ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1991, p. 819–823
0066-4804/91/050819-05$02.00/0

Vol. 35, No. 5

# Mercuric Reductase Activity and Evidence of Broad-Spectrum Mercury Resistance among Clinical Isolates of Rapidly Growing Mycobacteria

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Received 19 November 1990/Accepted 20 February 1991

**Resistance to mercury was evaluated in 356 rapidly growing mycobacteria belonging to eight taxonomic groups. Resistance to inorganic $Hg^{2+}$ ranged from 0% among the unnamed third biovariant complex of *Mycobacterium fortuitum* to 83% among *M. chelonae*-like organisms. With cell extracts and $^{203}Hg(NO_3)_2$ as the substrate, mercuric reductase (HgRe) activity was demonstrable in six of eight taxonomic groups. HgRe activity was inducible and required NADPH or NADH and a thiol donor for optimal activity. Species with HgRe activity were also resistant to organomercurial compounds, including phenylmercuric acetate. Attempts at intraspecies and intragenus transfer of HgRe activity by conjugation or transformation were unsuccessful. Mercury resistance is common in rapidly growing mycobacteria and appears to function via the same inducible enzyme systems already defined in other bacterial species. This system offers potential as a strain marker for epidemiologic investigations and for studying genetic systems in rapidly growing mycobacteria.**

Bacteria that were resistant to inorganic and organic mercury were first isolated from mercury-contaminated soil in Japan in the late 1960s. Since that time, mercury resistance has come to be recognized as widespread among procaryotic species isolated from human and environmental sources (4, 14, 23). In some areas (especially Japan), resistance to heavy metals such as mercury is as common as or more common than resistance to antibiotics (10–12, 14).

The mechanism of mercury resistance has been studied extensively (14). Two types of resistance have been described. Narrow-spectrum resistance is due to the presence of the cytoplasmic enzyme mercuric reductase (HgRe), which confers resistance to inorganic mercury, merbromin, and fluorescein mercuric acetate (FMA) by converting $Hg^{2+}$ to the volatile $Hg^0$. Broad-spectrum resistance involves a second enzyme, organomercurial lyase, which confers resistance to organomercurial compounds such as phenylmercuric acetate (PMA) by disrupting the carbon-mercury linkage. The majority of mercury-resistant isolates of *Escherichia coli* contain only the HgRe enzyme (narrow-spectrum resistance), whereas all isolates of *Staphylococcus aureus* contain both enzymes (broad-spectrum resistance) (14, 25).

Many of the mercury resistance determinants in both gram-positive and gram-negative bacteria appear to be on transposons and, as such, are capable of inserting into plasmids of numerous sizes and compatibility groups and into the chromosome (27). More recently, DNA sequencing has been performed on the mercury resistance operon from both gram-positive and gram-negative bacteria (3, 7). The extensive nature of prior studies into mercury resistance, its widespread prevalence among environmental species, and knowledge that the resistance is plasmid mediated suggests that mercury resistance would be an excellent genetic system to study in species of rapidly growing mycobacteria.

We have therefore undertaken to assess the prevalence of mercury resistance among eight taxa of rapidly growing mycobacteria and to demonstrate the presence of HgRe and organomercurial lyase as the mechanism of mercury resistance. Additionally, we have attempted to partially characterize the HgRe enzyme of rapidly growing mycobacteria.

(This work was presented in part at the Twenty-Second Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., May 1988 [19a].)

## MATERIALS AND METHODS

**Organisms.** Clinical isolates of rapidly growing mycobacteria submitted to the Mycobacteria/Nocardia Laboratory of the University of Texas Health Center at Tyler for antimicrobial susceptibility testing between 1982 and 1987 were randomly chosen. In addition, isolates obtained from surgical infections after augmentation mammaplasty (24) and cardiac bypass surgery (21) as previously reported were also included. Only single isolates were reported from apparent epidemics.

The control strains were *E. coli* AB1133 (mercury susceptible), *E. coli* AB1133(pR100) (narrow-spectrum mercury resistant), *S. aureus* ATCC 25923 (mercury susceptible), *S. aureus* RN860 (kindly provided by the Pasteur Institute; mercury resistant), *Mycobacterium scrofulaceum* W262C (mercury susceptible), and *M. scrofulaceum* W262 (mercury resistant). The last two isolates were kindly provided by J. Falkinham and were described previously (8).

