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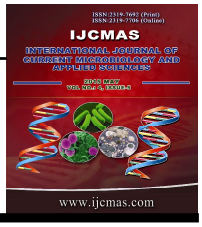

Original Research Article

The Relationship Between Biofilm Forming and Antibiotics Resistance of *Streptococcus mutans* Isolated From Dental Caries

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A B S T R A C T

Streptococcus mutans are believed to be the principal etiological agent of human dental caries. It has developed multiple mechanisms to colonize the tooth surface and has become a significant species in cariogenic biofilm. Microbial biofilm are more resistant to antimicrobial agents and therefore more difficult to control. In this article the correlation between biofilm forming and antibiotic susceptibility of *S. mutans* isolates was studied. We studies *S. mutants* isolated from dental caries identification by VITEK-2 compact system and antibiotics susceptibility by disk diffusion method. Detection of biofilms formation was followed by (quantifying bacterial adhesion test). The results showed *S. mutants* the high percentage (71.4%) in comparison with the other *streptococcus* species from total dental caries samples. Out of 54 (90%) *S. mutants* have ability to forming biofilm. No significant difference among positive and negative *S. mutants* isolated were found regarding to antibiotics susceptibility. The high over all proportion of *S. mutans* produce biofilm. *S. mutans* biofilm have been showed to be significantly more resistance to antibiotics.

Keywords

Streptococcus mutans, Dental caries, Biofilm forming, Antibiotics susceptibility

Introduction

Dental caries is one of the most common infectious diseases in the human oral cavity. destruction of enamel, dentin or cementum of teeth by cariogenic bacteria cause dental caries (Gamboa *et al.*, 2004). with dental caries occurs disturbed in balance inside mouth cause increases the amount of acidogenic and aciduric (acid-tolerating) bacteria leads to demineralization of enamel by rapid metabolism of dietary sugars to acid, resulting into reduction pH (Petersen *et al.*, 2005).

Streptococcus mutans is a Gram-positive, non-motile, non-spore forming, catalase negative, facultative anaerobic cocci bacterium commonly found in the human oral cavity, is a significant contributor to tooth decay (David *et al.*, 2011). This is due to their ability metabolize carbohydrates, such as glucose and sucrose, to produce acid and enhance biofilm formation with the early colonizing bacteria to induce dental caries (Featherstone, 2008).

Biofilms are surface adherent population of microorganisms consisting of cells, water and extra cellular matrix material (Sutherland, 2004). *Streptococcus mutans*, the principle cariogen for dental caries, co-exist with over 500 other species of bacteria as an interactive community known as the dental biofilm. The formed biofilm, provides an excellent adhesion site for the colonization and growth of many bacterial species (Paster *et al.,* 2001).

Biofilms enhance the virulence of the pathogen and have their potential role in various infections. Microbial biofilm are more resistant to antimicrobial agents and therefore more difficult to control, remain largely unexplored (Limsong *et al.,* 2004). Their inherent resistance to antimicrobial agents is at the root of many persistent and chronic bacterial infections. Biofilms have been reported to be less susceptible to antimicrobial agents and have reduced sensitivity to inhibitors. The resistance shown by biofilm to various antibiotics is a matter of concern (Thomas *et al.,* 2011).

The aim of present study was to determine the biofilm *S. mutans* isolated from dental caries, also to investigate the correlation between biofilm forming and antibiotic susceptibility of *S. mutans* isolates for the first time.

Materials and Method

Collection of samples

A total of (120) swabs was collected from dental caries from mouths of patients referring to central of dental faculty of dental, kufa university. These patients were of both sexes with the mean age of 26 year. Swabs for culture were placed in tubes contain transport media to maintain the bacterial swabs vital until being taken to the laboratory. The swab planted on blood agar, milis salivarius agar, Mmcconkey agar and manitol salt agar. All plates were incubated aerobically at 37 Cº for 24 hours.

Identification of *S.mutans*

After 24 hours incubated period, isolates were identified by colony characters, gram stain, catalase and oxidase test, optochin and bacitracin Susceptibility. These tests were performed on all samples of dental caries as per standard procedures. *S. mutans* identified was specified by using VITEK-2 compact system (bio merierx) according to the manufacturer's instructions.

