Microbial Resistance of Staphylococcus aureus Against Commonly Used Antibiotics

Asthma Nafeesa, Munir Ahmad Sheikh, 'Ikram-ul-Haq, Amer Jamil and Zahida Parveen

A total of 80 isolates of Staphylococcus aureus, 30 each from upper respiratory tract as well as from post operative wound infections were subjected to antibiogram studies. Staph. aureus isolates from upper respiratory tract were found 30, 53.33 and 43.33% resistant against ciprofloxacin, amoxycillin and chloramphenicol respectively. A similar pattern of antibiotic resistance was observed against the bacterial isolates from post operative sepsis. Susceptibility of ciprofloxacin, amoxycillin and chloramphenicol in such cases was found only to be 43.33, 10 and 3.33% respectively. The high bacterial resistance appears to be due to indiscriminate use of antibiotics.

Key words: Staphylococcus aureus, amoxycillin, ciprofloxacin, chloramphenicol, antibiotic resistance
Nafeesa et al.: Resistance against antibiotics

Introduction
Increased use of antibiotics in medical therapeutics and extension of the uses to areas other than the treatment and prophylaxis of the infectious diseases, have engendered serious problems. There is generally a positive correlation between antibiotic consumption and incidence of resistance to antibiotics used either for prophylaxis or therapy in human infections (Lourdeau et al., 1994). More and more strains of microorganisms are becoming resistant to the available anti-biotics on account of enhanced selective pressure of anti-microbials being used. This is particularly true for penicillins, the most widely used antibiotics (Alfonso, 1985). Usage of concentration of antibiotics lower than the minimal inhibitory concentrations (MIC) is also contributing to the pool of antibiotic resistant variant strains of bacteria. It has been generally found that sub-therapeutic/sub-optimal concentrations of drugs may play a potential role in the induction of resistance. Serious Staphylococcal infections remains a colossal challenge despite the advances in antibacterial therapy. The challenge is presented indeed by the induction of long standing, refractory infections (Timridge and Grayson, 1993).

The problem is magnified in this era of chemotherapy because so many virulent bacterial strains causing serious infections of upper respiratory tract, sepsis of superficial and deep surgical wounds etc. are rapidly becoming antibiotic resistant. This gloomy situation has arisen due to extensive use of antibiotics prescribed on hit and trial basis, without resorting prior to proper anti-biogram studies and usage of antibiotics below their MIC.

It has also been reported through various studies that bacteria usually become resistant to anti-microbial agents by three major mechanisms. (i) destruction of inactivation of drug, (ii) prevention of penetration to the target site within the microbe, (iii) alteration of the anti-microbial target site.

Variations in these mechanisms may also occur. Strains of bacteria that are resistant to antibiotics are particularly common among people who work in hospitals where antibiotics are in constant use. Staph. aureus, a common opportunistic pathogen, is carried in the nasal passage, develops resistance very frequently (Gerard et al., 1996). The research was undertaken on Staph. aureus isolates from clinical cases of upper respiratory tract infection/ ailments as well as pyogenic exudates from superficial and deep surgical wounds, so as to find the incidence of drug resistance.

Materials and Methods
The samples for the isolation of Staph. aureus were obtained aseptically from upper respiratory tracts, superficial and deep septic wounds of different patients using sterile cotton swabs (Curickshank, 1975). The swabs were placed on the infected areas, gently rolled and stored in sterilized tubes.

Isolation and Identification of Staphylococcus aureus: Samples were streaked on blood agar medium and incubated for 18 hours at 37°C. A smear was prepared from the greyish, pin-head size, convex, slimy colonies and stained by Gram staining method. The selective Gram positive cocci in random clusters were then sub cultured on staph 110 medium for further affirmation. Catalase and coagulase tests were performed to type the isolates of Staph. aureus. Catalase test was performed by using the method of Jawetz et al. (1997). The effervesence produced due to prompt release of O_{2} from H_{2}O_{2} (3% solution) by the bacterial catalase indicated a positive test for catalase activity. For coagulate test citrated (pooled) plasma of rabbits (diluted 1:5 with normal saline) was mixed with an equal volume of fresh broth culture and incubated at 37°C. Clot formation within 1 to 4 hour indicated positive reaction for coagulase (Jawetz et al., 1997).

