

Resistance to Mercury and Antimicrobial Agents in *Streptococcus mutans* Isolates from Human Subjects in Relation to Exposure to Dental Amalgam Fillings

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Resistance to cefuroxime, penicillin, tetracycline, and mercury is reported for 839 *Streptococcus mutans* isolates from 209 human study subjects. The MICs of these drugs did not differ for isolates from one dental amalgam group and two nonamalgam subsets: a group with no known exposure to amalgam and a group whose members had their amalgam fillings removed.

Amalgam fillings contain approximately 50% mercury (19). Mainly due to the mercury, dental amalgam has antibacterial properties (14, 15). Different genes for antimicrobial resistance are often genetically linked and spread together; selection pressure from a single agent can promote multiple resistance. Heavy-metal ion resistance (for example, against mercury [Hg], cadmium, and silver) has been found together with antimicrobial resistance determinants (2, 6, 13, 21, 24). It has been suggested that the environmental load of mercury, including that released from amalgam fillings, may promote and maintain antimicrobial resistance together with mercury resistance in human normal flora (20). The threat of pathogens acquiring this resistance is always present; one example is the transfer of penicillin resistance genes from oral streptococci to *Streptococcus pneumoniae* (3).

The oral streptococcus *Streptococcus mutans* is considered one of the most important cariogenic species of the human microbial flora (12). The suppression of *S. mutans* by antimicrobial agents, especially by locally administered chlorhexidine, is consequently of clinical importance (11, 17, 23). Chlorhexidine reduces the rate of caries significantly (11).

Oral bacteria might respond to mercury from dental amalgam by developing resistance. If they simultaneously develop resistance to other antimicrobial agents, then the use of mercury-containing dental amalgam would have to be reconsidered. The effect of dental amalgam on antimicrobial resistance has not been sufficiently studied in human populations.

In the present study, we have examined *S. mutans* to find the possible differences in mercury and antimicrobial resistance in oral flora among three adult human groups: a group whose members had had all amalgam fillings removed (designated the NAR group; $n = 62$, mean age 50 years, range 31 to 72 years), a group that had never been exposed to dental amalgam fillings (the NA group; $n = 48$, mean age 23 years, range 18 to 65 years), and a group having various numbers of amalgam fillings (the A group; $n = 99$, mean age 48 years, range 19 to 83 years).

We collected paraffin-stimulated whole saliva samples (5 ml) from 209 human study subjects. The fresh saliva samples were cultured within 1 h of arrival at the laboratory. The samples

were diluted 1:100 in physiological saline, and 20 μ l of this dilution was plated onto mitis salivarius agar, composed of mitis salivarius agar base (Difco Laboratories, Detroit, Mich.), 1% potassium tellurite (Merck OY, Espoo, Finland), 15% sucrose, and 0.1 U of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml.

Plates were incubated for 3 days at 35°C in a 5% CO₂ atmosphere to facilitate identification. Judging by colony appearance, approximately four colonies were picked. Dark, rough *S. mutans*-like colonies (5, 8) were identified as mutans streptococci (7).

Susceptibility to the following agents was tested: HgCl₂ (MIC, 2 to 128 μ g/ml; Merck Oy), chlorhexidine diacetate (MIC, 0.25 to 16 μ g/ml; Fluka BioChemica, Bushs, Switzerland), cefuroxime (MIC, 0.063 to 16 μ g/ml; Sigma), benzylpenicillin (MIC, 0.008 to 2 μ g/ml; Sigma), and tetracycline (MIC, 0.063 to 32 μ g/ml; Sigma).

The guidelines of the National Committee for Clinical Laboratory Standards were used for the agar dilution as described earlier (8), with the exceptions mentioned below. Preliminary tests showed that mitis salivarius agar without any supplements had to be used when testing mercury; Mueller-Hinton II agar (Becton Dickinson and Company, Cockeysville, Md.) supplemented with blood could not be used, since mercury reacts with the blood.

Bacteria were cultured on mitis salivarius agar with doubling concentrations of mercury chloride and on Mueller-Hinton agar supplemented with 5% sheep blood and doubling concentrations of the other antimicrobial agents. The plates were incubated at 35°C in a 5% CO₂ atmosphere and read after 24 h.

