HEAVY METAL AND DISINFECTANT RESISTANCE IN CLINICAL ISOLATES OF GRAM-NEGATIVE RODS

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INTRODUCTION

R plasmids have been shown to carry genetic determinants for resistance to a variety of chemical and physical agents. In addition to antibiotics, R plasmids confer resistance to toxic heavy metals, colicins and ultraviolet light (Falkow, 1975). R plasmids of enterobacteria have been shown to determine resistance to mercury, silver, nickel and cobalt (Smith, 1967; McHugh *et al.*, 1975) and a penicillinase plasmid of *Staphylococcus aureus* has been shown to mediate resistance to mercury, cadmium, arsenate, lead and zinc (Novick and Roth, 1968; Dyke *et al.*, 1970).

Resistance to disinfectants has not been extensively studied but there have been several reports of disinfectant resistance in Gram-negative rods (Stickler and Thomas, 1976; Stickler and Thomas, 1980; Nakahara and Kozukue, 1982). There has been one report of disinfectant resistance being plasmid -mediated (Sutton and Jacoby, 1978) and the plasmid also carried the genes for sulphonamide and gentamicin resistance.

This study was undertaken to determine whether known antibiotic resistant Gram-negative rods from clinical sources also carried resistant determinants to heavy metals and disinfectants.

MATERIALS AND METHODS

Bacterial strains used for the study were Gram-negative rods isolated from clinical specimens of urine, stool, blood and pus received by the Bacteriology Division, Institute for Medical Research, from January to December 1981.

Screening for resistance to mercury and silver was carried out by replicating using a multipoint inoculator (Denley-Teck Ltd., England) onto plates prepared by incorporating mercuric chloride ($HgCl_2$) into nutrient agar (Difco) and silver nitrate ($AgNo_3$) into tryptone yeast extract agar (McHugh *et al.*, 1975). Strains not inhibited by 10 mcg/ml of $HgCl_2$ (Nakahara *et al.*, 1977) and 40 mcg/ml of $AgNO_3$ (Annear *et al.*, 1976) were regarded as resistant.

Disinfectants tested were : Chlorhexidine gluconate (Hibitane, ICI Ltd.) ; a phenolic preparation containing 15% O-phenylphenol, 6.3% p-tert-amyphenol, 4.7% alcohol, 44.0% anhydrous soap (Amphyl, Sterling Drug Inc.); chloroxylenol (Dettol, Reckitt and Colman Ltd.); and a saponated cresol (Lysol).

Screening for resistance to the disinfectants was similarly carried out by multipoint inoculation onto plates of nutrient agar (Difco) incorporated with the disinfectants. Plates were prepared by adding various concentrations of the disinfectants to molten agar that had been cooled to 50°C.

The lowest concentration of the agent preventing colony formation after overnight incubation at 37°C was taken as the minimal inhibitory concentration (MIC) for that strain. The criterion that resistant strains had MIC's greater than the recommended use-dilution of the disinfectant was adopted (Stickler and Thomas, 1980). Thus, strains with MIC's greater than 500 mcg/ml of chlorhexidine gluconate, 2% of Amphyl, 5%

of chloroxylenol and 2% of Lysol were considered resistant.

Strains were tested for their ability to transfer heavy metal and disinfectant resistances to a recipient: *Escherichia coli* W1802 resistant to nalidixic acid by a conjugation method previously described (Khor and Jegathesan, 1980).

Mating mixtures of donor and recipient in a ratio of approximately 1:100 were set up and after overnight incubation at 37°C, appropriate dilutions were plated on selection plates. The selection plates consisted of nutrient agar (Difco) containing 50 mcg/ml of nalidixic acid and either 500 mcg/ml of chlorhexidine gluconate or 10 mcg/ml of $HgCl_2$. For transfer of Ag resistance the plates consisted of tryptone yeast extract agar containing 50 mcg/ml of nalidixic acid and 40 mcg/ml of $AgNo_3$.

RESULTS

Ninety-five multiply-resistant strains were selected from 730 Gram-negative rods isolated for screening for heavy metal and disinfectant resistance.

Table 1 shows the distribution of heavy metal and disinfectant resistance among the strains tested. 39 out of 95 (41%) of the bacteria tested were resistant to mercury, 20 (21%) were resistant to silver and 7 (7.3%) were resistant to chlorhexidine. Resistance to the other disinfectants tested was not found.

All resistant strains were examined for transferable resistance. 17 of the strains transferred mercury resistance and 10 strains transferred silver resistance. Chlorhexidine resistance was not shown to be transferable.

Table 2 shows the transferable resistance determinants carried by the clinical isolates.

Table 1

Distribution of heavy metal and disinfectant resistance among clinical isolates of Gram-negative rods.

Organism	No. tested	Mercury resistance		Silver resistance		Chlorhexidine Resistance
		No.	No. transferable	No.	No. transferable	No.
Salmonella spp.	43	13	6	8	5	1
E. coli	21	11	2	3	2	0
Klebsiella spp.	11	7	4	6	2	1
Shigella spp.	7	0	0	0	0	0
Proteus spp.	5	4	2	0	0	3
Citrobacter spp.	2	1	1	0	0	1
Enterbacter spp.	2	1	1	2	1	0
Pseudomonas spp.	2	1	0	1	0	1
Serratia spp.	1	0	0	0	0	0
Providencia spp.	1	1	1	0	0	0
Total	95	39	17	20	10	7

Table 2

Transferable resistance determinants among clinical isolates of Gram-negative rods.

