Resistance of the Normal Human Microflora to Mercury and Antimicrobials After Exposure to Mercury from Dental Amalgam Fillings

Charlotta Edlund, Lars Björkman, Jan Ekstrand, Gunilla Sandborgh-Englund, and Carl Erik Nord

The concentrations of mercury in saliva and feces and the resistance pattern of the gastrointestinal microflora were investigated for 20 subjects. Ten patients, with a mean number of 19 amalgam surfaces, had all amalgam fillings removed during one dental session. Ten subjects without amalgam fillings served as a control group. Saliva and fecal samples were collected before amalgam removal and 2, 7, 14, and 60 days afterward. Mercury levels in saliva and feces correlated significantly with the number of amalgam surfaces. No differences in the resistance pattern of the oral microflora were detected between the two groups. In the amalgam group there was an increase in the relative number of intestinal microorganisms resistant to mercury, ampicillin, cefoxitin, erythromycin, and clindamycin on days 7–14. This was not statistically significant in light of the normal variations of the control group. A significant correlation between the prevalence of mercury resistance and multiple antimicrobial resistance in intestinal bacterial strains was observed.

The normal human oral and intestinal microflora harbors a huge potential reservoir of resistant microorganisms that may cause infections at other sites of the body. Bacteria from the intestinal microflora dominate as causative agents in postoperative infections, and antimicrobial resistance in the microflora may seriously complicate the prophylaxis and treatment of intraabdominal infections [1, 2]. Previous studies have shown a correlation between consumption of antimicrobial agents and the emergence of drug resistance among bacteria in the normal intestinal microflora of outpatients and hospitalized patients [1–7]. However, this correlation is not strong enough to be the only factor promoting the spread of antimicrobial resistance.

Other factors in the environment, apart from antimicrobial agents, may have an impact on the resistance pattern. It has been shown that the use of organomercurials in liquid detergents and disinfectants promoted resistance to mercury among microorganisms in hospital settings [8]. The mechanism of mercury resistance is described as an enzymatic detoxification process of the reactive form Hg\(^{2+}\) to the less reactive form Hg\(^0\) [9]. Since mercury resistance occurs on transferable resistance elements that also carry antibiotic resistance genes [10–12], it may be possible that environmental mercury—including low doses derived from dental amalgam—could promote the emergence and spread of mercury resistance and antimicrobial resistance in the normal human microflora. Summers et al. [12] have shown that mercury released from amalgam fillings can cause enrichment of mercury resistance plasmids in the normal microflora of primates. This may be associated with an increased prevalence of antibiotic resistance in the normal microflora.

The aims of the present investigation were (1) to study mercury concentrations in saliva and fecal samples from patients after mercury exposure due to removal of dental amalgam, (2) to study the resistance patterns in the oral and intestinal microflora after mercury exposure, compared to such patterns in a control group without any history of amalgam fillings, and (3) to study the relationship between mercury resistance and antimicrobial resistance in isolated intestinal bacterial strains.

Materials and Methods

Patients. Twenty healthy patients who had not taken any antimicrobial agents during the previous 3 months participated in the study. Intake of fish of any kind was not allowed 1 month prior to and during the experimental period. The “amalgam group” consisted of 10 patients, 7 women and 3 men (mean age, 38 years; range, 26–52 years), with a mean of 19 amalgam surfaces (range, 13–34) on their teeth. The control group consisted of 10 subjects, 7 women and 3 men (mean age, 20 years; range, 18–23 years), without any present or previous amalgam fillings. The study was approved by the Ethics Committee of Huddinge University Hospital, at the Karolinska Institute (Stockholm). The subjects in the amalgam group had all their amalgam fillings removed during a single dental session. The fillings were replaced with composites, cast gold crowns, and inlays.

Sampling procedures. In both groups 6 saliva samples and 6 fecal samples were collected from each subject: 2 baseline samples were collected during the week before the removal of amalgam fillings (day 0), and the other 4 were collected 2, 7,
14, and 60 days after the exposure to mercury. The saliva samples were collected in sterile plastic tubes (free from mercury) in the morning, before breakfast and toothbrushing. Fecal specimens were collected in plastic bags and sterile plastic containers. All samples were homogenized, and aliquots for mercury analysis and microbiological analysis were frozen separately at −70°C until assayed.

