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The Relationship between Cell Surface Hydrophobicity and Antibiotic Resistance of Streptococcal Strains Isolated from Dental Plaque and Caries

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Abstract

Objective

Bacterial adhesion is governed by specific and nonspecific interactions such as hydrophobicity. Hydrophobic interactions play a role in the adherence of microorganisms to a wide variety of surfaces and facilitate biofilm formation due to bacterial adhesion. In this article the relation between cell surface hydrophobicity and antibiotic resistance was studied.

Materials and Methods

We studied antibiotic susceptibility of isolated *Streptococci* from dental plaque and caries (by disk diffusion method) and cell suface hydrophobicity (by microbial adhesion to hydrocarbon).

Results

The results indicated that the mean surface hydrophobicity of all *mutans* and other than *mutans Streptococci*, without considering their location (caries or plaque) showed significant differences (mean 78%, 59%, respectively). No significant differences among caries and plaque isolated *Streptococci* were found regarding the antibiotic susceptibility. Chi square test with contingency table showed significant qualitative correlation between classes of hydrophobicity (high, moderate and low) and antibiotic resistance (p= 0.001), but according to the regression models we could not find any linear correlation between cell surface hydrophobicity and the diameter of inhibition zone for each antibiotic separately.

Conclusion

The high overall proportion of hydrophobic bacteria found in this study suggests that cell- surface hydrophobicity may play a role in adherence of certain oral species to the tooth surface. If bacteria attach to surfaces and form biofilm they can be more resistant, but these two factors (antibiotic resistance and cell surface hydrophobicity) did not show a linear correlation in this study.

Keywords: Antibiotic resistance, Dental plaque and caries, Hydrophobocity, *Streptococcus mutans*

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Introduction

Dental plaque is an example of microbial biofilm with a very complex microbial composition. Dental plaque formation which results from adhesion of oral bacteria to tooth surfaces, can lead to two main oral diseases: dental caries and periodontal disease (1). Dental caries is a microbial disease that continues to pose a worldwide health problem. *Streptococcus mutans*, harboring the dental biofilm is the principal etiological factor of this disease and endocarditis. It`s ability to adhere to teeth surfaces is paramount for the progression of the disease (2).

Bacterial adhesion is governed by specific (ligand- receptor like) and nonspecific (physic-chemical) interactions. All the interactions forces depend on physicochemical properties of substratum and bacterial surfaces such as hydrophobicity (3). Hydrophobic interactions play a role in the adherence of microorganisms to a wide variety of surfaces (4) and facilitate biofilm formation due to bacterial adhesion (5).

It is well established that biofilm embedded bacteria are much more resistant to antibiotic treatment when compared to their planktonic counterparts (6).

The aim of present study was to determine the cell surface hydrophobicity of *S. mutans* and other *Streptococci*, isolated from plaque and caries lesions, also to investigate the correlation between surface hydrophobicity and antibiotic susceptibility of *Streptococcal* isolates for the first time.

Materials and Methods

Bacterial strains and growth conditions

A total of 40 *Streptococci* strains were isolated from dental caries (caries lesion class I) or plaque (normal teeth, without any periodontal disease) of patients referring to diagnostic center of dental school of Esfahan medical University & Khorasgan Branch of Islamic Azad University. These patients were of both sexes with the mean age of 22 years. The samples were prepared by means of sterile dentistry curette. The standard strain was *S. mutans ATCC 35668*. All strains were cultured on the media such as blood agar and mitis salivarius agar (Merck co.) in a CO2 enriched atmosphere, and the biochemical tests were done for their identification (7, 8). All the experiments were carried out in 2 replicates.

