Linkage of Mercury, Cadmium, and Arsenate and Drug Resistance in Clinical Isolates of *Pseudomonas aeruginosa*

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Of the 787 isolates, 99.8% were metal resistant, with most (99.5%) showing multiple resistance. Fifty-three percent of the isolates were both metal and drug resistant, whereas only 19% were metal resistant and drug sensitive.

It is well known that many bacterial isolates are resistant to heavy metal ions (2, 6, 9–11). For example, besides penicillin resistance, the penicillinase plasmid of *Staphylococcus aureus* carries genes determining resistance to several metallic ions such as Hg, Cd, As, Pb, and Zn (7). We also observed that resistances to Hg and Cd mediated by the penicillinase plasmid were controlled by quite different mechanisms (3). Furthermore, R-factor-mediated resistance to Hg, Co, and Ni has also been observed in *Escherichia coli* (8).

It is of interest that resistance to these metals is mediated by the same plasmid that determines resistance to drugs. Most of these metals have recently been listed as established or possible causes of environmental pollution. In addition, methyl mercury is known to cause Minamata disease, and cadmium is the causative agent of Itai-Itai disease in Japan.

Studies of the epidemiology, genetics, and biochemistry of drug-resistant bacteria indicate that the origin, selection, spread, and prevalence of drug-resistant microorganisms resulted from use of drugs. In contrast, several investigations suggest a correlation between resistance to metals and drugs and exposure of bacteria to a hospital environment (1, 5).

However, the nature of the factors contributing to these metal-resistant organisms has not yet been explained by epidemiological and genetic investigations. These metal-resistant isolates do not appear to originate by chance. We presumed that one of the factors selecting for metal-resistant organisms may be environmental contamination by these metals.

Our previous studies of the metal resistance of 415 isolates of *S. aureus* derived from clinical lesions (4) indicated that the frequency of metal resistance in isolates of *S. aureus* was higher than that of drug resistance. Most of these metal-resistant isolates were multiply metal resistant and also multiply drug resistant. Furthermore, 8.0% of the cultures tested were metal resistant but drug sensitive.

In the current investigation, we assayed both metal and drug resistances in 787 *P. aeruginosa* isolates from clinical lesions and attempted to demonstrate a relation between the two phenomena. The four metals tested (Hg, Cd, As, and Pb) were provided as HgCl₂, CdCl₂, Na₂HAsO₄, and Pb(CH₃COO)₂, respectively. Effective concentrations of the metals were determined experimentally, whereas the concentrations of the test drugs were selected arbitrarily. Those cultures not inhibited by 100 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.

Figure 1 shows a clear-cut bimodal distribution of susceptibility to three of the metals, but only a single peak of resistance to Pb. It can be seen that resistance was demonstrable in media containing the following concentrations of metals (in micrograms per milliliter): HgCl₂, 10; CdCl₂, 400; and Na₂HAsO₄, 400.

The frequencies of resistance to these concentrations of Hg, Cd, and As were 75.1, 96.5, and 98.8%, respectively (Table 1). A greater fraction of the isolates were resistant to each metal than to each antibiotic.

Cultures with resistance to all three metals (triple) were isolated most frequently, followed by those resistant to two metals (double) and then those resistant to one (single) (Table 2). Among the doubles, those resistant to Cd and As were isolated most frequently. In sum, a total of 99.8% of the cultures tested comprised metal-resistant isolates, and 99.5% of these were multiply metal resistant. In addition, 53.2% of these multiply metal resistant isolates were also multiply drug resistant. The number that were metal sensitive and drug resistant was small, comprising only 0.2% of the isolates. This contrasts with 19.4% of the isolates that
were metal resistant but drug sensitive. A similar result was obtained in *S. aureus* (4).

In conclusion, the results of this study may be summarized as follows: (i) the frequency of metal resistance was higher than that of antibiotic resistance; (ii) most of these metal-resistant isolates were multiply metal resistant and also multiply drug resistant; and (iii) there were many isolates that were metal resistant but drug sensitive.

These results should be noted in view of the fact that these metals are a cause of environmental pollution.

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**LITERATURE CITED**