**Mercury analysis.** The total concentration of mercury in saliva was analyzed by cold vapor atomic absorption spectrometry (AAS), after addition of a mixture of CdCl₂ and SnCl₂ [13]. The saliva samples were solubilized in sodium hydroxide prior to analysis [14]. Total mercury in feces was analyzed in duplicate after acid digestion in polytetrafluoroethylene vessels by nitric acid in a microwave oven. After digestion, mercury concentration in the samples was analyzed with use of a hydride-generation system (FIAS 200, Perkin Elmer, Norwalk, CT) and AAS (Perkin Elmer 3100). As reductant, 5-mM sodium tetrahydroborate was used, and the carrier solution was 0.1-M HCl.

**Microbial procedures.** The saliva and stool specimens were suspended in prereduced peptone–yeast extract medium, diluted 10-fold to 10⁻⁷ and inoculated with an autotipette on selective media (blood agar, cystine-lactose-electrolyte-deficient agar, esculin agar, Sabouraud dextrose agar, kanamycin-vancmycin-blood agar, neomycin-vancmycin-blood agar, egg yolk–neomycin agar, Clostridium difficile–selective agar, Veillonella-selective agar, Rogosa selective Lactobacillus agar, and Bifidobacterium-selective agar), as described by Heimdahl and Nord [15]. In addition, the different dilutions were inoculated on agar plates containing 5% defibrinated horse blood and 50-μM HgCl₂ (equivalent to 10.0 μg of Hg per mL) and on Antibiotic Sensitivity Medium II agar plates (AB BIODISK, Solna, Sweden) containing 5% defibrinated horse blood and seven different antimicrobial agents at defined concentrations.

The antimicrobial agents and the corresponding breakpoints used were as follows: ampicillin (Astra, Södertälje, Sweden), 16 μg/mL; cefuroxime (Glaxo, Greenford, Middlesex, United Kingdom), 16 μg/mL; cefoxitin (Merck Sharp & Dohme International, Rahway, NJ), 16 μg/mL; doxycycline (Pfizer, Brussels, Belgium), 8 μg/mL; erythromycin (Astra), 4 μg/mL; clindamycin (Upjohn, Kalamazoo, MI), 2 μg/mL; and chloramphenicol (Parke-Davis Pharmaceutical Research Division, Warner Lambert, Ann Arbor, MI), 16 μg/mL. The breakpoints were chosen according to the National Committee for Clinical Laboratory Standards (NCCLS) [16, 17]. The aerobic agar plates were incubated for 24 hours at 37°C, and anaerobic plates were incubated for 48 hours at 37°C in anaerobic jars (Gas Pak, BBL, Cockeysville, MD). Hg media were incubated both aerobically and anaerobically, and the antimicrobial media were incubated anaerobically.

After incubation, different colony types were counted, isolated in pure culture, and identified to the genus level by morphological, biochemical, and gas-chromatographic analysis. The total numbers of aerobic and anaerobic saliva and intestinal microorganisms were determined, and the microorganisms from mercury agar and the different antimicrobial media were enumerated. The microorganisms were counted from plates with 20–200 cfu. The coefficient of variation of cfu obtained from duplicate agar plates that were inoculated with feces from a specific dilution was 8%. All resistant strains were quantified in relation to the total number of anaerobic microorganisms, and the percentage of microorganisms resistant to 50-μM HgCl₂ and other antimicrobial media was analyzed for each sample. Differences in numbers (i.e., total counts or counts of a particular microorganism) of ≥2 log₁₀ were considered to represent a significant change.

**Minimum inhibitory concentrations.** The MICs of the different antimicrobial agents were determined for all isolated intestinal Escherichia coli, enterococci, and Bacteroides strains by the agar dilution method and use of Antibiotic Sensitivity Medium II and an inoculum of 10⁶ cfu/mL. Control strains used were Enterococcus faecalis American Type Culture Collection (ATCC) 29212, E. coli ATCC 25922, and Bacteroides fragilis ATCC 25285. The susceptibility to 5-, 10-, 25-, 50-, and 100-μM HgCl₂ was also analyzed for these strains, with use of E. coli ATCC 25922 (MIC, 25-μM Hg) as a control strain. An inoculum of 10⁶ cfu/mL was shown to be relevant for determination of mercury resistance. Inoculation of ≥10⁵ cfu/mL resulted in false resistance due to the inoculum effect. For the aerobic strains the breakpoint of HgCl₂ was >50 μg/mL, chosen according to previous studies [12]. For the anaerobic Bacteroides strains, the breakpoint of HgCl₂ was set to >25 μg/mL.