Perhaps surprisingly, we found no differences in the MICs of the studied antimicrobial agents between the samples of the three subject groups (Table 1). The antimicrobial resistance profiles for chlorhexidine, cefuroxime, penicillin, and tetracycline are in line with the results of our previous study (8). Similar levels of resistance have also been found by Baker and Thornsberry (1) and Teng et al. (22) (1998) for penicillin and by Liebana et al. (10) for penicillin, cefuroxime, and tetracycline.

In conclusion, mercury derived from dental amalgam fillings did not select resistant *S. mutans* strains. Based on these results, we can speculate on at least three topics. First, the amounts of mercury found in saliva might not be high enough to cause any selection pressure on *S. mutans*. Second, certain protective

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TABLE 1. In vitro susceptibilities of 839 clinical isolates of mutans streptococci to mercury and 279 consecutive isolates of these to four other antimicrobial agents

Antimicrobial agent	Group ^a											
	A			NA			NAR					
	No. of isolates	MIC (µg/ml)			No. of isolates	MIC (µg/ml)			No. of isolates	MIC (µg/ml)		
	Range	50%	90%		Range	50%	90%		Range	50%	90%	
Mercury(II) chloride	455	4–16	16	16	150	16–32	16	16	234	8–32	16	32
Chlorhexidine	166	≤0.25–4	0.5	1	57	≤0.25–4	0.5	1	56	≤0.25–4	0.5	1
Cefuroxime	166	≤0.063–0.5	≤0.063	≤0.063	57	≤0.063–0.5	≤0.063	≤0.063	56	≤0.063	≤0.063	≤0.063
Penicillin	166	0.032–0.063	0.032	0.032	57	0.032–0.063	0.032	0.032	56	0.016–0.063	0.032	0.032
Tetracycline	166	0.125–1	1	1	57	0.25–1	1	1	56	0.25–1	1	1

^a A, amalgam fillings in mouth; NA, no known exposure to dental amalgam; NAR, amalgam fillings removed.

factors—which exist also in human saliva—have been found that are able to influence mercury resistance, especially in gram-negative bacteria. It seems that Ca²⁺ and Mg²⁺ ions can directly protect at least gram-negative cells from the toxic effects of Hg (4). Third, it is also known that the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine), which is widely present in cells, can increase cellular resistance to mercury ions in the gram-negative *Escherichia coli* (9). We are not aware of any corresponding studies of gram-positive bacteria, but *S. mutans* is known to import glutathione (18). Thus, it may be speculated that the agents mentioned can also protect gram-positive bacteria against mercury. Although exposure to mercury from dental amalgam did not select for resistant strains of *S. mutans*, the situation may be different in other streptococci and gram-positive oral genera. In gram-negative bacteria, the results are contradictory (16, 20). Further systematic studies of multifactorial causal complexes of mercury and antimicrobial resistance in bacteria are needed.