Clinical isolates of rapidly growing mycobacteria were identified to species and appropriate subgroups by standard methods, many at the Mycobacterial Reference Section of the Centers for Disease Control (Atlanta, Ga.) (1, 18, 22).

**Mercury susceptibility testing.** Susceptibility testing to inorganic and organic Hg was performed by a disk diffusion method on Mueller-Hinton agar (MHA) plates (23). Samples of 20 μl of the solution of each compound were added to separate 6-mm paper disks. Mercury compounds tested included $HgCl_2$ ($1 \times 10^{-2}$ M), merbromin ($5 \times 10^{-3}$ M), *para*-chloromercuribenzoate (pCMB) ($5 \times 10^{-3}$ M), thimerosol ($2.5 \times 10^{-5}$ M), PMA ($5 \times 10^{-5}$ and $1 \times 10^{-5}$ M), and FMA ($5 \times 10^{-3}$ M). Zones of inhibition were measured after 72 h of incubation. Resistant and susceptible break-

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points were <20 and ≥25 mm, respectively, for $HgCl_2$, thimerosol, PMA, and FMA. For pCMB and merbromin the resistance breakpoint was ≤15 mm and the susceptibility breakpoint was ≥20 mm.

**HgRe activity.** HgRe was obtained by using whole-cell extracts of acetone-treated cells as previously described (2, 13). Briefly, organisms were grown in Mueller-Hinton broth (MHB) on a shaker at 140 rpm at 35°C for 3 to 7 days. $HgCl_2$ in a final concentration of 10, 25, or 100 μM was added as an inducer after 24 h of incubation. Cells were spun down, washed twice with acetone, and evaporated to dryness at 4°C. The cells were suspended in modified 0.05 M phosphate buffer (pH 7.0) (8) for 5 to 7 days at 4°C for extraction of HgRe. For organomercurial lyase extraction, the concentration of EDTA in this buffer was increased from 0.5 to 5.0 mM as recommended by Schottel (16) and Williams and Silver (26). The cells were then removed by centrifugation, and the supernatant (cell extract) was used as the enzyme source.

HgRe activity was determined by measuring the loss of radioactive $^{203}Hg$ from an assay mixture containing modified 0.05 M phosphate buffer (pH 7.0), 1 mM β-mercaptoethanol (β-ME) or 0.5 mM L-cysteine, 200 μM NADPH or NADH, and 0.5% 2-octanol. The 1.0-ml final volume contained 450 μl of enzyme preparation. $Hg^{2+}$ (4 μM) as $HgCl_2$ and $^{203}Hg(NO_3)_2$ (5 μCi/ml) was added as a substrate. Room air was slowly bubbled through the mixtures, which were held at 37°C in a water bath. Duplicate 25-μl samples were removed at timed intervals and added to 4.0 ml of scintillation counting fluid (BioSafe II; Research Products International Co., Mt. Pleasant, Ill.). The radioactivity remaining in the samples was then assayed by liquid scintillation spectroscopy on an LKB Rack Beta scintillation spectrometer (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). Assays were repeated an average of three to four times for each strain; some strains were assayed up to 11 times (range, 2 to 11 assays per strain).

For organomercurial lyase activity, we used a similar assay involving the same 0.05 M phosphate buffer modified by increasing the EDTA concentration to 5 mM. The substrate in this assay was $^{203}Hg$-labeled PMA at a concentration of 4 μM. The 1.4-ml reaction mixture included 0.5 mM L-cysteine, 200 μM NADPH, and 1.183 ml of cell extract as an enzyme source. At timed intervals, duplicate 100-μl samples were removed and added to 4.0 ml of scintillation counting fluid and assayed as noted above.

Protein concentrations in the enzyme preparations were measured by using the bicinchoninic acid reagent (Pierce Chemical Co., Rockford, Ill.) (19) on a Diode Array Spectrophotometer with the "Quant I" software package (Hewlett-Packard, Avondale, Pa.). Enzyme activity was expressed as nanomoles of mercury volatilized per minute per milligram of protein.