**Statistical analyses.** Data on microbial resistance to mercury and antimicrobial agents were related to the total number of cfu, and the percentages of microflora resistant to mercury and antimicrobial agents were calculated. Generally these distributions were skewed, and thus data was log-transformed. Analysis of variance with a repeated-measures design was used to estimate the significance of the two independent factors (group and time) and the interaction term between these factors. By inclusion of the control group in the analyses, the normal variation was taken into consideration. Data on mercury concentrations in saliva and fecal samples were analyzed by Wilcoxon’s signed rank test for paired samples and the Mann-Whitney U test. Mercury resistance and antimicrobial resistance in isolated bacterial strains were analyzed by the χ² test.

**Results.**

**Mercury concentrations in saliva.** In the amalgam group, median values (range) of mercury concentrations in saliva on days 0, 7, and 60 were 31 (3–207), 4 (1–78) and 0.2 (<0.1–1) ng/g, respectively. Mercury concentrations in saliva at all sampling periods were significantly related to the number of amalgam surfaces, as recorded at the first dental visit (P < .05). The median value (range) of mercury concentrations in saliva from the control group on day 0 was 0.02 (<0.02–2)
ng/g. There was a statistically significant difference between mercury levels of the amalgam group on days 0 and 7, respectively, vs. mercury levels in the control group on day 0 ($P < .001$). Within the amalgam group, there was a significant decrease of mercury levels in saliva samples collected on days 0 and 7, compared with those on day 60 ($P < .001$).

Mercury concentrations in feces. The median levels of mercury in fecal samples collected on days 0, 7, and 60 in the amalgam group were 120, 370, and 20 ng/g (wet weight of feces); on day 0 in the control group, the median level was 11 ng/g (figure 1). There were statistically significant higher mercury levels in feces collected from the amalgam group on days 0, 7, and 60 than in feces from the control group on day 0 ($P < .001$, $P < .001$, and $P < .05$, respectively). Within the amalgam group, there was a significant decrease in intestinal mercury levels from day 0 to day 60, as well as from day 7 to day 60 ($P < .001$). Mercury concentrations in feces collected before the amalgam removal correlated significantly to the number of amalgam surfaces ($P < .05$).

Microbial analyses. Three sampling periods were investigated, as follows. Mean values were determined for the two baseline samples (week 0), the samples collected 7 and 14 days after removal of amalgam (week 2), and the samples collected 2 months after mercury exposure (week 9). The compositions of the saliva and intestinal microflora of both groups were within the normal range, and there were no significant differences in the number of microorganisms between the groups or within the groups.

Frequency of mercury resistance and antimicrobial resistance in oral microflora. The percentages of mercury-resistant microorganisms of the total saliva microflora during weeks 0, 2, and 9 in the amalgam group and control group were calculated. The respective median values (range) were 56% (11%–100%), 48% (11%–80%), and 58% (0.7%–86%) in the amalgam group and 50% (20%–66%), 42% (5.1%–94%), and 56% (4.2%–100%) in the control group. There were no statistically significant differences between or within the groups. The percentages of microorganisms resistant to ampicillin, cefuroxime, and chloramphenicol were very low; median values were zero in both groups. Resistance to cefoxitin, doxycycline, erythromycin, and clindamycin did not vary significantly between or within the two groups. When all samples from both groups were analyzed together ($n = 120$), the median percentages (range) of oral microflora resistant to these agents were 0.04% (0 to 44%), 4.3% (0 to 26%), 1.4% (0 to 52%), and 0.7% (0 to 18%), respectively.

Frequency of mercury resistance and antimicrobial resistance in intestinal microflora. The prevalence of mercury-resistant microorganisms in the total fecal anaerobic microflora is shown in figure 2. In the amalgam group, there was an increase in the number of mercury-resistant microorganisms following mercury exposure. However, this increase was not statistically significant when the normal variations of the control group were considered and when analysis of variance with a repeated-measures design was undertaken. In addition, there were no statistically significant differences between the two groups.

