



# Prevalence of Mercury-Resistant and Antibiotic-Resistant Bacteria found in Dental Amalgam

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## Abstract

Mercury and antibiotic resistance has long been a subject of interest in microbiology that a vast of literature consisting of studies that looked at its genetics and molecular mechanisms. The aim of this study was to isolate and identify Mercury-resistant and antibiotic resistant bacteria and determine the Hg-resistant isolates were also antibiotic resistant. Twenty four bacterial isolates (54% Gram-negative and 46% Gram-positive) from saliva samples with amalgam fillings were screened for Mercury and resistance by cultivation in an  $HgCl_2$ -containing medium. Surviving organisms were identified using the conventional method of identification and susceptibility to antibiotics was determined by Kirby-Bauer disc diffusion. All of the twenty four isolates were able to grow in Mercury-containing medium and were considered as Hg-resistant bacteria. The rate of susceptibility of the bacterial isolates against the antibacterial disk showed high mean percentage in each antibiotic disk, showing that there is a growing trend of susceptibility to antibiotic which might result to antibiotic resistance. The result of the study showed that Mercury-resistant and antibiotic-resistant bacteria can be isolated from the oral saliva samples of the amalgam filled individual. This is a matter of interest for science and medicine since more and more bacterial species acquire genes that confer them resistance and presents new challenges for treating the associated conditions it cause and in eliminating the bacteria themselves.

**Keywords:** Amalgam, antibiotic-resistant bacteria, mercury-resistance, microflora.

## Introduction

Dental amalgam is a commonly used dental restorative material used since early in the 19<sup>th</sup> century and has been controversial because of its mercury content<sup>1</sup>. Many dental amalgams are made by mixing one part of liquid mercury with one part of a mixture of other metals; mainly silver, but also tin, copper, and small amounts of zinc<sup>2</sup>. Amalgam has been the restoration of choice for many years due to its low cost, ease of application, strength, durability, and bacteriostatic effects. Thus, according to various studies, dental amalgam is responsible for at least 60-95% of mercury deposits in human tissues<sup>3</sup> and mercury compounds are well known for their immunosuppressive activity<sup>4</sup>. In addition, the elements other than mercury contained with dental amalgam all have their own, different profiles in terms of essentiality and/or toxicology<sup>5</sup>.

It has been shown that mercury from dental amalgam can induce resistant bacteria<sup>6,7,8</sup>. The fact that mercury from dental amalgams may be promoting an increase in antibiotic resistance in oral bacteria is obviously of concern to dental practitioners, as antibiotics are used in the treatment of a number of oral infections e.g. periodontitis and abscesses. This leads to a general antibiotic resistance in oral bacteria and in other body sites<sup>8</sup>, which is particularly true when the antibiotic resistance genes are contained within the same mobile element as the mercury resistance operon<sup>9,10</sup>. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the

development of antibiotic resistance and the association between increased rates of antimicrobial use and resistance has been well documented<sup>11</sup>.

The aim of this study is to determine if mercury and antibiotic-resistant bacteria are maintained within the oral cavity of persons having dental amalgams and to determine whether it results in an increase in the prevalence or oral load of mercury-resistant oral bacteria and to investigate whether it exhibit resistance to antibiotics.

## Material and Methods

**Collection and Processing of Saliva Samples:** Saliva samples were obtained from healthy constituents of MSU-IIT. Consent and ethical approval were given and were asked for history of antibiotic exposure during the previous 2 weeks (including both therapeutic and employment exposures).

Saliva samples of 2 ml were aseptically collected and placed immediately into 4 ml Ringer's solution in a sterile 7 ml container that included five sterile 2-3 mm diameter glass beads. The samples were taken to the laboratory for immediate processing.

Samples from each patient were vortexed for 30 seconds. Serial 1 in 10 dilutions (up to  $10^{-5}$ ) of the sample were prepared in Nutrient Broth.

**Screening for Mercury-Resistant Bacteria:** Serial dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ) of the sample were prepared. In order to isolate individual colonies, 100  $\mu$ l of the dilutions were spread over the Nutrient Agar with 16  $\mu$ M HgCl<sub>2</sub>. Four plates were used for each dilution: two were incubated at 37°C anaerobically for 48h and two aerobically for 48h. 100  $\mu$ l aliquots of dilutions ( $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ) of each sample were duplicated and inoculated under anaerobic and aerobic conditions at 37°C to determine the total aerobic and anaerobic viable counts.

Following incubation, only plates with 30 and 300 colonies were considered. Colonies growing on the Hg-containing and Hg-free media were enumerated.