**HgRe heat stability.** Replicate 450-μl samples of each enzyme were held on ice and then exposed individually to designated temperatures for 10 min and replaced on ice. All samples were then held at −70°C until assayed for HgRe activity. Results are expressed as percentages based on the activity of the 22°C controls.

**Conjugation and transformation studies.** Conjugational transfer of mercury resistance was attempted with strains of *Mycobacterium fortuitum* biovar. *fortuitum* and *M. chelonae*-like organisms by using the membrane filter methods of Sasaki et al. (15). Recipient strains selected were doxycycline resistant ($Doxy^r$) and mercury susceptible ($Hg^s$), whereas donor strains were $Hg^r$ and $Doxy^s$. Cell suspensions of donor (approximately $1.5 \times 10^7$ CFU/ml) and recipient strains were mixed in ratios of 1:1, 1:5, and 1:10 in 2 ml of 0.05 M phosphate buffer (pH 7.0) and collected on a 0.22-μm-pore-size membrane filter (Milipore Corp., Bedford, Mass.). The cells were washed and placed on MHA plates and incubated at 30°C for 24 h. The filter membranes were then placed in test tubes, and the cells were washed free of the filters. Samples (100 μl) of each cell suspension were spread on triplicate MHA plates containing 10 mM $HgCl_2$ plus 10 μg of doxycycline per ml, 10 mM $HgCl_2$ alone, or 10 μg of doxycycline per ml alone. Recipient and donor cells were spread on plates of selective media like those used for spontaneous mutation controls.

For transformation studies, donor and recipient strains were cultured in MHB. After 24 to 72 h, glycine was added to *M. fortuitum* cultures to a final concentration of 1%, and D-cycloserine was added to *M. chelonae*-like organism cultures to a final concentration of 0.1 mg/ml. Cultures were incubated an additional 24 h, and the cells were harvested by centrifugation.

Donor $Hg^r$ and $Doxy^s$ cultures were used as sources of DNA, which was prepared as described by Shoemaker et al. (17). Recipient $Hg^s$ and $Doxy^r$ cultures were converted to spheroplasts for transformation as described by Jacobs et al. (6). After the transformation procedure, 100-μl samples of treated recipient spheroplasts were spread on triplicate plates of MHA, MHA supplemented with 100 μM $HgCl_2$, and MHA supplemented with 10 μg of doxycycline. Plates were evaluated for transformants after 7 days of incubation at 35°C.

TABLE 1. Incidence of $HgCl_2$ resistance among 356 clinical isolates of eight taxonomic groups of rapidly growing mycobacteria

| Species and subgroup | No. tested | No. resistant | % Resistant |
|---|---|---|---|
| *M. chelonae* | | | |
| Subspecies *chelonae* | 20 | 6 | 30 |
| Subspecies *abscessus* | 45 | 9 | 20 |
| *M. chelonae*-like organisms | 84 | 70 | 83 |
| *M. smegmatis* | 25 | 2 | 8 |
| *M. fortuitum* | | | |
| Third biovariant complex | 35 | 0 | 0 |
| Biovariant *peregrinum* | 25 | 7 | 28 |
| Biovariant *fortuitum* | | | |
| Breast strains | 24 | 11 | 46 |
| Cardiac bypass strains | 33 | 3 | 9 |
| Other strains | 64 | 11 | 17 |
| Unidentified species | 1 | 1 | 100 |

## RESULTS

**Susceptibility testing.** Three hundred fifty-six isolates belonging to eight different taxa were tested for susceptibility to inorganic mercury ($HgCl_2$). Resistance was identified in 35% of the isolates, with a range from 0% among the isolates of the unnamed third biovar complex of *M. fortuitum* to 83% among the isolates of *M. chelonae*-like organisms. The findings are summarized in Table 1. Results for 53 of the isolates of *M. fortuitum* biovar. *fortuitum* and the third biovariant complex have been previously reported (23). In addition, the results for the sternal isolates and 50 of the control strains ("other strains" in Table 1) were reported in