The same pattern was observed in analysis of the proportion of mercury-resistant aerobic bacteria (of the total aerobic microflora, mainly enterobacteria and enterococci). For all fecal samples ($n = 120$), the median percentages (range) of microorganisms resistant to the different antimicrobial agents tested were as follows: ampicillin, 0.1% (0 to 100%); cefuroxime, 0.2% (0 to 100%); cefoxitin, 0.2% (0 to 100%); doxycycline, 0.02% (0 to 44%); erythromycin, 1.0% (0 to 100%); clindamycin, 0.3% (0 to 100%); and chloramphenicol, 0.0% (0 to 0.3%). In the amalgam group, the number of strains resistant to ampicillin, cefoxitin, erythromycin, and clindamycin increased from week 0 to week 2 and subsequently decreased from week 2 to week 9.

In the control group, no particular trends of increases or decreases in the percentage of resistant microflora could be detected during the investigation period. Using analysis of variance with a repeated-measures design including both groups, we found no significant alterations of resistance to the different antimicrobial agents between or within the two groups.

Frequency of mercury-resistant strains. A total of 236 E. coli strains, 200 enterococcal strains (77 E. faecalis, 95 Enterococcus faecium, and 28 Enterococcus durans), and 256 Bacteroides strains were isolated and classified as susceptible or resistant to mercury. The distribution of mercury resistance in the amalgam and control groups is shown in table 1. For aerobic strains there were no significant differences with regard to mercury resistance in the two groups, while among the anaerobic intestinal microflora (represented by Bacteroides species) there was a significantly higher number of mercury-resistant strains in the amalgam group than in the control group ($P < .001$, $\chi^2$ test).

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**Figure 1.** Mercury concentrations in fecal samples in the amalgam (Hg) group (10 patients) and control group (10 subjects without amalgam fillings). Horizontal bars indicate median values. For the Hg group, the numbers on the horizontal axis indicate the number of days after removal of the amalgam fillings; 0 refers to the baseline sampling. The asterisks indicate values that were significantly different from those on day 60 and in the control group ($P < .01$, Wilcoxon's signed rank test for paired samples and Mann-Whitney U test). The dagger indicates values that were significantly different from those on days 0 and 7 and in the control group ($P < .01$, Wilcoxon's signed rank test for paired samples and Mann-Whitney U test).
Minimum inhibitory concentrations. The MICs of ampicillin, cefuroxime, cefoxitin, erythromycin, and doxycycline were determined for E. coli strains isolated from the amalgam group (n = 124) and from the control group (n = 112), as well as for enterococcal isolates from the amalgam group (n = 97) and from the control group (n = 103). For Bacteroides strains (amalgam group, n = 104; control group, n = 152), the MIC of the antianaerobic agent clindamycin was also determined. The MIC$_{50}$ and MIC$_{90}$ values for strains isolated during the different sampling periods are shown in Table 2. In the amalgam group there was a trend toward higher MIC$_{50}$ and MIC$_{90}$ values for E. coli and Bacteroides strains during week 2 (1–3 dilution steps) in comparison with values for weeks 0 and 9.

Relationship between mercury resistance and the prevalence of antimicrobial resistance. The isolated E. coli (n = 236), enterococci (n = 200), and Bacteroides strains (n = 256) were classified as susceptible or resistant to mercury as well as to the different antimicrobial agents tested, according to breakpoints recommended by the NCCLS. Since there were no statistically significant differences in MICs between strains from the two groups and since mercury-resistant strains appeared frequently in both groups, the relationship between mercury resistance and antimicrobial resistance was analyzed for all strains together (Table 3). Significantly more mercury-resistant than mercury-susceptible E. coli strains were also resistant to ampicillin and doxycycline. Similarly, a significantly higher number of mercury-resistant enterococcal strains also were resistant to cefuroxime and cefoxitin, in comparison with mercury-susceptible strains. In the Bacteroides group, the number of mercury-resistant strains that also were resistant to ampicillin, cefuroxime, and erythromycin was higher than that of mercury-susceptible strains ($\chi^2$ test).

Correlation between the incidence of mercury resistance and multiple antimicrobial resistance. There was a significant correlation between the prevalence of mercury resistance and that of multiple antibiotic resistance in bacterial strains isolated in the present study. Mercury-resistant bacteria, in comparison with mercury-susceptible strains, were significantly more likely to have resistance determinants for two or more of the seven antimicrobial agents tested. This was true for E. coli, enterococci, and Bacteroides species (Table 4).