Biofilm forming

Suspension of tested strain was incubated in the glass tubes containing brain – heart infusion broth aerobically at 35ºC for 48 hours. Then supernatant was discarded, the glass tubes has been stained by 0.1% safranin solution, washed with D.W. three times and dried. In the case of biofilm forming, a grainy red structure on the test tube bottom was found.

Antibiotic Susceptibility Test

Kirby- Bauer method for antibiotics sensitivity test was determined following the method described by Claus and Berkeley (1984). Brain heart infusion broth (5 ml) was inoculated with a loop full of bacteria isolates. The culture was incubated at 37ºC for 24 hours. The inoculated broth was compared with the turbidity standard and density of the test suspension was adjusted to be equal to 0.5 tube of Maccfarland. The inoculated broth transferred to Mueller-Hinton agar plates by sterile cotton swab was used to streak the inoculums on the plate surface in two difference plates. The inoculated plates were left to dry for a few

minutes at room temperature with sterile forceps selected antibiotic disks (Amikacin, Amoxicillin, Cefotaxim, Ciprofloxacin, Erythromycin, Gentamyacin and Tetracycline) were placed on the inoculated plates and incubated at 37ºC for 24 hours in an inverted position. After overnight incubated, the diameters of inhibition zone was measured by a ruler in (mm), results were determined according to National Committee for laboratory standards.

Statistical analysis

The results were analyzed statistically by Chi-square (X^2) test at the level of significant when P-value ≤ 0.01.

Results and Discussion

One hundred and twenty samples were collected from central of dental in faculty of dental, kufa - university. In the study one hundred and two samples (85%) were positive culture for any microorganism. microscopic examination and biochemical test was applied on all 102 isolates. The streptococci are gram- stain colonies to dark pink, due to mannitol-1-phosphate dehydrogenase-mediated hydrolysis of mannitol (Brown *et al.*, 1973) to the acid by these bacteria (Carlsson, 1968). The results in figure 1 show *streptococcus spp*. represented the highest percentage of the rates of isolations, which 84 isolates (70%) while other microorganism has accounted for 18 isolates from total samples collection. *Streptococci* identification according to species by using VITEK-2 compact system. Based on the results of vitek-2 system, *S. mutans* was comprised the high percentage (71.4%) in comparison with the other *Streptococcus* species (table 1), followed by *S. mitis* 10.7%, while *S. sangins* has accounted for (8.3%) and (4.7%) for each *S. sorbinus* and *S. salivarius*. The statistical analysis showed a significance differences ($P \leq 0.01$) among *streptococcus* species.

Figure 2 showed that 54 (90%) of *S. mutans* isolates have the ability to biofilm forming while 6 (10%) isolates don't forming biofilm. The result indicated signification difference in produce biofilm of *S. mutans* ($P \leq 0.01$).

Figure 3 showed that most of both positive and negative biofilm *S. mutans* isolated were resistant to Amoxicillin and Erythromycin while susceptibilities to Cefotaximn and Ciprofloxacin. No significant different between positive and negative biofilm *S. mutans* isolated were found in antibiotic susceptibility.

In the present study investigation of 120 samples collected from dental caries. A total of 84 (70%) *Streptococcus* species were identified in this study (Figure 1). The results agree with Muna (2011) So Young (2007) in which found 73.8 % and 81% respectively. *streptococcus* species particularly mutans *Streptococci* are the most found in human and the correct identification and differentiation among them is an important step to understand the early phases of colonization of the oral cavity Teresa *et al.* (2007).

Table 1 showed that most frequent *Streptococci* spp. was *S. mutans* (71.4%), followed by followed by *S. mitis* 10.7%. According to the other epidemiological data in Iraq, *S. mutans* and *S. mitis* were detected in 40% and 10.7% respectively collected from 100 patients with dental caries by use vitek-2 system (Nizar, 2014). It has been observed during the study, *S. mutans* is one of the most important oral bacterial which play a major role in dental caries, bacteraemia and bacterial endocarditis among predisposed patient Natagta (2006).

In this study, biofilm forming on polymeric surfaces was measured by quantifying bacterial adhesion test using brain heart infusion broth with 0.1 safranin. According to appear agraing red structure under test tube as report positive biofilm forming.