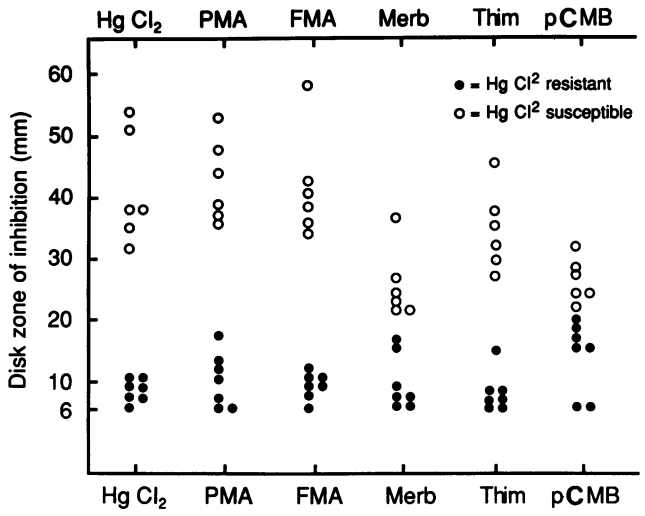


FIG. 1. Comparison of disk zone sizes with $HgCl_2$ and five organomercurial compounds of mercury-susceptible and -resistant strains of *M. fortuitum* biovar. *fortuitum*. Merb, merbromin; Thim, thimerosol.

an epidemiologic study of mycobacterial cardiac bypass surgical infections (21) and the results from the isolates from breasts in an epidemiologic study of augmentation mammaplasty surgical infections (24).

Twenty-nine strains that were resistant to inorganic mercury and 34 strains that were susceptible were tested for susceptibility to organic mercury compounds. Neither isolate of *Mycobacterium smegmatis* resistant to $HgCl_2$ was resistant to the organomercurial compounds. Of the remaining 27 mercury-resistant strains of six different taxa, all were resistant to organomercurial compounds, with a similar pattern that included resistance to PMA. None of the 34 strains susceptible to $HgCl_2$ were resistant to organomercurial compounds. A comparison of zone sizes for resistant and susceptible strains of *M. fortuitum* biovar. *fortuitum* is shown in Fig. 1.

**HgRe activity.** Enzyme activity in mercury-resistant control strains was associated with a volatilization rate of at least 0.2 nmol of Hg per min per mg of protein, whereas susceptible strains had activities of 0.1 nmol of Hg per min per mg of protein or less. Intermediate activities between these two values were seen in susceptible strains only when the protein concentration was very low.

With this definition, 17 of 17 strains of *M. fortuitum* biovar. *fortuitum* that were resistant to mercury by disk diffusion had HgRe activity comparable to that of the mercury-resistant bacterial control strains (mean mycobacterial volatilization rate of 0.48 nmol with a range of 0.2 to 1.27 nmol of Hg volatilized per min per mg of protein). Fourteen (82%) of these strains had activities that exceeded 0.3 nmol of Hg per min per mg of protein. Four strains susceptible to mercury by disk diffusion had enzyme activity of less than 0.1 nmol of Hg per min per mg of protein.

Similar results were obtained with most of the other species. Enzyme activity was detected in all 4 resistant strains of *M. fortuitum* biovar. *peregrinum*, all 4 strains of *M. chelonae* subsp. *chelonae*, all 4 strains of *M. chelonae* subsp. *abscessus*, the strain of unidentified rapidly growing mycobacteria, and all 10 strains of *M. chelonae*-like organisms.

TABLE 2. Comparative rates of $^{203}Hg^{2+}$ volatilization by whole-cell extracts of cultures grown in the presence (induced) or absence (uninduced) of 25 or 100 μM $HgCl_2$ as an inducer