Discussion

It is well known that mercury from dental amalgam fillings is a major contributor to the total body burden of mercury in the general population [18, 19]. Measurements of mercury released from dental amalgam fillings and daily dose calculations have been widely discussed and are based mainly on human exposure by elemental mercury vapor inhalation. The average daily retention of elemental mercury from amalgam fillings has been reported to range from 1.7 to 20 $\mu$g/24 hours [18–24]. However, the fraction of mercury released from amalgam fillings that reaches the gastrointestinal tract is not well known. In the present study, both saliva and fecal concentrations of mercury had a significant positive correlation to the number of amalgam surfaces, which is in accordance with previous investigations of mercury levels in plasma, urine, and feces [22, 23, 25–27]. The concentrations of mercury in saliva in the present study were 100-fold higher in the baseline samples from subjects with

<table>
<thead>
<tr>
<th>Susceptibility (S) or resistance (R) to Hg</th>
<th>E. coli</th>
<th>Enterococci</th>
<th>Bacteroides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>Control</td>
<td>Total</td>
<td>Hg</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>S</td>
<td>112</td>
<td>103</td>
<td>215</td>
</tr>
<tr>
<td>R</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>112</td>
<td>236</td>
</tr>
</tbody>
</table>

* Significantly different from the number in control group; $P < .001$ ($\chi^2$ test).
Table 2. MIC\textsubscript{50} and MIC\textsubscript{90} values (\(\mu g/mL\)) for \textit{E. coli}, enterococci, and \textit{Bacteroides} strains isolated from the amalgam (Hg) group and the control group.

<table>
<thead>
<tr>
<th>Isolates ((n) per group), antimicrobial agent tested</th>
<th>MIC\textsubscript{50}/MIC\textsubscript{90} for Hg group strains, per week of isolation</th>
<th>MIC\textsubscript{50}/MIC\textsubscript{90} for all isolates, per group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 2</td>
</tr>
<tr>
<td>\textit{E. coli} (Hg: 124; control: 112)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2/4</td>
<td>2/8</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2/4</td>
<td>2/8</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1/8</td>
<td>1/16</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>32/32</td>
<td>32/32</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Enterococci} (Hg: 97; control: 103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1/1</td>
<td>0.5/1</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>4/64</td>
<td>4/32</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>32/&gt;64</td>
<td>32/&gt;64</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.062/8</td>
<td>0.062/8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>\textit{Bacteroides} species (Hg: 104; control: 152)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>16/128</td>
<td>32/128</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>128/512</td>
<td>256/512</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>8/32</td>
<td>8/32</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>4/8</td>
<td>4/8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2/8</td>
<td>4/16</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1/2</td>
<td>1/32</td>
</tr>
</tbody>
</table>

Dental amalgam fillings than in those from subjects without amalgam fillings. Two months after removal of the amalgam fillings, the saliva mercury levels had decreased significantly, and these levels were still at least 10-fold higher than those of the control group. In spite of the high mercury concentrations, no selection for mercury resistance or antimicrobial resistance could be detected in the oral microflora among subjects in the amalgam group.

Summers and co-workers reported a significant increase in mercury resistance of oral streptococci after insertion and removal of dental amalgam fillings in monkeys [12], a finding we could not confirm with humans in the present study. Previous studies have reported low to moderate levels of antimicrobial resistance in human oral microflora [7, 28]. In the present study, resistance to cefoxitin, doxycycline, erythromycin, and clindamycin occurred in both groups.

High total mercury levels in feces were recorded for subjects in the amalgam group, especially 1 week after removal of the amalgam fillings. In a previous study, the fecal excretion of mercury was estimated to be 27–190 \(\mu g/24\) hours for nine subjects with amalgam fillings [23]. These findings correspond well with those of the present study, on the basis of the assumption that each subject excretes \(~100–200\) grams of feces per 24 hours. The fecal mercury levels approached those of the control group 2 months after removal of dental amalgam fillings, although they were still significantly higher.

