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Microbial Resistance of *Staphylococcus aureus* Against Commonly Used Antibiotics

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A total of 60 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

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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 (Loulergue *et al*., 1994). More and more strains of micro-organisms 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 plays 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 (Turnidge and Grayson, 1993).

The problem is magnified in this era of chemotherapy because so many virulent bacterial strains causing serious infections of upper respir atory 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*., 1995).

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 asceptically 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 biotype, the isolates of *Staph. aureus*. Catalase test was performed by using the method of Jawetz *et al*. (1997). The effervescence produced due to prompt release of O_2 from H_2O_2 (3% solution) by the bacterial catalase indicated a positive test for catalase activity. For coagulase 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 coagulase positive Staphylococcal isolates 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 (Difco) 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 studded into the medium. In each plate four disks were radially placed at almost 30 to 36 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 autodata processor through bottom of the plates. Results are reported as 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 (30 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 Amyes *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 (1994) who suggested cefpodoxime and Bay Y3118, (recently developed antibiotics),

Table 1: Zone Diameter Interpretative Chart

Sr. No.	Antibiotic	Disc Potency (μg)	Control Zone Diameter (mm) *Staph. aureus* ATCC 25923	Zone Diameter Interpretative Standard (cm)	
				Resistant	Intermediate Susceptible
1	Ciprofloxacin	5	22-30	<15	16-20 >21
2	Amoxycillin	30	29-35	<20	21-28 >29
3	Chloramphenicol	30	19-26	<12	13-17 >18

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

Source	Amoxycillin			Chloramphenicol			Ciprofloxacin		
	S	I	R	S	I	R	S	I	R
Upper respiratory tract isolates n=30	16.66	30.00	53.33	0	56.66	43.33	46.66	23.33	30.00
Post operative pus n=30	10.00	0	90.00	3.33	30.00	66.66	43.33	13.33	43.33

S = sensitive I=intermediate R=Resistant

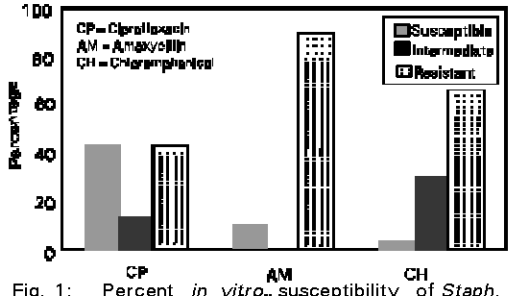

Fig. 1: Percent *in vitro* susceptibility of *Staph. aureus* isolates from post operative sepsis

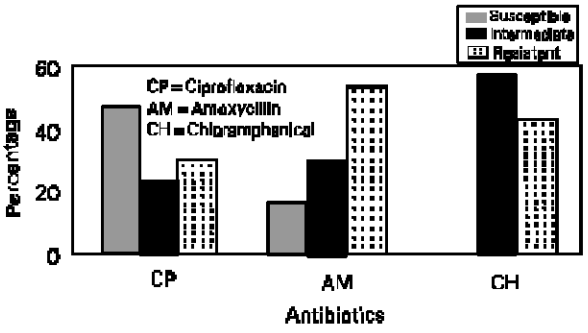

Fig. 2: Percent *in vitro* susceptibility of *Staph. aureus* isolates from upper respiratory tract infections

were highly effective against upper respiratory tract pathogens. Doss *et al*. (1995) commented that for infections caused by *Staph. aureus*, the dosage of ciprofloxacin should be adequate to ensure inhibitory concentration at the site of infection. Doses below the MIC, induce mutation in susceptible isolates of *Staph. aureus*. As per findings of the present studies, the higher percentage of resistant *Staph. aureus* strains may be due to largely the indiscriminate use of this antibiotic in concentration lower than the MIC values. Goldstein (1988) observed that ciprofloxacin was generally more active against aerobic isolates of bite wounds as compared to other antibiotics. The wide variation observed in the antibiotic sensitivity of the isolates of *Staph. aureus* collected between 1986 to 1997, showed that the resistance has gradually developed over the passage of time due to frequent use in sub-optimal concentrations of ciprofloxacin.

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 as encouraging results 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. Petit *et al*. (1995) found similar results i.e. 31 to 100% of the isolates from post-operative recuperations 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 is 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; Magalhaes *et al*., 1990). Secondly, this paradox in findings may be ascribed to the higher intake of antibiotics by the human beings. According to Loulergue *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*. (1995) who reported that 74 % of the hospital isolates of *Staph. aureus* were resistant to chloramphenicol. Similar results have also been observed by Akpede *et al*. (1995) who reported 40.6% isolates of *Staph. aureus* as resistant, isolated from childhood septicemia. 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. Similary, Kurek and Niemczyk (1987) observed 55% 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 instances have been discussed and fairly elucidated by Wenzel (1995).

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