| Species and strain | Rate of $^{203}Hg$ volatilization[a] (nmol/min/mg of protein) | | Increase in rate (fold) |
|---|---|---|---|
| | Uninduced | Induced | |
| *Escherichia coli* AB1133 | | | |
| $Hg^r$ (plasmid pR100) | 0.015 ± 0.007 | 0.69 ± 0.21 | 46 |
| $Hg^s$ | 0.02 ± 0.0 | —[b] | |
| *Staphylococcus aureus* | | | |
| $Hg^r$ (RN860) | 0.02 ± 0.02 | 0.32 ± 0.06 | 16 |
| $Hg^s$ (ATCC 25923) | 0.085 ± 0.007 | —[b] | |
| *M. scrofulaceum* | | | |
| $Hg^r$ (W262) | 0.0 ± 0.0 | 1.04 ± 0.68 | >104 |
| $Hg^s$ (W262C) | 0.07 ± 0.06 | —[b] | |
| *M. fortuitum* biovar. *fortuitum* | | | |
| $Hg^r$ (Mff250) | 0.015 ± 0.02 | 0.59 ± 0.31 | 39 |
| $Hg^s$ (TMC 1529) | 0.12 ± 0.02 | —[b] | |
| *M. fortuitum* biovar. *peregrinum* | | | |
| $Hg^r$ (Mfp394) | 0.0 ± 0.0 | 0.50 ± 0.17 | >50 |
| $Hg^s$ (Mfp33) | 0.04 ± 0.01 | —[b] | |
| *M. chelonae*-like organisms | | | |
| $Hg^r$ (Mo50) | 0.01 ± 0.002 | 0.68 ± 0.08 | 68 |
| $Hg^s$ (Mo32) | 0.02 ± 0.002 | —[b] | |
| *M. chelonae* subsp. *chelonae* | | | |
| $Hg^r$ (Mcc269) | 0.03 ± 0.02 | 0.61 ± 0.13 | 20 |
| $Hg^s$ (Mcc244) | 0.02 ± 0.02 | —[b] | |
| *M. chelonae* subsp. *abscessus* | | | |
| $Hg^r$ (Mca99) | 0.04 ± 0.02 | 0.71 ± 0.10 | 18 |
| $Hg^s$ (Mca375) | 0.0 ± 0.0 | —[b] | |
| Unidentified species ($Hg^r$) | 0.03 ± 0.01 | 0.22 ± 0.10 | 7 |

[a] Initial volatilization rate (mean ± standard deviation) after 5 min of exposure to the substrate in the presence of 200 μM NADPH and 1.0 mM β-ME.

[b] Unable to grow under inducing conditions, because the concentrations of $HgCl_2$ (25 to 100 μM) used for induction killed the cells.

HgRe activity could not be demonstrated in the two mercury-resistant isolates of *M. smegmatis*. These strains differed from mercury-resistant isolates of the other species in that they were not resistant by disk diffusion to any of the organomercurial compounds.

HgRe activity was inducible in all mycobacterial strains, as it was in the bacterial and *M. scrofulaceum* control strains. Essentially no activity was evident when the organism had not been grown in the presence of mercury. Volatilization rates with and without induction for mercury-susceptible and -resistant strains of the three control species and the six taxonomic groups of rapidly growing mycobacteria with HgRe activity are shown in Table 2. The enzyme

TABLE 3. Need for cofactors for Hg reductase activity in whole-cell extracts of *M. scrofulaceum* W-262 and strains of *M. fortuitum* biovar. *fortuitum* with and without predialysis of the enzyme[a]

| Addition to enzyme | Reaction rate (nmol of $^{203}Hg^{2+}$ volatilized/min/mg of protein) | | | | | |
|---|---|---|---|---|---|---|
| | *M. scrofulaceum* W262 | | *M. fortuitum* Mff250 | | *M. fortuitum* Mff267 | |
| | Dialysis | No dialysis | Dialysis | No dialysis | Dialysis | No dialysis |
| Nothing | <0.01 | 0.11 | 0.14 | 0.43 | 0.03 | 0.38 |
| 1.0 mM β-ME | <0.01 | <0.01 | 0.08 | 0.51 | 0.22 | 0.35 |
| 200 μM NADH | 0.07 | 1.11 | 0.62 | 0.47 | 0.60 | 1.02 |
| 200 μM NADPH | 0.03 | 1.48 | 0.51 | 0.41 | 0.22 | 0.45 |
| 200 μM NADH and 1.0 mM β-ME | 0.64 | 1.80 | 0.70 | 0.61 | 3.79 | 1.34 |
| 200 μM NADPH and 1.0 mM β-ME | 1.36 | 2.59 | 0.05 | 0.47 | 0.11 | 0.45 |

[a] With dialysis, one of the two strains of *M. fortuitum* (Mff267) and *M. scrofulaceum* W262 required both β-ME and an NAD hydrogen donor for optimal activity.

activities in all of the mercury-resistant strains were comparable.