In the present study, the relative amount of intestinal microorganisms resistant to 50-\(\mu M\) \(\text{HgCl}_2\) peaked 7 days after removal of the amalgam fillings; the median value was 6.1%, compared with 1.3% in samples collected before the mercury exposure (figure 2). If analyzed separately for the amalgam group, this increase would be considered statistically significant. However, the normal variations in the prevalence of mercury and antimicrobial resistance in the normal human microflora are considerable. This was demonstrated by the fluctuations in the control group, as well as in other studies [3, 6, 7, 29]. The increase in the number of mercury- and antimicrobial-resistant microorganisms in the present study may thus well be within the normal range.

Summers et al. demonstrated a marked increase in the proportion of aerobic mercury-resistant bacteria in the intestinal microflora of six monkeys after installation and removal of amalgam fillings [12]. However, the normal variations were high, no control group was included, and the results from this study have been questioned [30]. It has been suggested that the majority of fecal mercury appears in the form of inorganic mercury bound as very insoluble sulphide derivatives [22, 31]. This might be one explanation for why the obvious selection of mercury-resistant fecal microorganisms is observed infrequently, in spite of high intestinal concentrations of mercury.

Concerning the frequency of mercury-resistant strains in the two groups, it was shown that mercury-resistant \textit{Bacteroides} strains (MIC of \(\text{HgCl}_2\), >25 \(\mu M/\text{L}\)) were significantly more frequent in the amalgam group than in the control group. Since \textit{Bacteroides} is one of the dominating species of organisms in the intestinal microflora, this observation may indicate selection for mercury resistance in subjects with amalgam fillings. The breakpoint for \(\text{HgCl}_2\) in the screening part of the present study,
in which no statistically significant differences between the two groups were observed, was 50 μmol/L, a level that inhibited the growth of 247 of the 256 isolated Bacteroides strains. Hence, it cannot be excluded that an existing selection of mercury resistance in the intestinal micro flora due to mercury exposure would have been detected if another breakpoint had been selected.

Other studies have demonstrated that members of the family Enterobacteriaceae carry plasmids encoding resistance to both mercury and antimicrobial agents [10–12, 32]. This was confirmed in the present study; a positive correlation between mercury resistance and antimicrobial resistance was found for E. coli, enterococci, and Bacteroides species isolates (table 3). The incidence of multiresistance was also found to be more common in mercury-resistant strains than in mercury-susceptible strains (table 4).

In conclusion, the results of the present study show that mercury resistance in enterobacteria, enterococci, and Bacteroides species is significantly linked to multiresistance to antimicrobial agents. However, mercury exposure from amalgam fillings does not seem to be a major factor in the selection of mercury and antimicrobial resistance in the human oral and intestinal micro flora. Nevertheless, it cannot be excluded that dental procedures involving amalgam therapy may have an impact on the individual level (e.g., in immunocompromised patients) and contribute to an increase in prevalence of antimicrobial-resistant microorganisms.

### Table 3. Relationship between mercury resistance and the incidence of antibiotic resistance in 236 E. coli, 200 enterococcal, and 256 Bacteroides strains isolated from the intestinal microflora of the 20 subjects.

<table>
<thead>
<tr>
<th>Antimicrobial agent tested</th>
<th>E. coli*</th>
<th>Enterococci*</th>
<th>Bacteroides species*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>209</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>R</td>
<td>6</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>215</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>215</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>192</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>R</td>
<td>23</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>R</td>
<td>215</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>R</td>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
</tbody>
</table>

* No. of strains susceptible (S) or resistant (R) to Hg and the different antimicrobial agents, according to the breakpoints (see text).


### Table 4. Relationship between the incidence of mercury resistance and the incidence of antimicrobial multiresistance in intestinal E. coli and enterococcal and Bacteroides strains isolated from the 20 subjects.

<table>
<thead>
<tr>
<th>Resistance of isolates against no. of antibiotics*</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>0 or 1</td>
<td>192</td>
</tr>
<tr>
<td>≥2</td>
<td>23</td>
</tr>
<tr>
<td>Enterococci</td>
<td></td>
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<tr>
<td>0 or 1</td>
<td>26</td>
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<td>≥2</td>
<td>0</td>
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<td>Bacteroides species</td>
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<tr>
<td>0 or 1</td>
<td>62</td>
</tr>
<tr>
<td>≥2</td>
<td>74</td>
</tr>
</tbody>
</table>

* Among the seven antimicrobials tested in the present study (see text).

1. Hg S = susceptible to mercury; Hg R = resistant to mercury.

1. P < .001 (χ² test).

1. P < .05 (χ² test).