The result showed in figure 2 represent high percentage of *S. mutans* (90%) have ability to produce biofilm by forming grainy red color layer on test tube bottom. The result agreed with study by (16) demonstrated that all the *S. mutans* (100%) were isolated from dental caries are capable to forming biofilm.

Gibbons and Etherden (1983) found very few *S. mutans* isolates with moderate biofilm. These differences can be explained by difference in growth condition such as pH, ionic forces and the number of subculture (Grivet *et al.,* 2000). Biofilm facilitates the adherence of microorganisms to biomedical surfaces and protect them from host immune response and antimicrobial therapy, in addition, the production of biofilm may promote the colonization and lead to increase the infection rate of causative agent, then may be difficult to be treated as they exhibit multidrug resistance (Anderson *et al.,* 2006).

Table.1 The isolation percent of Streptococcus species from oral cavity of patients with dental caries

***Streptococcus* species.**	**No. of isolates**	**Percentage (%)**
S. mutans	60	71.4
S. mitis	9	10.7
S. sangius	7	8.3
S.salivarius	4	4.7
S.sorbinus	4	4.7
Total	**84**	**100%**

Figure.1 The percentage of *Streptococcus* spp. isolation from collection sample

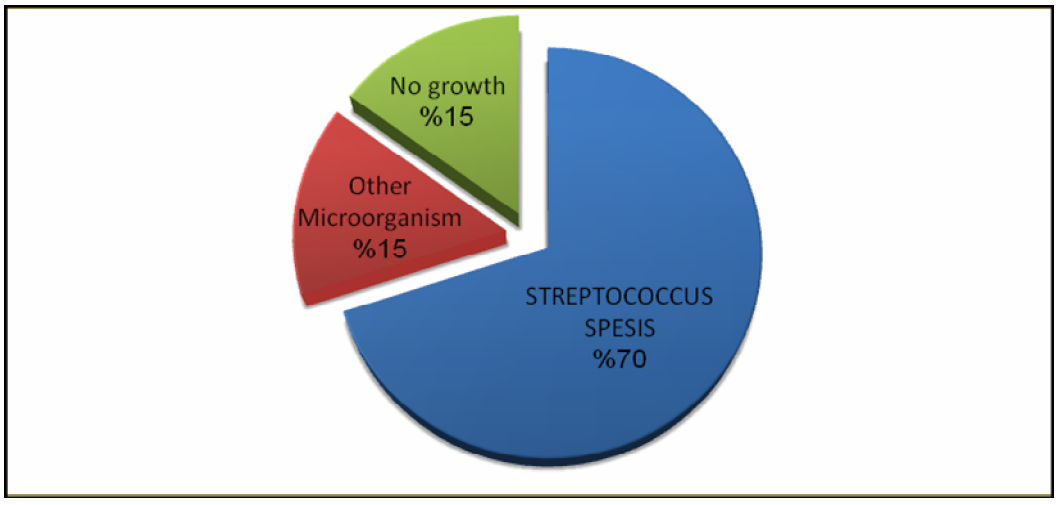


Figure.2 The percentage of each Positive and Negative biofilm among isolated *S. mutans*