HgRe activity in the rapidly growing mycobacteria generally required an electron donor (NADH or NADPH) and a thiol donor for activity, although dialysis of the cell extracts was often needed to show this requirement. With strain Mff250, e.g., the enzyme activity of nondialyzed cell extracts was the same whether NADPH and β-ME were added or not. After dialysis, however, enzyme activity was seen only after the addition of the electron donor. Similar results were seen with strain Mff267, but with the need for both NADH and β-ME for optimal activity. These results are compared with those for the control strain *M. scrofulaceum* W262 in Table 3. L-Cysteine gave similar results to β-ME when used as the thiol donor.

**HgRe heat stability.** To further characterize the HgRe enzymes of rapidly growing mycobacteria, the heat sensitivity range was studied. The pattern of heat stability of these mycobacterial HgRe resembles that of other gram-positive bacteria (e.g., *S. aureus*) in that it is relatively heat labile, although it exhibits some species differences. This activity differed from that of the HgRe of the gram-negative bacterium *E. coli*, which is heat stable and has activity at 80 to 90°C (12) (Table 4).

**Conjugation and transformation studies.** Both intergeneric and intrageneric matings between one $Hg^r$ strain of *M. fortuitum* biovar. *fortuitum* (Mff284) and two $Hg^r$ strains of *M. chelonae*-like organisms (Mo63 and Mo71) with five mercury-susceptible, doxycycline-resistant recipient strains of the same species by the membrane filter technique failed to provide any stable recombinants. A small number of colonies grew on the doxycycline-mercury plates, but on repeat transfer to the same selective medium these colonies either failed to grow or grew minimally. Transfer to nonselective medium resulted in recovery of rapidly growing doxycycline-resistant, mercury-susceptible colonies.

Transfer of mercury resistance by transformation to mercury-susceptible recipient spheroplasts was unsuccessful.

TABLE 4. Heat stability patterns of mycobacterial and bacterial mercuric reductase[a]

| Temp (°C) | % Enzyme activity | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 22 | 100 | 100 | 100 | 100 |
| 50 | 86 | 96 | 98 | 92 |
| 60 | 2 | 93 | 100 | 98 |
| 70 | 0 | 4 | 72 | 100 |
| 80 | 0 | 0 | 0 | 39 |
| 90 | 0 | 0 | 0 | 13 |

[a] Enzyme activity is defined as nanomoles of Hg volatilized (given here as percentages of 22°C control values) after 10 min of incubation at the indicated temperature. Organisms showing pattern A enzyme activity were *S. aureus* RN860, *M. fortuitum* biovar. *fortuitum* Mff244, and *M. fortuitum* biovar. *peregrinum* Mfp394. Those showing pattern B activity were *M. scrofulaceum* W262 and *M. chelonae* subsp. *abscessus* Mca139. *M. chelonae*-like organism strain Mo50 showed pattern C activity, and *E. coli* AB1133(pR100) showed pattern D activity.

## DISCUSSION

These results demonstrate that mercury resistance in rapidly growing mycobacteria, with the exception of that in strains of *M. smegmatis*, appears to be due to the presence of HgRe. This enzyme is inducible and generally requires an electron donor and a thiol donor for optimal activity. These HgRe enzymes also exhibit a trimodal heat sensitivity pattern, with loss of activity between 60 and 80°C. Thus the mechanism of mercury resistance appears to be the same as has been defined in numerous other gram-positive bacterial species (12, 16).

All of the isolates of rapidly growing mycobacteria with HgRe activity exhibited broad-range resistance to organomercurial compounds, including PMA, FMA, pCMB, merbromin, and thimerosal. This suggested that these strains also contained organomercurial lyase. Efforts to demonstrate $Hg^0$ volatilization from the organomercurial compound PMA were equivocal; hence the mechanism of organomercurial detoxification is not known. Strains of *E. coli* that contain only HgRe are resistant to merbromin and FMA but are unable to volatilize organomercurial compounds. Strains of *Pseudomonas aeruginosa* with only HgRe are resistant to merbromin and FMA as well as pCMB but are also unable to hydrolyze them (20). The mechanism for this resistance is unknown. No bacterial isolates with HgRe activity only (narrow-spectrum resistance) have been shown to be resistant to PMA. Resistance to PMA in the rapidly growing mycobacteria suggested that organomercurial lyase activity was also present.