The catalase and coagulase isolates were stored in mixture of sterile broth and glycerol (1:1) in refrigerator till further analysis.

Anti-biogram studies: The susceptibilities of 60 coagulate positive Staphylcoccal colonies from different human infections were determined by disk diffusion method on Mueller Hinton medium (Merck) according to guide lines of the National Committee for Clinical laboratory standard (N.C.C.S, 1990). The Staph. aureus ATCC 25923 (American Type Culture Collection, Rockville) was used as an antibiotic susceptibility quality control.

Inoculum for pure culture of Staph. aureus was prepared using fresh nutrient agar medium slants. Four or five colonies were transferred to trypticase soy broth (Oxoid) and incubated at 37°C till it achieved turbidity as that of Barium sulphate standard (N.C.C.S, 1990). The turbidity of inoculum was adjusted with sterile saline solution.

For disk diffusion method, test plates were prepared with Mueller Hinton Medium (Merck) and inoculated with inocula of samples and Staph. aureus standard. Antimicrobial disks (Becton and Dickinson) of ciprofloxacin, amoxycillin and chloramphenicol were placed on the surface of medium with the help of sterilized forceps and pressed lightly so that disk was properly studdied into the medium. In each plate four disks were radially placed at almost 30 to 50 mm distance to avoid overlapping of the zones of inhibition. The plates were incubated at 37°C for 18 hours. Zone diameters were measured with autodates processor through bottom of the plates.

Results are reported a susceptible, intermediate and resistant according to ranges recommended by N.C.C.S (1990) as shown in Table 1.

Results and Discussion
Ninety seven cases, suffering from different clinical intensities of respiratory tract infection and post operative pyogenic complications, were investigated for the incidence of drug resistance in Staph. aureus. Total of 60 isolates (39 clinical and 21 each) were found positive for Staph. aureus after catalase and coagulase tests. The results of in vitro antibiotic tests are presented in Table 2. It was observed that ciprofloxacin was most effective antibiotic against respiratory tract infections. Out of total, 40.66, 23.33 and 30% were sensitive, moderately sensitive and showed no zone of inhibition (resistant) respectively. Ciprofloxacin also showed highest anti-bacterial activity against isolates of post-operative sepsis (Fig. 1). It inhibited 43.33% isolates while 13.33 and 43.33% isolates were moderately inhibited and resistant respectively against ciprofloxacin.

The findings are evidently concordant with those of Amayes et al. (1994) reported that ciprofloxacin is to be effective against Staph. aureus. Similar results were also observed by Fass et al. (1995) who indicated that 93% of the Staph. aureus isolates have developed resistance within the period of nine years. According to this study there was no quinolone resistant strain encountered in 1986 but the susceptibility of the Staph. aureus isolates decreased gradually with the passage of time. The results are comparable with those narrated by Forsgren and Walder (1984) who suggested cephalodoxime and BAY 13118, (recently developed antibiotics),
Table 1: Zone Diameter Interpretative Chart

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibiotic</th>
<th>Disc Potency (µg)</th>
<th>Control Zone Diameter (mm)</th>
<th>Zone Diameter Interpretative Standard (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATCC 29213</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Staph. aureus</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ciprofloxacin</td>
<td>5</td>
<td>22-30</td>
<td>&lt; 16</td>
</tr>
<tr>
<td>2</td>
<td>Amoxycillin</td>
<td>30</td>
<td>29-35</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>3</td>
<td>Chloramphenicol</td>
<td>30</td>
<td>19-25</td>
<td>&lt; 12</td>
</tr>
</tbody>
</table>

Table 2: In vitro susceptibility (%) of Staph. aureus isolates from upper respiratory tract infections and post operative sepsis