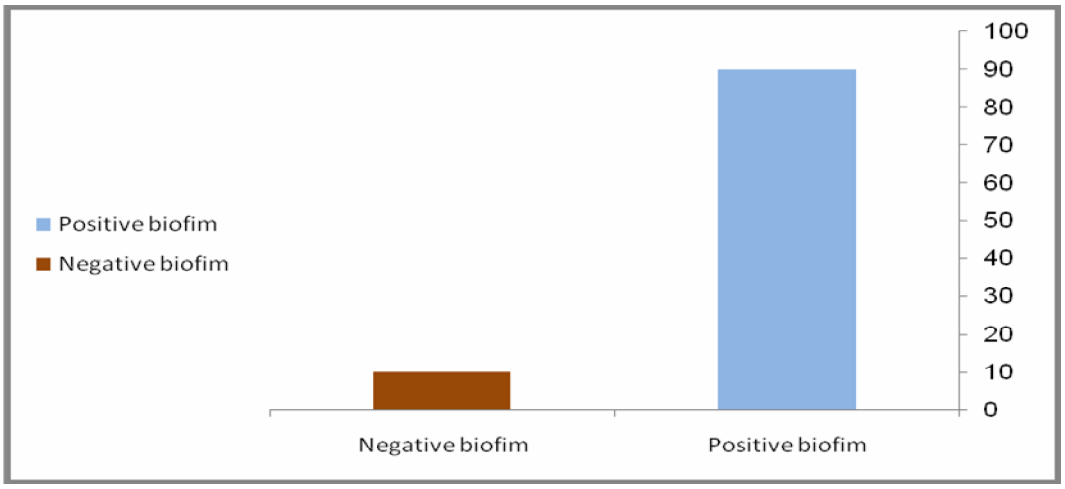
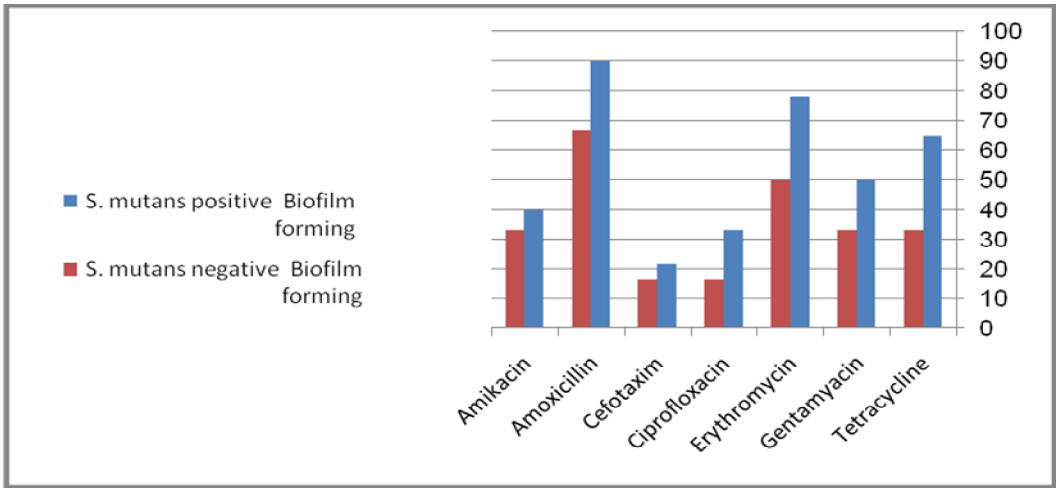


Figure.3 The percentage of resistance and susceptibility positive and negative biofilm *S. mutans* isolates to antibiotics



Antimicrobial susceptibility test was conducted for all positive and negative biofilm *S. mutans* isolates against 7 antibiotics. To investigate the most effective therapy to this type of bacteria, in addition to that the different between *S. mutans* biofilm forming and *S. mutans* no biofilm isolates was observed and discussed in attempt to compare between these two groups of isolates. Result shown in figure 3 indicated that most *S. mutans* isolates were resistance to Amoxicillin and Erythromycin with 90% and 78% from all isolates. This result was agreed with (Muna, 2011) in which found rate resistance for each Amoxicillin and Erythromycin was 87.5%. The result of this study also showed an increase resistance to tetracycline 65%.

Gamal (2014) who found that rate resistance to tetracycline 35% from all *S. mutans* isolates. This difference may be due to the influence of many factors like the age of the patient, the season of samples collection and

how much this antibiotics was used in community and this is different from person to person.

The result of the this study also shows resistance to aminoglycosides, the ration of resistance to gentamyacin 50% while the percentage resistance to amikacin is less than 40%, it is believed that this resistance aminoglycosides which is due to the formation of enzyme by resistant bacteria modifies the antibiotic because of loss of outer membrane proteins, which reduces the permeability of the antibiotic inside the bacterial cell (Alia, 2006).

The study showed that the Cefotaxim was more effective on *S. mutans* followed by Ciprofloxacin and the rate *S. mutans* isolates to both antibiotics of 88% and 66.5% respectively.

In conclusion, the result of this study showed that high over all proportion of *S. mutans* produce biofilm. Bacterial biofilm have been showed to be significantly more resistance to antibiotics.

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