Cell surface hydrophobicity

The ability of bacterial cells to adhere to hydrocarbon (Octan: Merck co.) was used as a measure of their hydrophobicity as described by Rosenberg et al (9, 10). Briefly, bacteria were harvested during the exponential growth phase by centrifugation at 4000 xg for 15 mins, washed twice with PBS (pH=7) and resuspended in the same buffer. The suspension was adjusted to approximately 10^8 CFU/ml cell densities (by optical density at 640 nm (A1)). Samples (3.5 ml) of bacterial suspensions were placed in tubes and 500 µl of Octan were added, mixed using a vortex mixer for 2 mins and allowed to stand until the phase separated (10-15 mins). The lower aqueous phase was carefully removed and it`s optical density was determined at 640 nm (A2) with spectrophotometer (Schimadzu model). The hydrophobicity index (HPBI) was calculated as: HPBI= [(A1- A2)/A1] x 100

Isolates with a HPBI greater than 70 % was arbitrarily classified as highly hydrophobic. Isolates with HPBI between 50 and 70 classified as moderate and isolates with HPBI lower than 50 classified as low hydrophobic (11).

Antibiotic susceptibility test

Susceptibility of isolated strains was determined by disk diffusion method on sheep blood Muller Hinton agar media. The diameter of inhibition zone was measured after 24 hours incubation at 37ºC and strains were classified as Resistant (R), Intermediate (I) and Sensitive (S) (in comparison with Padtan teb zone size interpretive chart).

Antibiotic disks (Padtan teb co.) used in this test were as follows: Penicillin-G (10 µg), Vancomycin (30 µg), Cephalothin (30 µg), Cephalexin (30 µg), Gentamycin (10 µg) and Streptomycin (10 µg).

Statistical analysis

All the statistical analysis was carried out with SPSS software. One way analysis of variance

(ANOVA) was used to check the reproducibility of each test. Unpaired T test was used to examine unpaired differences in adherent bacteria counts, and the percentage of bacterial adherence to Octan.

Non- parametric chi square test and regression analysis were used to correlate adherent bacteria counts with antibiotic response. For all statistical analysis the probability of type I error less than or equal to 0.05 was considered as statistically significant.

Results

Cell surface hydrophobicity

The hydrophobicity of the bacterial surfaces was determined by measuring the percentage of adhesion to Octan (Figure 1). There was significant differences in the mean of HPBI between the caries and plaque isolated *Streptococci* (79.7% & 44.3% respectively), *mutans* and other than *mutans Streptococci* (without considering their location, caries or plaque, mean 78% & 59%, respectively), (p= 0.001). However no remarkable difference was noticed between the *S. mutans* & other than *mutans Streptococci* isolated from caries lesions (83.5%, 75.4% respectively) and plaque (52%, 41.5% respectively). Caries isolated *Streptococci* showed high (83%) and moderate (17%) HPBI, but among the plaque isolated *Streptococci*, 67%, 20% and 13% of isolates showed high, moderate and low hydrophobicity, respectively (not indicated in the figure).

The mean hydrophobicity index of standard strain *S. mutans 35668* was about 81.75%, therefore, there was no significant difference in the hydrophobicity of the standard strain and highly hydrophobic isolated strains in this research (HPBI>70).

