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MULTIPLE DRUG RESISTANCE FACTORS AMONG INDIGENOUS CLINICAL *STAPHYLOCOCCI*

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108 *Staphylococcus aureus* isolates, collected from various clinical sources of Karachi, were screened for their resistance to 8 different antibiotics. Among these cultures, 98 were found resistant to single or multiple antibiotics in different combinations. Generally, the isolates showed the highest frequency of resistance to ampicillin and the lowest to chloramphenicol, followed by tetracyclin and gentamycin. A total of 14 different patterns of antibiotic resistance were observed at a level as high as 500 μg/ml. Some of the antibiotic determinants were cured by acridine orange treatment, indicating the widespread of antibiotic resistance through plasmids among *Staphylococcus aureus.*

Key words: Plasmids, Indigenous staphylococci, Acridine orange.

INTRODUCTION

Wide dissemination of drug resistance plasmids among *S. aureus* strains represents the main cause of high incidence of antibiotic resistant strains of *S. aureus* [5]. Clinical Staphylococci have been associated with a wide variety of human infection combined with the fact that drug resistance determinants in these organisms are not only borne on non conjugative plasmids [1,5] but also on conjugative plasmids [7]. Hence it is interesting to study the cause and mode of antibiotic resistance in indigenous Staphylococci. This paper includes the study of the incidence of multiple drug resistance among clinical *S. aureus,* and the correlation of drug resistance and plasmids with the help of curing methods.

MATERIALS AND METHODS

Becterial isolates. S. aureus isolates were collected from different clinical centres of Karachi.

Antibiotics. 8 antibiotics were used in these studies: Ampicillin(A), Chloramphenicol(C), Gentamycin(G), Kanamycin(K), Neomycin(N), Polymyxin(P), Streptomycin(S) and Tetracyclin hydrochloride(T). Stock solutions (10 mg/ml) of antibiotics were made in distilled water. Chloramphenicol was dissolved in ethanol. All solutions were sterilized by millipore (0.45 mμ) filters and refrigerated.

Media. Difco (USA) nutrient broth and agar were used for the screening of cultures for antibiotic resistance. Cultures were maintained in tryptone agar that consists of bactotryptone 17 g; bacto-agar 5 g and distilled water 1 lit.

Antibiotic resistance/sensitivity tests. The cultures obtained were further identified on the basis of pigment production and coagulase activity.

The pure isolates were subjected to antibiotic resistance screening by replica plate method [6]. For this purpose, a broth culture of the test strain was plated on nutrient agar plate to obtain isolated colonies. A few colonies were picked on to a master plate, incubated overnight and replicated on nutrient agar plates containing different concentrations of all the antibiotics. The highest concentration of an antibiotic showing growth of all the replicated clones was taken as the resistant level of the strain for that particular antibiotic.

Acridine orange mediated plasmid curing. Method of Hirota [2] was followed for this purpose. A small inoculum ($2 \times 10^2 - 5 \times 10^2$ bacteria) was added to varying concentrations of acridine orange broth and incubated at 37° overnight. Cultures containing the highest concentration of acridine orange in which growth was clearly visible were diluted and spread on nutrient agar plates. Where cultures contained R factors, colonies from the nutrient agar plates were replicated to nutrient agar plates containing appropriate antibiotic.

RESULTS

Antibiotic resistance. The results of antibiotic resistance obtained after using the levels of 25, 50, 100, 250 and 500 μg per ml of each of the antibiotics are indicated in Table 1.

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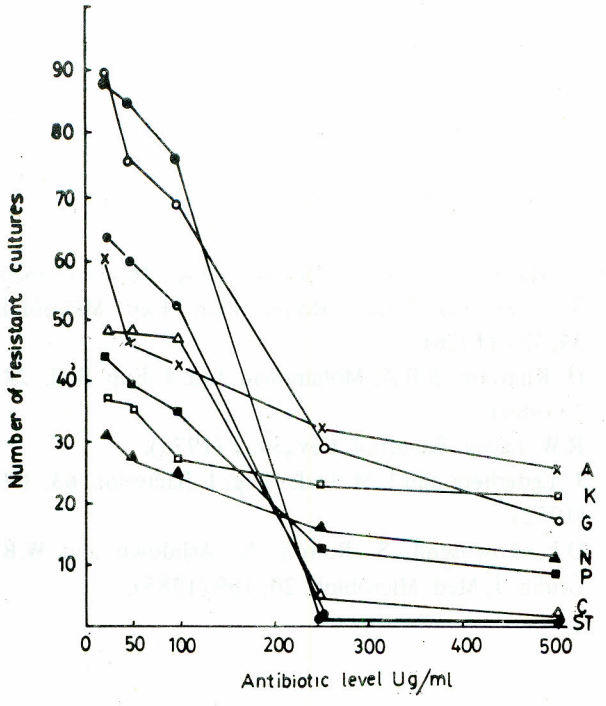

Fig. 1. Comparison of drug resistance of cultures at different levels of antibiotics.