<table>
<thead>
<tr>
<th>Source</th>
<th>Amoxycillin</th>
<th>Chloramphenicol</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (S)</td>
<td>16.66</td>
<td>53.33</td>
<td>46.66</td>
</tr>
<tr>
<td>I (I)</td>
<td>30.00</td>
<td>43.33</td>
<td>23.33</td>
</tr>
<tr>
<td>R (R)</td>
<td>30.00</td>
<td>66.66</td>
<td>30.00</td>
</tr>
</tbody>
</table>

Fig. 1: Percent in vitro susceptibility of Staph. aureus isolates from post operative sepsis

Fig. 2: Percent in vitro susceptibility of Staph. aureus isolates from upper respiratory tract infections

The drug found next in the order of resistance shown by Staphylococcal isolates was amoxycillin. The results of in vitro antibiotic sensitivity of Staph. aureus strains isolated from upper respiratory tract and post operative pus infections are shown in Table 2. Amoxycillin did not exhibit a sufficient result in the study and the sensitive strains comprised only 16.66 and 10% of the total isolates tested respectively. 90 percent of the isolates from post operative sepsis were found resistant against amoxycillin. Pelti et al. (1995) found similar results i.e. 31 to 100% of the isolates from post-operative recoveries were resistant to amoxycillin. The results are also in accordance with the findings of Forsgren and Walder (1994) who observed amoxycillin to be poorly effective against respiratory tract infections. It was concluded that 80-90% strains of Staph. aureus produced β-lactamase. Similarly, Bonfiglio and Livermore (1993) have also reported that β-lactamase production protected Staph. aureus against amoxycillin. Nevertheless, these results are considerably different from those of Araki et al. (1985), who reported high anti-bacterial activity of amoxycillin against Staph. aureus isolates of animal origin. Similarly, Rojas et al. (1983) indicated that 69.4% Staphylococcus isolates of animal origin were sensitive to amoxycillin. This difference in the results may be due to the reason that the isolates of Staph. aureus of animal origin are generally more sensitive to the human counterparts (Rojas et al., 1983; Magazhaes et al., 1990). Secondly, this paradox in findings may be ascribed to the higher intake of antibiotics by the human beings. According to Louergue et al. (1994), there is generally a positive correlation existing between antibiotic consumption and incidence of resistance in Staphylococci whether the intake is intended for prophylaxis or therapy. Chloramphenicol was the third antibiotic for which in vitro anti-biotic susceptibility of isolates of Staph. aureus was studied. It was found that 66.66% isolates from post operative sepsis were resistant, 30% were moderately sensitive, and only one out of 30 was found susceptible to chloramphenicol (Fig. 1). In case of isolates of upper respiratory tract 43.33% were resistant whereas 53.66% were moderately sensitive (Fig. 2). The findings were simulating to those of Teixeira et al. (1986) who reported that 74% of the hospital isolates of Staph. aureus were resistant to chloramphenicol. Similar results have also been observed by Alpede et al. (1995) who reported 40.6% isolates of Staph. aureus as resistant, isolated from childhood sepsis. However the results of this study were discordant.
with those of Akay (1986) who found 61% of the Staphylococcus isolates from mastitic milk as sensitive to chloramphenicol. Similarly, Kurek and Niemczyk (1987) observed 95% of the isolates as chloramphenicol sensitive. Likewise, Rao and Char (1987) found that 75% of the Staph. aureus isolates of animal origin were sensitive. The different outcomes may be reviewed in the context that the isolates of the animal origin are relatively more susceptible to the antibiotics as compared to those of human origin (Rojas et al., 1983; Magalhaes et al., 1990). High bacterial resistance appears to be consequence of the indiscriminate use of antibiotics as well as unsatisfactory consideration given to good hygienic measures in hospitals (Ludwig et al., 1995). The associated problems concerning Staphylococci are particularly serious and are further complicated by the fact that a large proportion of nosocomial strains are antibiotic resistant. Furthermore, such staphylococcal infections are readily transmitted among susceptible populations by the individuals who have acquired them during hospitalization. Such critical incisions have been discussed and fairly elucidated by Wenzel (1995).

References