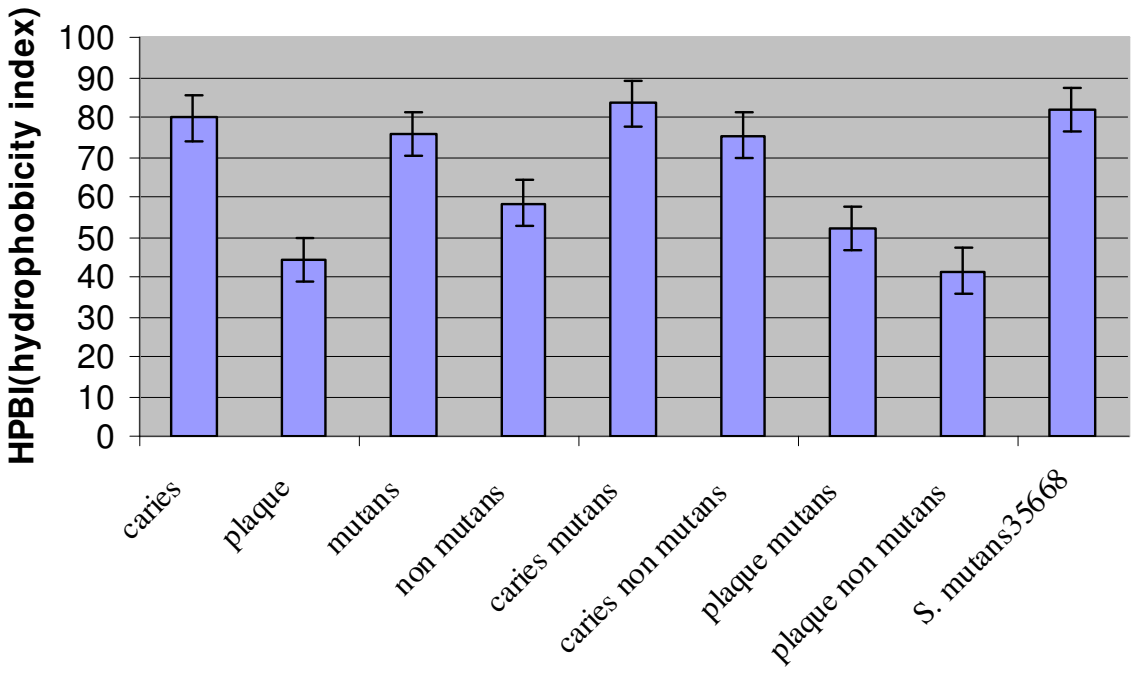

Figure 1. The mean of hydrophobicity index between the isolates of each group. Error bars represent the significant differences between the groups ($p < 0.05$).

Antibiotic susceptibility

The antibiotic susceptibility of *Streptococci* isolates to their therapeutic agents was determined. The results showed that most of the isolated *Streptococci* were resistant to Gentamycin and Streptomycin (Figure 2).

Susceptibility to Cephalosporines and Vancomycin was found to be more than other antibiotics and response to Penicillin was moderate. No significant differences between isolates were found in antibiotic susceptibility.