Frequency of antibiotic resistance. A comparative account of the drug resistance of cultures, at different levels is given in Fig. 1. Generally, the cultures, showed the highest frequency of resistance to ampicillin and lowest to chloramphenicol, followed by tetracyclin and gentamycin. There was a slight decrease in the number of ampicillin resistant cultures at the levels of 50-500 μg/ml compared with the level of 25 μg/ml. At 250 μg/ml level, the cultures showed a considerable decrease in the resistance frequency of almost all antibiotics.

Antibiotic resistance patterns. The resistant cultures showed 14 different patterns of antibiotic resistance at a level as high as 500 μg/ml (Table 2). Among these, ampicillin resistance (A) was found to be the most common, followed by AS, AP and ASNK.

Loss of drug resistance after plasmid curing. Two representative multiple drug resistant *S. aureus* cultures (patterns 1 and 2) were selected for plasmid curing. Out of 200 colonies each from the 2 treated cultures some had lost the resistance to one or the other antibiotics. However, a total loss of chloramphenicol resistant cultures was observed in one of the cultures.

DISCUSSION

The results indicate that drug resistance among indigenous clinical Staphylococci is very common. Out of a total of 108 isolates, 98 were found to be resistant to single or multiple drugs. A total of 14 different resistance patterns were observed among the resistant. Resistance to ampicillin was shown to be the most common. Maximum number of the resistant cultures were found at the level of 25 μg/ml. Number of kanamycin resistant cultures was found to be the lowest up to 100 μg/ml. In view of the overall high incidence of multiple drug resistance among *S. aureus,* the possibility of the presence of R plasmids was explored. The loss of resistance to single or multiple antibiotics after acridine orange treatment of cultures points out to the

Table 1. Occurrence of antibiotic resistance of *S. aureus* at 5 different concentrations.

Antibacterial drug	Number of resistant cultures at concentrations of (μg/ml)				
	25	50	100	250	500
Ampicillin	60	46	44	34	26
Chloramphenicol	48	48	47	4	3
Gentamycin	90	75	68	28	16
Kanamycin	37	36	29	27	26
Neomycin	32	29	26	16	11
Polymyxin	44	40	36	13	9
Streptomycin	87	85	76	1	1
Tetracyclin	64	60	52	1	1

Table 2. Drug resistance pattern at 500 μg/ml in *S. aureus* isolated from different clinical specimen

Pattern No.	Resistance pattern	No. of cultures	Culture of the pattern (%)
1	SATCGNKP	1	1.02
2	SAGNKP	1	1.02
3	SPANK	1	1.02
4	SANK	4	4.08
5	SAK	2	2.04
6	SAN	1	1.02
7	SP	2	2.04
8	SK	1	1.02
9	AN	1	1.02
10	AK	1	1.02
11	SA	2	2.04
12	S	10	10.2
13	F	11	11.2
14	A	8	8.1

observed drug resistance being plasmid borne. Neither of the two cultures showed a loss of resistance to all the drugs simultaneously. This shows that different plasmids determine the resistance against different antibiotics. Three distinct antibiotic resistant plasmids have also been reported earlier in *S. aureus* [3]. Loss of chloramphenicol resistance in all the colonies of one of the cured cultures could be due to the fact that the corresponding plasmid may be very small and fragile which could not resist the curing treatment in all the cells. Low percentage of curing in other resistant determinants was observed in the present studies. Analogous results have been reported by Khatoon *et al.* [4] in the case of KR61 plasmid of *Aerobacter aerogenes* after its transfer to *E. coli* AB2463. Since curing was not observed in all the resistance determinants, it may be presumed that some plasmids were present in integrated state and hence stable as has been suggested by Hirota [2].

In view of these interesting findings, further work on the possible occurrence of conjugative plasmids in the isolates is under way.

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