases caused by the strains sensitive to gentamicin (GS-MRSA), as documented evidence showed that community-acquired strains were more susceptible to gentamicin than nosocomially acquired isolates (Nimmo *et al.*, 2000).

The appearance of gentamicin-resistant MRSA (GR-MRSA) is of great concern, as *Staph. aureus* continues to show its resilience against antibiotics and gradually with time build resistance against them (Pavillard *et al.*, 1982).

According to this study, the overall incidence of *Staph. aureus* was found to be higher in wound pus compared to ear pus. The reasons may be that *Staph. aureus* constitutes the normal flora of skin, or the wounds are more exposed and there is greater chance of dissemination, contamination and secondary infections, as compared to ear pus. These results are similar to the findings by other researchers who reported that the most common infections caused by MRSA include wound infections (Boyce, 1998) and that highest incidence of *Staph. aureus*, amongst clinical samples, was in pus samples (Abu Saud, 1996).

Based on the antibiogram results, the present study has revealed that all of the 8 MRSA isolates were susceptible to vancomycin (a useful antibiotic against gram-positive pathogens). Similar findings have been reported earlier (Trakulsomboon *et al.*, 2001; Jawetz *et al.*, 1995).

An increasing evidence has indicated the reduced susceptibility of *Staph. aureus* to vancomycin (vancomycin-resistant MRSA). Such strains have been reported in Thailand, USA, France and Korea (Martin, 1997; Trakulsomboon *et al.*, 2001; Hiramatsu *et al.*, 1997; Centers for Disease Control and Prevention, 1997; Ploy *et al.*, 1998; Kim *et al.*, 2000).

The interesting aspect revealed in this study is the effectiveness, (as the case may be), of gentamicin on the MRSA isolates, where, among the pus isolates (both wound and pus) 4 were resistant to gentamicin - GR-MRSA (2 each for wound and pus), whereas 4 were GS-MRSA (1 from wound and 3 from ear pus samples). This is indicative of an increase in resistance to this drug that until recently was as effective as vancomycin (Collignon *et al.*, 1998; O'Brien *et al.*, 1999; Riley *et al.*, 1998). This change in resistance pattern of *Staph. aureus* may be due to indiscriminate use of antibiotics, non-compliance of patients, (as reported by physicians), the easy access and availability of various drugs in the market without prescription (Haneef and Khan, 1990).

The study under discussion has revealed variations in sex-wise incidence of *Staph. aureus* in wound and ear pus. In wound pus, incidence of *Staph. aureus* was higher in males (72%) than in females (28%), which may be due to the reason that males, especially in our society, are involved in numerous outdoor activities and their work too exposes them to an increased chance of contamination and infection. Furthermore, improper use of ointments on minor cuts and abscesses during haircutting and shaving, poor postoperative care and surgical wounds may also cause the spread of infections (Boyce, 1998; Ahmed *et al.*, 1998).

The incidence of ear pus infections caused by *Staph. aureus*, this study, however, showed an opposite trend i.e. a higher incidence was recorded among females (75%) than in males (25%). The reason may be that females traditionally, frequently pierce their ears, sometimes more than once, and decorate them with numerous ornamental jewelry or artificial jewelry, which may cause a sepsis to occur.

In the community, *Staph. aureus* infections incidence may be reduced by proper hygiene and greater awareness with the family physician playing a key role. To prevent spread of MRSA in general and GR-MRSA in particular, carriers among hospital personnel should be identified, nasal colonization should be decreased and only those antibiotics prescribed that have been tested through culture sensitivity and proven to be effective.

The prevalence of gentamicin-resistant methicillin-resistant *Staph. aureus* is of great concern and may be attributed to indiscriminate use of antibiotics, non-compliance of patients, over-the-counter availability of drugs, improper health facilities, care and guidance. Clinicians, pharmacists and the general public at large must be taken into confidence to spread the awareness of this problem in order to combat the spread of resistant strains. Moreover, better immune system development is also essential. This can be obtained through proper diet, exercise, low stress, adequate rest and use of antibiotics only if absolutely necessary, and that too, as a last resort.

Table 1: Incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among wound and ear pus samples

Clinical specimen (Number)	*Staphylococcus aureus* Positive (%)			Methicillin-Resistant *Staphylococcus aureus (%)*
	Total	Male	Female	
Wound (54)	25 (46%)	18(72%)	7 (28%)	3 (12%)
Ear (21)	8 (38%)	2(25%)	6 (75%)	5 (63%)
Total (75)	33 (44%)			8 (10.7%)

Table 2: Antibiogram of 3 MRSA isolates from wound pus

Antibiotics	Sensitive	Resistant
Vancomycin	3	0
Amikacin	2	1
Gentamicin	1	2
Methicillin	0	3

Table 3: Antibiogram of 3 MRSA isolated from ear pus

Antibiotics	Sensitive	Resistant
Vancomycin	5	0
Amikacin	5	0
Gentamicin	3	2
Methicillin	0	5

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