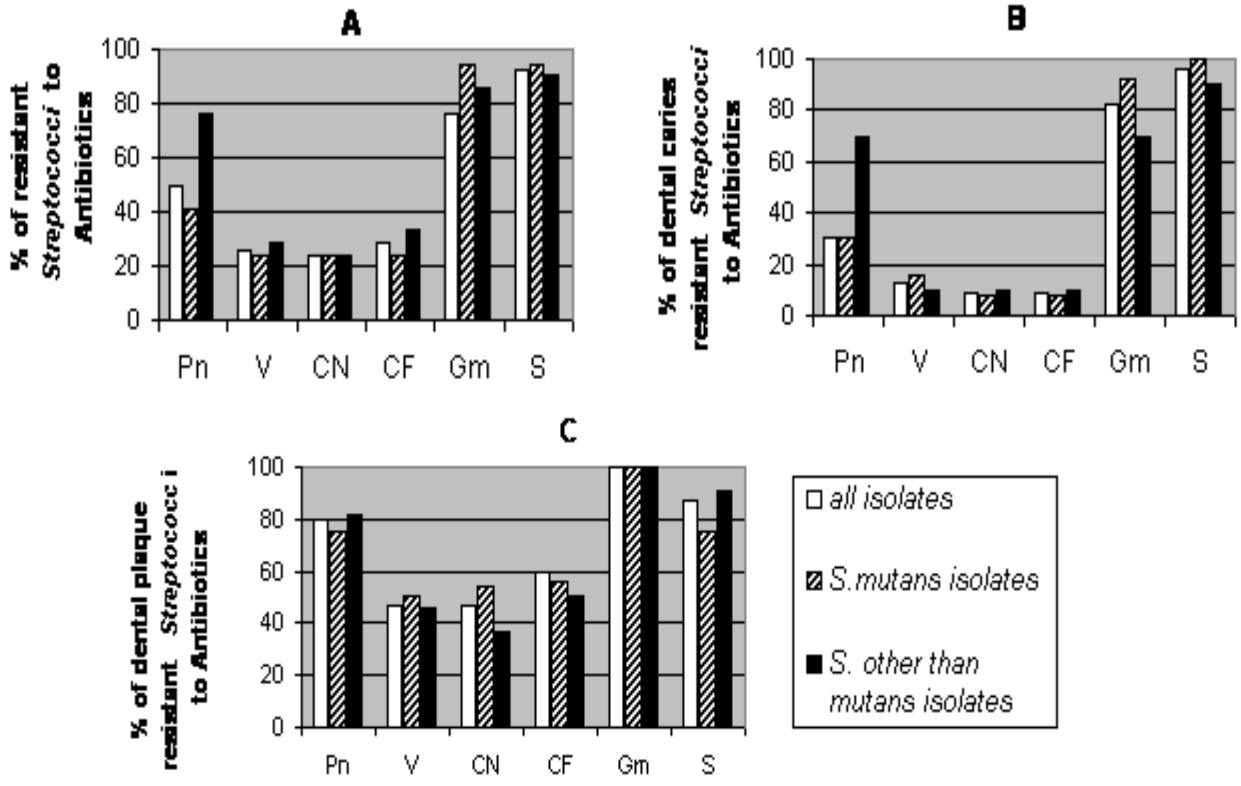

Figure 2. The percentage of resistant *Streptococcal* isolates to their therapeutic agents among, A: All oral *Streptococci* isolates, B: All dental caries *Streptococci* isolates, C: All dental plaque *Streptococci* isolates.
Pn: Penicillin, V: Vancomycin, CN: Cephalexin, CF: Cepfalothin, Gm: Gentamycin, S: Streptomycin

The relationship between cell surface hydrophobicity and antibiotic resistance

In general, non parametric statistical analysis such as chi square test with contingency table showed significant qualitative correlation between classes of hydrophobicity (high, moderate and low) and antibiotic resistance (% of resistant bacteria) (p= 0.001), but according to regression models we could not find any linear correlation between cell surface hydrophobicity and the diameter of inhibition zone for each antibiotic separately.

Discussion

Bacteria attach to teeth of humans in a highly selective manner and attachment is thought to be the first step leading to colonization. Several studies have suggested that the hydrophobic properties of bacteria may be an important factor in their adherence to host tissue (12). Many authors have used hydrophobicity to predict bacterial adhesion. adhesion to hexadecane (5, 12).

In this study, the HPBI of bacterial surfaces was measured by quantifying bacterial adhesion to Octan because, according to Rosenberg (1983), cells bound to Octan with extremely high affinity and adherence to hexadecane was lower than Octan (10). However this method has been shown to be precise and reproducible as long as experimental parameters are carefully defined. Adhesion to hydrocarbon allowed us to define highly hydrophobic strains *(S. mutans)* showing high adhesion to hydrocarbon. These results concur with those of other studies using the same protocol, showing that *S. sanguis, S. mutans* and *S. oralis* are highly hydrophobic *Streptococci* (5, 12).

Gibbons and Etherden (12) found that *S. mutans* was only slightly hydrophobic; we also found very few *S. mutans* isolates with moderate hydrophobicity. These differences can be explained by differences in growth condition such as pH, ionic force and the number of subculture (5, 12).

In this investigation caries isolated *Streptococci* were more hydrophobic than plaque isolates. It is known that cell surface hydrophobicity may change as a function of the physiological state of bacteria (10). The results support the hypothesis that hydrophobic interactions play a major role in bacterial adherence on human tooth surface and subsequent plaque accumulation and dental caries.

This article results agree with those of other investigators about antibiotic susceptibility. No significant differences were found between the isolates regarding the antibiotic susceptibility. Most of the isolates were resistant to Gentamycin and Streptomycin. Cephalosporines and Vancomycin were the most active antibiotics and Penicillin was a moderate antibiotic.

Wayne and Thomson reported that several oral *Streptococci* strains were resistant to Erythromycin, Streptomycin, and Tetracyclin. In their research Vancomycin and Penicillin were the most active antibiotics, that about Penicillin is not in agreement with our results, because of the increasing resistance genes in the environment. They also showed that *Streptococcal* strains were much less susceptible to Gentamycin and Streptomycin (13).

The high overall proportion of hydrophobic bacteria found in this study suggests that cell-surface hydrophobicity may play a role in adherence of certain oral species to the tooth surface. If bacteria attach to surfaces and form biofilm they can be more resistant, but these two factors (antibiotic resistance and cell surface hydrophobicity) did not show a linear correlation in this study.

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