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Isolation of Chlorhexidine-Resistant *Pseudomonas aeruginosa* from Clinical Lesions

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The chlorhexidine resistance of 317 strains of *Pseudomonas aeruginosa* isolated from hospital patients was determined. The distribution pattern of their susceptibility to chlorhexidine clearly revealed two peaks, and the frequency of resistance to chlorhexidine was 84.2%.

Chlorhexidine is one of the most useful disinfectants used in hospitals. For example, over the past 5 years, Jikei University Hospital has continued to use 400 to 500 bottles (500 ml) containing 5% of the disinfectant per month. The effect of chlorhexidine has been described by many authors (5, 7, 10). Species differences in resistance to chlorhexidine are well known, and several authors have reported finding *Alcaligenes faecalis* (4), *Proteus mirabilis* (6, 9, 14), *Proteus rettgeri* (1, 8), *Pseudomonas aeruginosa* (2, 3), *Pseudomonas cepacia* (15), and *Providencia* (14) resistant to chlorhexidine.

It is well known that in many bacteria, resistance to antibiotics and metal ions is associated with plasmids (11). The role of plasmids in drug resistance has been widely studied, and extrachromosomal determinants are a main cause of the increase in the number of drug-resistant bacteria. However, the factors selecting for these metal-resistant bacteria have not yet been identified. We believe that metal-resistant microorganisms do not arise by chance, but that there must be selectional factors beyond mere drug resistance. One of these selectional factors may be environmental pollution by these metals. To investigate this possibility, we previously tested the drug and metal resistance of clinical strains of *P. aeruginosa* and observed that the frequency of metal resistance was the same as, or higher than, that of antibiotic resistance (13). Weiss et al. (16) reported that many plasmids carried not only resistance to $HgCl_2$, but also resistance to several organomercurials such as phenylmercuric acetate, Mercurochrome, fluorescein mercuric acetate, and thimerosal. It is of interest that $HgCl_2$ and thimerosal were used for a long time as disinfectants, and Mercurochrome was used for treatment. We think the Hg-resistant bacteria result mainly from industrial pollution and usage of agricultural chemicals. Furthermore, we need to think about Hg-resistant bacteria emerging due to the use of mercury in disinfectants and treatments in hospitals, particularly in the case of clinical isolates. We have investigated susceptibility to chlorhexidine used as a disinfectant, with the idea that bacteria resistant to disinfectants may exist. We have found chlorhexidine-resistant strains of *P. aeruginosa* isolated from clinical lesions. The distribution and frequency of chlorhexidine resistance were higher than those of antibiotic and mercury resistance.

Strains of *P. aeruginosa* were isolated from the Jikei University Hospital in 1980 and tested for their resistance to six antibiotics, three metals, and chlorhexidine. Those cultures not inhibited by 50 μg of streptomycin, tetracycline, chloramphenicol, or kanamycin per ml or by 25 μg of gentamicin or dibekacin per ml were regarded as resistant to each of the antibiotics. The three metals tested (Hg, Cd, and As) were provided as $HgCl_2$, $CdCl_2$, and Na_2HAsO_4, respectively. Effective concentrations of the metals were described in a previous paper (12).

Figure 1 shows the distribution of chlorhexidine resistance obtained by the agar dilution method from 317 clinical isolates of *P. aeruginosa*. The bimodal distribution curve of susceptibility to chlorhexidine showed a clear-cut distinction between susceptible and resistant populations. The resistance was differentiated by a chlorhexidine concentration of 50 μg/ml. In addition, in our study, seven strains of chlorhexidine-resistant *P. aeruginosa* grew in 200 μg of chlorhexidine per ml (0.02%), the same amount of concentration used daily by doctors. In view of the widespread use of chlorhexidine, this result is of obvious interest.

The frequency of resistance to chlorhexidine was 84.2% among the 317 isolates (Table 1). The frequency of the chlorhexidine-resistant strains was higher than the frequency of drug- and mercury-resistant strains, but was lower than the frequency of Cd- and As-resistant strains (Table 1).

We also tested the susceptibilities of the above isolates (five chlorhexidine-sensitive, five

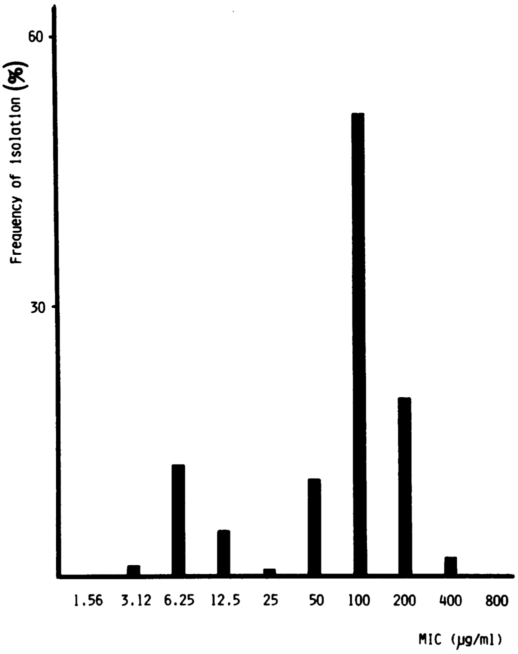

FIG. 1. Distribution pattern of resistance to chlorhexidine in 317 strains of *P. aeruginosa*. The chlorhexidine resistance revealed a clear-cut bimodal distribution. From this pattern, those cultures not inhibited by 50 μg of chlorhexidine per ml were regarded as resistant to chlorhexidine.