Because of the location of the mercury resistance genes on plasmids or transposons, mercury resistance among most bacterial species is readily transferable. However, we were unable to transfer mercury resistance among strains of *M. fortuitum* or *M. chelonae*-like organisms. The resistance appears to be highly stable, since all attempts to produce loss of this determinant by multiple passaging or with curing agents have been unsuccessful (23).

Previous attempts at mating of auxotrophs of *M. smegma-*

*tis* with presumed chromosomal determinants have been done by Mizuguchi et al. (9). However, they noted several mating types, and only recombinant experiments between compatible strains were successful. It is not known whether similar mating types are present in *M. fortuitum* and *M. chelonae*-like organisms and what effect this would have on attempted conjugal or transformational DNA exchange.

Mercury resistance is clinically important, since mercury-containing compounds (e.g., merbromin) are still used as topical antiseptics and at least one nosocomial outbreak of *M. chelonae* disease has been reported due to a contaminated solution of merbromin used in presurgical care (5).

Epidemiologic studies of rapidly growing mycobacteria for a given species have suffered from a lack of phenotypic markers. Epidemic nosocomial disease involving sternal wound infection (21), augmentation mammaplasty (24), and respiratory tract disease and dialysis (1) has been reported for *M. fortuitum*, *M. chelonae*, and *M. chelonae*-like organisms. The variable incidence of mercury resistance (approximately 20%) for the first two species makes it a potentially useful phenotypic marker in epidemiologic studies, and it has helped in the separation or clustering of strains involved in such outbreaks. Isolates from the large 1976 North Carolina sternal wound outbreak involving *M. chelonae* subsp. *abscessus* were all mercury resistant, strongly suggesting that a single strain was involved in the outbreak (21).

Mercury resistance in rapidly growing mycobacteria appears to be mediated by HgRe, which thereby can serve as a phenotypic marker in epidemiologic studies of outbreaks of rapidly growing mycobacterial infections.

## ACKNOWLEDGMENTS

We thank Keitha Jackson and Dorotha Harper for their clerical expertise and attention to detail in the preparation of the manuscript.

This study was funded in part by a research grant from the University of Texas.