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REFERENCES

- Baker, C. N., and C. Thornsberry. 1974. Antimicrobial susceptibility of *Streptococcus mutans* isolated from patients with endocarditis. *Antimicrob. Agents Chemother.* **5**:268–271.
- De La Cruz, F., and J. Grinsted. 1982. Genetic and molecular characterization of Tn21, a multiple resistance transposon from R100.1. *J. Bacteriol.* **151**: 222–228.
- Dowson, C. G., T. J. Coffey, C. Kell, and R. A. Whiley. 1993. Evolution of penicillin resistance in *Streptococcus pneumoniae*; the role of *Streptococcus mitis* in the formation of a low affinity PBP2B in *S. pneumoniae*. *Mol. Microbiol.* **9**:635–643.
- Farrel, R. E., J. J. Germida, and P. Ming Huang. 1993. Effects of chemical speciation in growth media on the toxicity of mercury(II). *Appl. Environ. Microbiol.* **59**:1507–1514.
- Fujiwara, T., E. Sasada, N. Mima, and T. Ooshima. 1991. Caries prevalence and salivary mutans streptococci in 0-2-year-old children of Japan. *Community Dent. Oral Epidemiol.* **19**:151–154.
- Heikkilä, E., M. Skurnik, L. Sundström, and P. Huovinen. 1993. A novel dihydrofolate reductase cassette inserted in an integron borne on a Tn21-like element. *Antimicrob. Agents Chemother.* **37**:1297–1304.
- Järvinen, H., K. Pienihäkkinen, P. Huovinen, and J. Tenouvo. 1995. Susceptibility of *Streptococcus mutans* and *Streptococcus sobrinus* to antimicrobial agents after short-term oral chlorhexidine treatments. *Eur. J. Oral Sci.* **103**: 32–35.
- Järvinen, H., J. Tenouvo, and P. Huovinen. 1993. In vitro susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents. *Antimicrob. Agents Chemother.* **37**:1158–1159.
- Latinwo, L. M., C. Donald, C. Ikediobi, and S. Silver. 1998. Effects of intracellular glutathione on sensitivity of *Escherichia coli* to mercury and arsenite. *Biochem. Biophys. Res. Commun.* **242**:67–70.
- Liebana, J., A. Castillo, J. Peis, P. Baca, and G. Piedrola. 1991. Antimicrobial susceptibility of 1042 strains of *Streptococcus mutans* and *Streptococcus sobrinus*: comparison from 1985 to 1989. *Oral Microbiol. Immunol.* **6**:146–150.
- Lindqvist, B., S. Edward, P. Torell, and B. Krasse. 1989. Effect of different caries preventive measures in children highly infected with mutans streptococci. *Scand. J. Dent. Res.* **97**:330–337.
- Loesche, W. J. 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **50**:353–380.
- Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Silver. 1977. Linkage of mercury, cadmium, and arsenate and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **33**: 975–976.
- Nunez, L. J., G. Schmalz, J. Hembree, and L. D. Hulett. 1976. Influence of amalgam, alloy, and mercury on the in vitro growth of *Streptococcus mutans*. II. Comparison of amalgams and alloys. *J. Dent. Res.* **55**:893–899.
- Ørstavik, D. 1985. Antibacterial properties of and element release from some dental amalgams. *Acta Odontol. Scand.* **43**:231–239.
- Österblad, M., J. Leisteuvo, T. Leisteuvo, H. Järvinen, L. Pyy, J. Tenouvo, and P. Huovinen. 1995. Antimicrobial and mercury resistance in aerobic gram-negative bacilli in fecal flora among persons with and without dental amalgam fillings. *Antimicrob. Agents Chemother.* **39**:2499–2502.
- Rask, P.-I., C. G. Emilson, B. Krasse, and H. Sundberg. 1988. Effect of preventive measures in 50-60-year-olds with a high risk of dental caries. *Scand. J. Dent. Res.* **96**:500–504.
- Sherrill, C., and R. C. Fahey. 1998. Import and metabolism of glutathione by *Streptococcus mutans*. *J. Bacteriol.* **180**:1454–1459.
- Skinner, E. W., and R. W. Phillips. 1969. The science of dental materials, 6th ed., p. 303 and 332. W. B. Saunders Co., Philadelphia, Pa.
- Summers, A. O., J. Wireman, M. J. Vimy, F. L. Lorscheider, B. Marshall, S. B. Levy, S. Bennett, and L. Billard. 1993. Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. *Antimicrob. Agents Chemother.* **37**:825–834.
- Tanaka, M., T. Jamamoto, and T. Sawai. 1983. Evolution of complex resistance transposons from an ancestral mercury transposon. *J. Bacteriol.* **153**: 1432–1438.
- Teng, L.-J., P.-R. Hsueh, Y.-C. Chen, S.-W. Ho, and K.-T. Luh. 1998. Antimicrobial susceptibility of viridans group streptococci in Taiwan with emphasis on the high rates of resistance to penicillin and macrolides in *Streptococcus oralis*. *J. Antimicrob. Chemother.* **41**:621–627.
- Tenouvo, J., P. Häkkinen, P. Paunio, and C. G. Emilson. 1992. Effects of chlorhexidine-fluoride gel treatments in mothers on the establishment of mutans streptococci in primary teeth and development of dental caries in children. *Caries Res.* **88**:236–243.
- Wireman, J., C. A. Liebert, T. Smith, and A. O. Summers. 1997. Association of mercury resistance with antibiotic resistance in gram-negative fecal bacteria of primates. *Appl. Environ. Microbiol.* **63**:4494–4503.