chlorhexidine-resistant, and seven highly chlorhexidine-resistant strains of *P. aeruginosa*) to chlorhexidine in broth. Bacterial suspensions of chlorhexidine-sensitive, chlorhexidine-resistant, and highly chlorhexidine-resistant strains (about 10^5 organisms per ml) were inoculated into broths containing various concentrations of chlorhexidine. After 24 h of incubation, the cultures of these strains in broth were plated on agar plates. The number of living cells was indicated as colony-forming units. Figure 2 shows three strains with different susceptibility, namely, *P. aeruginosa* JP1177 as an example of chlorhexidine sensitive, *P. aeruginosa* PU21 as an example of chlorhexidine resistant, and *P. aeruginosa* JP1777 as an example of highly chlorhexidine resistant, respectively. The maximal concentration of chlorhexidine under which chlorhexidine-resistant strains were able to grow was 100 μg/ml, about 10 times as much as that for chlorhexidine-sensitive strains. Seven cultures not inhibited by 200 μg of chlorhexidine per ml by the agar dilution method were also able to demonstrate growth in 200 μg of chlorhexidine per ml in broth.

All *P. aeruginosa* strains tested against chlorhexidine were also tested against six antibiotics to investigate the correlation between chlorhexidine resistance and antibiotic resistance. Of 267 chlorhexidine-resistant *P. aeruginosa* strains,

TABLE 1. Frequency of isolation of drug-, metal-, and chlorhexidine-resistant strains of *P. aeruginosa*

Determination	No. of strains[a]	% of strains
Drug[b]		
SM	168	53.0
TC	60	18.9
CP	163	51.4
KM	227	71.6
GM	38	12.0
DKB	40	12.6
Metal		
Hg	240	75.7
Cd	308	97.2
As	311	98.1
Disinfectant		
CH[c]	267	84.2

[a] Of a total of 317.

[b] SM, Streptomycin; TC, tetracycline; CP, chloramphenicol; KM, kanamycin; GM, gentamicin; DKB, dibekacin.

[c] CH, Chlorhexidine.

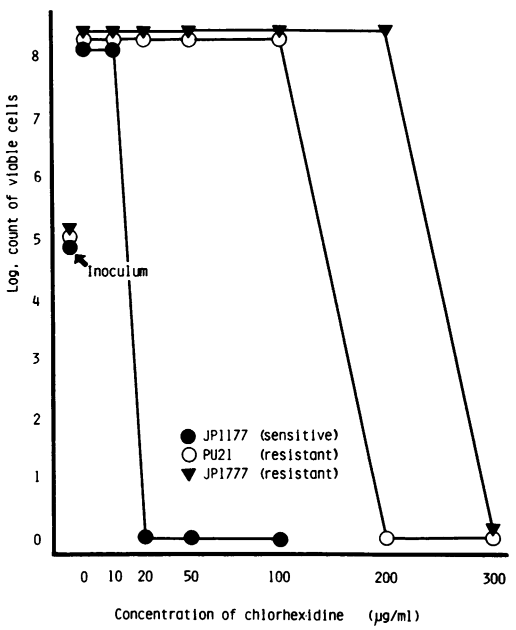

FIG. 2. Resistance of chlorhexidine-resistant, chlorhexidine-sensitive, and highly chlorhexidine-resistant strains of *P. aeruginosa* to chlorhexidine. Symbols: ●, log counts of viable organisms of a chlorhexidine-sensitive strain (*P. aeruginosa* JP1177) when cultured overnight at 37°C in broths containing various concentrations of chlorhexidine; ○, chlorhexidine-resistant strain (*P. aeruginosa* PU21) cultured in the same media; ▼, highly chlorhexidine-resistant strain of *P. aeruginosa* JP1777 cultured in the same media. In all cases the initial inocula were adjusted to a level of about 10^5 organisms per ml.

TABLE 2. Patterns of resistance of chlorhexidine-resistant *P. aeruginosa* strains to antibiotics

Type of resistance[a]	No. of strains[a]	Isolation frequency among tested strains[b]
Quintuple		
SM, TC, CP, KM, DKB	2 (0.7)	22 (8.2)
SM, TC, KM, GM, DKB	20 (7.5)	
Quadruple		
SM, TC, CP, KM	19 (7.1)	37 (13.8)
SM, CP, GM, DKB	12 (4.5)	
SM, KM, GM, DKB	6 (2.2)	
Triple		
SM, TC, KM	16 (6.0)	82 (30.8)
SM, CP, KM	49 (18.4)	
TC, CP, KM	17 (6.4)	
Double		
SM, CP	5 (1.9)	63 (23.6)
SM, KM	37 (13.9)	
TC, KM	6 (2.2)	
CP, KM	15 (5.6)	
Single		
CP	10 (3.7)	23 (8.6)
KM	13 (4.9)	
Sensitive	40 (15.0)	40 (15.0)

[a] For abbreviations, see Table 1, footnote *a*.

[b] Figures in parentheses represent percentage of chlorhexidine-resistant strains.

204 (76.4%) were multiple drug resistant (Table 2).

The results of this study may be summarized as follows: (i) the distribution curve of chlorhexidine resistance shows a clear-cut distinction between "resistant" and "sensitive" populations; (ii) the frequency of chlorhexidine resistance was higher than that of antibiotic and mercury resistance; (iii) most of these chlorhexidine-resistant isolates were multiple antibiotic resistant.

It is very difficult to determine whether *P. aeruginosa* has always been resistant or whether resistant strains are now being selected by the widespread use of chlorhexidine. The extremely high frequency of 84.2% of clinical strains of *P. aeruginosa* which were resistant to chlorhexidine should be noted from the viewpoint of pseudomonad infection and contamination of chlorhexidine in the hospital. The frequency of chlorhexidine resistance in clinical isolates from hospitals needs to be determined annually.

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