## REFERENCES

1. **Band, J. D., J. I. Ward, D. W. Fraser, N. J. Peterson, V. A. Silcox, R. C. Good, P. R. Ostroy, and J. Kennedy.** 1982. Peritonitis due to a *Mycobacterium chelonei*-like organism associated with intermittent chronic peritoneal dialysis. J. Infect. Dis. **145**:9–17.
2. **Bhaduri, S., and P. H. Demchich.** 1983. Simple and rapid method for disruption of bacteria for protein studies. Appl. Environ. Microbiol. **46**:941–943.
3. **Brown, N. L., T. K. Misra, J. N. Winnie, A. Schmidt, M. Seiff, and S. Silver.** 1986. The nucleotide sequence of the mercuric resistance operons of plasmid R100 and transposon Tn501: further evidence for *mer* genes which enhance the activity of the mercuric ion detoxification system. Mol. Gen. Genet. **202**:143–151.
4. **Falkinham, J. O., III, K. L. George, B. C. Parker, and H. Gruft.** 1984. In vitro susceptibility of human and environmental isolates of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum* to heavy-metal salts and oxyanions. Antimicrob. Agents Chemother. **25**:137–139.
5. **Foz, A., C. Roy, J. Jurado, E. Arteaga, J. M. Ruiz, and A. Moragas.** 1978. *Mycobacterium chelonei* iatrogenic infections. J. Clin. Microbiol. **7**:319–321.
6. **Jacobs, W. R., Jr., M. Tuckman, and B. R. Bloom.** 1987. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. Nature (London) **327**:532–534.
7. **Laddaga, R. A., L. Chu, T. K. Misra, and S. Silver.** 1987. Nucleotide sequence and expression of the mercurial-resistance operon from *Staphylococcus aureus* plasmid pI258. Proc. Natl. Acad. Sci. USA **84**:5106–5110.
8. **Meissner, P. S., and J. O. Falkinham III.** 1984. Plasmid-encoded mercuric reductase in *Mycobacterium scrofulaceum*. J. Bacteriol. **157**:669–672.
9. **Mizuguchi, Y., K. Suga, and T. Tokunaga.** 1976. Multiple mating types of *Mycobacterium smegmatis*. Jpn. J. Microbiol. **20**:435–443.
10. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Mitsuhashi.** 1977. Mercury resistance and R plasmids in *Escherichia coli* isolated from clinical lesions in Japan. Antimicrob. Agents Chemother. **11**:999–1003.
11. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, and S. Mitsuhashi.** 1977. Frequency of heavy-metal resistance in bacteria from inpatients in Japan. Nature (London) **266**:165–167.
12. **Nakahara, H., J. L. Schottel, T. Yamada, Y. Miyakawa, M. Asakawa, J. Harville, and S. Silver.** 1985. Mercuric reductase enzymes from *Streptomyces* species and Group B Streptococcus. J. Gen. Microbiol. **141**:1053–1059.
13. **Nash, D. R., R. J. Wallace, Jr., V. A. Steingrube, and P. A. Shurin.** 1986. Isoelectric focusing of β-lactamases from sputum and middle ear isolates of *Branhamella catarrhalis* recovered in the United States. Drugs **31**(Suppl. 3):48–54.
14. **Robinson, J. B., and O. H. Tuovinen.** 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analysis. Microbiol. Rev. **48**:95–124.
15. **Sasaki, Y., N. Taketomo, and T. Sasaki.** 1988. Factors affecting transfer frequency of pAMβ1 from *Streptococcus faecalis* to *Lactobacillus plantarum*. J. Bacteriol. **170**:5939–5942.
16. **Schottel, J. L.** 1978. The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of *Escherichia coli*. J. Biol. Chem. **253**:4341–4349.
17. **Shoemaker, S. A., J. H. Fisher, W. D. Jones, Jr., and C. H. Scoggin.** 1986. Restriction fragment analysis of chromosomal DNA defines different strains of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **134**:210–213.
18. **Silcox, V. A., R. C. Good, and M. M. Floyd.** 1981. Identification of clinically significant *Mycobacterium fortuitum* complex isolates. J. Clin. Microbiol. **14**:686–691.
19. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. J. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**:76–85.

19a. **Steingrube, V. A., R. J. Wallace, Jr., L. S. Steele, and D. R. Nash.** 1988. Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, abstr. U-17, p. 135.

20. **Summers, A. O., and S. Silver.** 1978. Microbial transformation of metals. Am. Rev. Microbiol. **32**:637–672.
21. **Wallace, R. J., Jr., J. M. Musser, S. I. Hull, V. A. Silcox, L. C. Steele, G. D. Forrester, A. Labidi, and R. K. Selander.** 1989. Diversity and sources of rapidly growing mycobacteria associated with infections following cardiac bypass surgery. J. Infect. Dis. **159**:708–716.
22. **Wallace, R. J., Jr., D. R. Nash, M. Tsukamura, Z. M. Blacklock, and V. A. Silcox.** 1988. Human disease due to *Mycobacterium smegmatis*. J. Infect. Dis. **158**:52–59.
23. **Wallace, R. J., Jr., L. C. Steele, G. D. Forrester, J. M. Swenson, and S. I. Hull.** 1984. Susceptibilities of *Mycobacterium fortuitum* biovariant *fortuitum* and the unnamed third biovariant complex to heavy-metal salts. Antimicrob. Agents Chemother. **26**:594–596.
24. **Wallace, R. J., Jr., L. C. Steele, A. Labidi, and V. A. Silcox.** 1989. Heterogeneity among isolates of rapidly growing mycobacteria responsible for infections following augmentation mammaplasty despite case clustering in Texas and other southern coastal states. J. Infect. Dis. **160**:281–288.
25. **Weiss, A. A., S. D. Murphy, and S. Silver.** 1977. Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. J. Bacteriol. **132**:197–208.
26. **Williams, J. W., and S. Silver.** 1984. Bacterial resistance and detoxification of heavy metals. Enzyme Microb. Technol. **6**:530–537.
27. **Witte, W., L. Green, T. K. Misra, and S. Silver.** 1986. Resistance to mercury and to cadmium in chromosomally resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29**: 663–669.