**Screening for Antibiotic -Resistant Bacteria:** Antibiotic resistance was evaluated using the Kirby-Bauer disc diffusion method. The concentrations of antibiotics were: Nitrofurantoin, Chloramphenicol, Gentamicin and erythromycin, 0.008-8.0 mg/L; Vancomycin, 0.0625-16 mg/L; Tetracycline, 0.016-128 mg/L. The antimicrobial impregnated disks were placed with sterile forceps on the agar surface in such a way that each disk was at least 24mm away from each other to avoid the overlapping zone inhibition. The plates were then incubated at 37°C for 24 hours and observed for diameter of zone of inhibition<sup>12</sup>.

**Presumptive Identification of Bacterial Isolates:** Oral isolates that were able to grow on the nutrient agar containing 16  $\mu$ M of HgCl<sub>2</sub> or greater were regarded as being mercury resistant and were identified on the basis of Gram's stain, morphology, atmospheric requirements and standard biochemical tests (Catalase test, MSA, EMB and SCA test).

## Results and Discussion

**Mercury-Resistant Bacteria:** Twenty-four (24) bacterial isolates were selected for further identification, thirteen were Gram-negative (*Enterobacteriaceae* spp. And *Escherichia coli*) and eleven were Gram-positive bacteria (*Staphylococcus* spp., *Streptococcus pyogenes*, and *Streptococcus pneumonia*). These bacterial isolates were able to grow in MHA with HgCl<sub>2</sub> which may suggest that it is mercury resistant. Some bacteria developed a mercury resistance mechanism, based on a group of genes located in an operon (*mer* operon)<sup>13</sup>. An extensively studied resistance system based on clustered genes in an operon allows bacteria to detoxify Hg<sup>2+</sup> into volatile metallic mercury through its enzymatic reduction. Bacterial mercury resistance has been found in a wide range of Gram-negative and Gram-positive bacteria<sup>13</sup>. Mercury resistance is an adaptation of bacteria that is correlated not only with the ability to live in mercury containing environments, but to survive other forms of toxicity, such as antibiotics<sup>14</sup>.

**Antibiotic-Resistant Bacteria:** Figure 1 shows the comparative profile of the mean zone of inhibition of each characterized sample or isolates against the antibacterial disk.

Chloramphenicol has the highest mean of inhibition for *E.coli*. Vancomycin on the other hand shown the minimum total mean of inhibition for *E.coli* compared to other antibiotic disk. Tetracycline yielded the most effective antiseptic disk with 45% of inhibition rate.

The rate of susceptibility of the bacterial isolates against the antibacterial disk showed high mean percentage in each antibiotic disk (figure 2), showing that there is a growing trend of susceptibility to antibiotic which might result to antibiotic resistance. Susceptible isolates are supposed to have undergone strain mutation that provided partial secondary inhibition layers of the isolates called satellites. This mutation could be the results of the wild strains that have evolve resistance to the antibacterial disk causing over growth of the strain allowing partial layer inhibition.

The fact that mercury resistance is often associated with antibiotic resistance also has a genetic cause. Many transposons responsible for mercury resistant include genetic elements called integrons, which contain the genes that confer antibiotic resistance<sup>14,15</sup>. However, it is important to consider that different genes give resistance to different classes of antibiotics and not all are included in Hg-resistance transposons.

## Conclusion

The result of the study has shown high significance on the presence of mercury-resistant bacteria present in the oral flora from the saliva samples collected as well as the presence of antibiotic-resistant bacteria. Development of resistance from the isolated bacteria may pose a problem as this resistance may be because of the indiscriminate and inappropriate use of oral antibiotics.

Also, there is a significant difference in between groups of the isolates inhibitory and resistant reaction that shows that there is a different approach and assessment of every antibiotic to different form of isolates. The knowledge of susceptibility testing patterns of the different bacterial strains will be of guidance to dental practitioners to choose appropriate and cautious antibiotics for treatment of any oral infections and disease.

## References

1. Koral S.M., The Scientific Case Against Amalgam, *International Academy of Oral Medicine and Toxicology*, (2002)
2. Green Facts Organization, Tooth filling materials: Dental Amalgams and Alternative Materials, *Scenihhr/Scher Source Documents* (2008)
3. Mutter J., Is Dental Amalgam Safe for Humans? The opinion of the scientific committee of the European Commission, *Journal of Occupational Medicine and Toxicology*, 6, 2 (2011)

