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

JORMA LEISTEVUO,[1,2]* HELINÄ JÄRVINEN,[1] MONICA ÖSTERBLAD,[1] TIINA LEISTEVUO,[1,3] PENTTI HUOVINEN,[1] AND JORMA TENOVUO[2]

National Public Health Institute, Antimicrobial Research Laboratory,[1] and Department of Physical Medicine and Rehabilitation, Turku University Hospital,[3] FIN-20521 Turku, and Institute of Dentistry, Turku University, FIN-20520 Turku,[2] Finland

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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 administrated 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 μl 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% CO_2 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: $HgCl_2$ (MIC, 2 to 128 μg/ml; Merck Oy), chlorhexidine diacetate (MIC, 0.25 to 16 μg/ml; Fluka BioChemica, Bushs, Switzerland), cefuroxime (MIC, 0.063 to 16 μg/ml; Sigma), benzylpenicillin (MIC, 0.008 to 2 μg/ml; Sigma), and tetracycline (MIC, 0.063 to 32 μg/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% CO_2 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

* Corresponding author. Mailing address: National Public Health Institute, Antimicrobial Research Laboratory, P.O. Box 57, FIN-20521 Turku, Finland. Phone: 358-2-2519-255. Fax: 358-2-2519-254.

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 Ca^{2+} and Mg^{2+} 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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