Mrganism	Source	Resistance determinants	Resistance determinants transferred
Salmonella stanley	Stool	Hg	Hg
S. typhimurium	Stool	Hg	Hg
S. krefeld	Stool	AgChHg	AgHg/Hg
S. cerro	Stool	Ag	Ag
S. stanley	Stool	AgHg	Ag/Hg
S. emek	Stool	Ag	Ag
S. weltevreden	Stool	AgHg	AgHg/Hg
S. krefeld	Stool	Hg	Hg
Kelbseilla spp.	Urine	AgHg	AgHg
Klebsiella spp.	Urine	AgHg	Hg
Klebsiella spp.	Urine	AgHg	Ag/AgHg
Klebsiella spp.	Urine	AgHg	Hg
E. coli	Urine	AgHg	AgHg/Hg
E. coli	Urine	AgHg	Ag/AgHg
Citrobacter spp.	Pus	ChHg	Hg
Providencia spp.	Urine	ChHg	Hg
Enterobacter spp.	Pus	AgHg	Ag/Hg
Proteus spp.	Pus	ChHg	Hg
Proteus spp.	Urine	ChHg	Hg

Key : Ag = Silver, Ch = Chlorhexidine, Hg = Mercury.

DISCUSSION

Even extremely high dilutions of the heavy metals are known to be lethal for micro-organisms. Various forms of mercury, like mercurochrome, merthiolate, metaphen and phenylmercuric nitrate have been used in medicines for many years. However, they were shown to be unreliable and their present use is restricted mainly to the maintenance of sterility in biologicals and other industrial products. Silver has also been used in medicines for several hundred years. The inorganic silver salts are efficient germicides and in spite of its caustic and irritating properties, silver nitrate is still used as a germicide.

Thus it was not altogether unexpected that in our study, 39 out of the 95 strains tested were resistant to mercuric chloride and 20 strains to silver nitrate. 17 strains possessed transferable mercury resistance and 10 strains had transferable silver resistance. In a study on mercury and antibiotic resistance of *E. coli* isolated from hospital patients (Nakahara *et al.*, 1977), mercury resistance was found in 58.6% of the isolates.

It is difficult to know what criterion to employ to decide whether an organism is resistant or sensitive to an antimicrobial agent. With antibiotics, organisms are simply designated resistant if they are able to

multiply in the concentration of antibiotic attainable at the infection site. A similar criterion is clearly not applicable for disinfectants. Stickler and Thomas (1980) used the criterion that if the MIC value of the organism was greater than the concentration of the agent normally recommended for routine use, then the isolate was designated as resistant. Applying this criterion, we were able to detect only 7 strains (7.3%) resistant to one disinfectant, chlorhexidine. Nakahara and Kozukue (1982) reported that 84.2% of their *Pseudomonas aeruginosa* strains were resistant to chlorhexidine. However, they considered 50 mcg/ml of chlorhexidine as the concentration that differentiated resistant from sensitive strains. In our study, 500 mcg/ml was taken as the differentiating value.

Stickler and Thomas (1980) studied 802 Gram-negative bacteria causing urinary tract infections for resistance to chlorhexidine, cetrimide, glutaraldehyde, phenyl mercuric nitrate, resiguard and benzalkonium chloride. They showed that approximately 10% of the total number of isolates mainly of the genera *Proteus, Providencia* and *Pseudomonas* exhibited resistance to the cationic agents. Our study revealed that the disinfectant resistant organisms were of the genera *Proteus* (3), *Pseudomonas* (1), *Citrobacter* (1), *Klebsiella* (1) and *Salmonella* (1).

We were unable to demonstrate transfer of the chlorhexidine resistance to the sensitive *E.coli*. We were thus unable to show that the determinant for chlorhexidine resistance was plasmid-mediated. Sutton and Jacoby (1978) demonstrated the transfer of hexachlorophene resistance together with sulphonamide and gentamicin resistance between two *Ps.aeruginosa* strains. Failure to demonstrate transfer of chlorhexidine resistance in our strains does not mean that the resistance is not plasmid-mediated. Many factors affect the conjugal transfer of a determinant from one organism to another like incompatibility between the donor and recipient, and experimental conditions like the pH, media, temperature and length of incubation.

Our study established that 41% of clinical isolates of Gram-negative bacteria multiple-resistant to antibiotics also carried determinants specifying resistance to mercury, 21% to silver and that 7.3% carried determinants for chlorhexidine resistance. Thus, it can be seen that resistance to heavy metals is quite common among the clinical isolates and though not very widespread, resistance to disinfectants does exist. These results demonstrate the potential problem that can arise. There should be a rational use of disinfectants and heavy metals to ensure that clinical isolates do not become resistant to these antibacterial agents in addition to antibiotics.

SUMMARY

Ninety-five clinical strains of Gram-negative bacteria were examined for resistance to mercury, silver and disinfectants. 41% of the strains possessed resistance to mercury, 21% to silver and 7.3% of the strains were resistant to chlorhexidine. Mercury resistance was shown to be plasmid–mediated in 17 strains and silver resistance in 10 strains. Chlorhexidine resistance was not shown to be transferable.

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