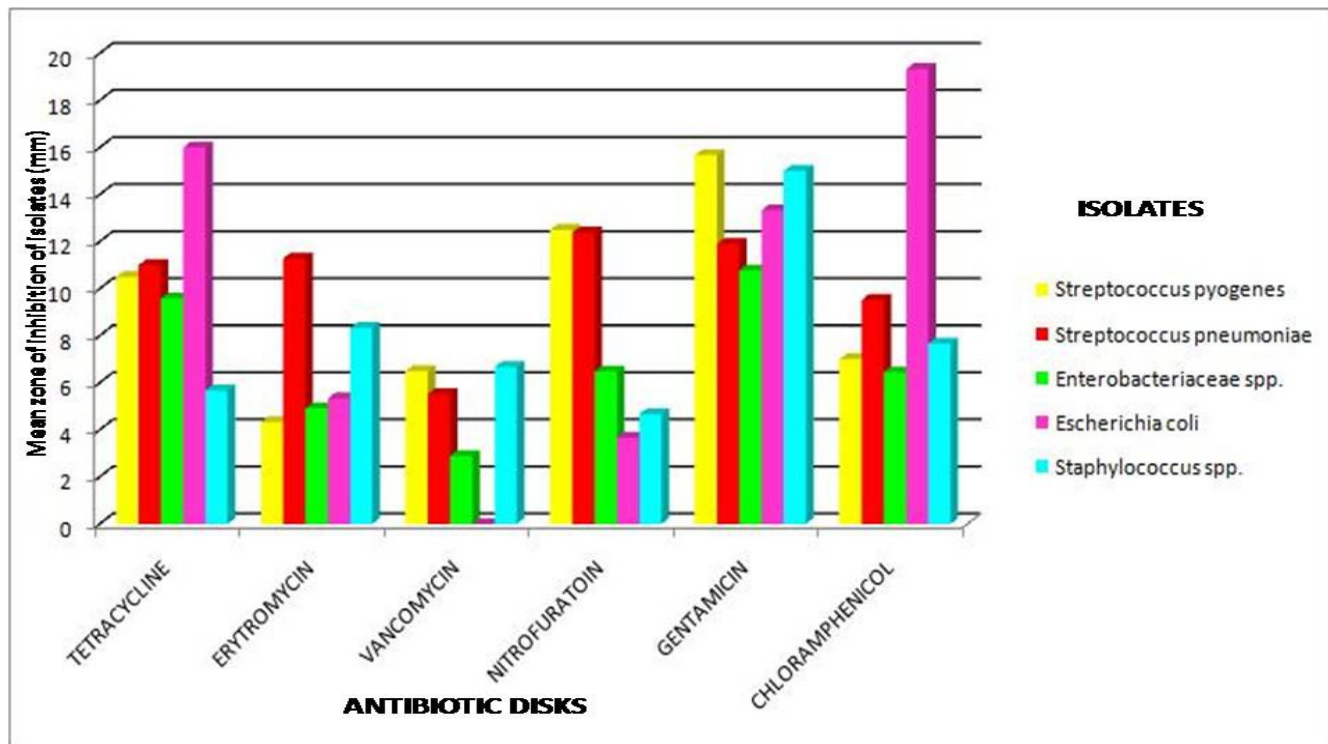


Figure-1

The mean zone of inhibition (mm) of each antibacterial disk against each of the characterized isolates

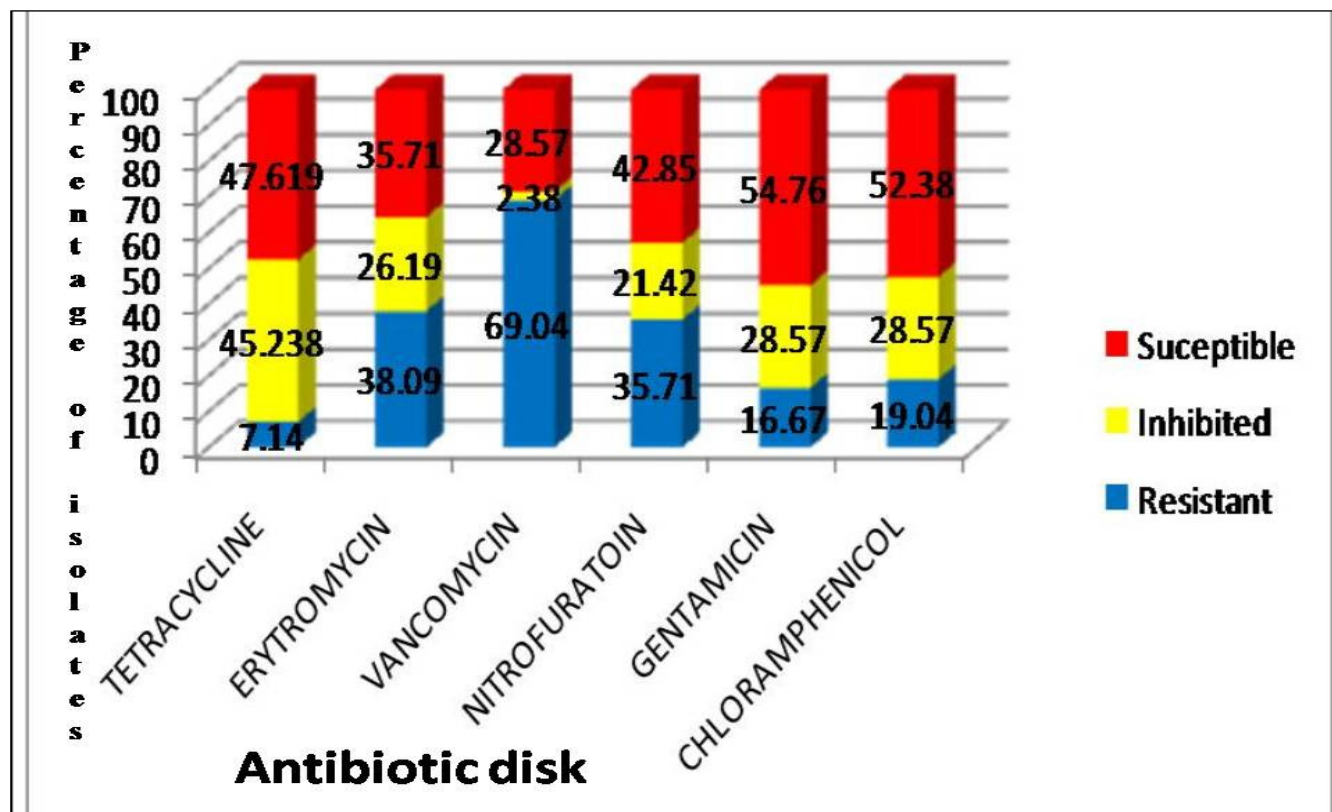


Figure-2

Percentage Rate of Susceptibility, Resistance and Inhibited Reaction of Isolates against the Six Antibacterial Disks

4. Havarinasab S. and Hultman P., Organic mercury compounds and autoimmunity, *Autoimmunity Rev.*, **4**, 270-5 (2005)
5. Scenihhr, The safety of dental amalgam and alternative dental restoration materials for patients and users: Preliminary Report. Scientific Committee on Emerging and Newly Identified Health Risks Plenary, Available on [http://ec.europa.eu/health/ph\\_risk/risk\\_en.htm](http://ec.europa.eu/health/ph_risk/risk_en.htm) (2007)
6. Liebert C.A., Wireman J., Smith T. and Summers A.O., The Impact of mercury released from dental "silver" fillings on antibiotic resistances in the primate oral and intestinal bacterial flora, *Met. Ions Biol Syst.*, **34**, 441-460 (1997)
7. Lorscheider F.L., Vimy M.U., Summers A.O. and Zwiars H., The dental amalgam mercury controversy-inorganic mercury and the CNS; genetic linkage of mercury and antibiotic resistances in intestinal bacteria, *Toxicology*, **97**, 12-22 (1995)
8. Summers A.O., Wireman J., Vimy M.U., Lorscheider F.L., Marshall B. and Levy S.B., 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 (1993)
9. Davis U., Roberts A.P., Ready D., Richards H, Wilson, M, Mullany P. Linkage of a novel mercury resistance operon with streptomycin resistance on a conjugative plasmid *Enterococcus faedium*, *Plasmid*, **54**, 26-38 (2005)
10. Skumik D., Ruimy R., Ready D., Ruppe E., Bemedebauduin C., Djossou F., Guillemot D., Pier G.B., Andremont A. Is exposure to mercury driving force for the carriage of antibiotic resistance genes? *Journal Med Microbiol.*, **59**, 804-807 (2010)
11. Radha K., Mahima R., Ramanathan G., and Thangapandian V. Survey on Drug Resistant Pattern of Clinical Isolates and Effect of Plant Extract on the Drug Resistant Pattern, *ISCA Journal of Biological Sciences*, **1(3)**, 14-19 (2012)
12. Nakade D.B., Antibiotic sensitivity of common Bacterial Pathogens against selected Quinolones, *ISCA Journal of Biological Sciences*, **1(1)**, 77-79 (2012)
13. Sotero-Martins A., de Jesus M.S., Lacerda M, Moreira J.C., Filgueiras A.L.L., and Barrocas P.R.G. A conservative region of the Mercuric Reductase Gene (MERA) as a Molecular Marker of Bacterial Mercury Resistance, *Brazilian Journal of Microbiology*, **39**, 307-310 (2008)
14. Bass L., Liebert C.A., Lee M.D., Summers A.O., White D.G., Thayer S.G., and Maurer J.J. Incidence and Characterization of Integrons, Genetic Elements, Mediating Multiple-Drug Resistance, in Avian *Escherichia coli*, *Antimicrobial Agents and Chemotherapy*, **43**, 2925-2929 (1999)
15. Essa A.M.M., Julian D.J., Kidd S.P., Brown N.L., and Hobman J.L. Mercury Resistance Determinants Related to Tn21, Tn1696, and Tn5053 in Enterobacteria from the Preantibiotic Era, *Antimicrobial Agents Chemother*, **47**, 1115-1119 (2003)