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The Role *of milleri Streptococci* in the Formation of Cariogenic Biofilm: Bacteriological Aspects

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Abstract

It is remarkable that the biofilm (adherence) mode of growth of bacteria on the tooth surfaces in the oral cavity has been well documented as one of the important causes of dental caries. This study has been undertaken to detect the ability of *milleri Streptococci* in the formation of bacterial cariogenic biofilm qualitatively and quantitatively by spectrophotometric assay with ELISA reader, and to detect, in terms of BICs and MBECs, biofilm antimicrobial susceptibility test for biofilm producer isolates of *milleri Streptococci* to ciprofloxacin. Forty swabs obtained from patients admitted to Operative Dental Clinic in the College of Dentistry in Al-Ramadi City were studied during the period from December 2009 to March 2010. Lancefield's group sero-grouping was done. Quantitative biofilm formation by spectrophotometric method was achieved. Antimicrobial susceptibility test for the study isolates at two physiological stages planktonic and sessile states was done. Out of 40 study specimens, 25 (62.5%) were culture positive cases. Among culture positive cases, 33 mixed bacterial infection cases were observed consist of both of *Staphylococcus* spp. and *Streptococcus* spp. while α-hemolytic *Streptococci* were identified as pure single culture in 25 (100%) cases. α-hemolytic *Streptococci* isolates which were submitted to Lancefield's group classification were identified as group F *Streptococci*. Out of 25 bacterial study isolates, 20 (80%) were biofilm producers distributed into 17 (68%) as strong biofilm producer and 3 (12%) as weak producer. In biofilm antimicrobial susceptibility test for sessile cells of Group F *Streptococci* against ciprofloxacin, the biofilm inhibitory concentration required were from (10X - 100X MIC)µg/ml of this antimicrobial agent to inhibit bacterial biofilm. The minimal biofilm inhibitory concentrations from (100 X to 1000 X MIC) MICs µg/ml were enough to eradicate bacterial biofilm.It is concluded that most of group F *milleri Streptococci* isolated from patients with dental caries produced cariogenic biofilm. Also, in term of BICs and MBECs, the biofilm producer isolates were required 10-50 X MICs of ciprofloxacin to inhibit bacterial biofilm and 100-1000 X MICs to remove of bacterial biofilm in patients with dental caries.

Keywords: Cariogenic biofilm, dental caries, *milleri Streptococci*.

1. Introduction

It is well realized that bacterial biofilm is an aggregate of microorganisms in which cells are stick to each other and/or to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) (Hall-Stoodley *et al.,* 2004). On the other hand, dental caries is an infectious and transmissible disease, and the primary infection can often come from family members or caregivers (Florio *et al.*, 2004). There is a significant challenge for many patients to be able to modify their risk factors in order to create an oral environment that will lead to a re-establishment of a healthy bacterial population within the oral biofilm (Featherstone, 2003).

It is well known that dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *Streptococcus mutans* and *Streptococcus sanguis*), salivary polymers and bacterial extracellular products and it is consider a form of biofilm on the surfaces of the teeth (Rogers, 2008). It is well known that *S. mutans* is an "obligate" biofilm-forming bacterium (Burne, 1998a) and the primary etiological agent of human dental caries. This bacterium has also a primitive role in infective endocarditis (IE) (Gauduchon, 2000). It has a variety of mechanisms to colonize the tooth surfaces to become a corner stone bacterial species in cariogenic biofilms (Burne,1998b). The production of acids by *S. mutans* causes dissolution of minerals in tooth enamel and formation of dental caries. The bacteria in biofilm can produce lactic acid through the fermentation of dietary sugars such as sucrose and carbohydrates. In addition, oral streptococci can metabolize sucrose to produce insoluble glucans that promote the formation of biofilm extracellular polymeric slime (EPS) matrix (PaesLeme *et al.*, 2006).

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Thus, sucrose has a negative synergy with respect to caries in that it promotes both biofilm formation and acid production by cariogenic bacteria such as *S. mutans* (Paul Stoodley *et al.,* 2008). .

In fact, depending on the organism and type of antimicrobial and experimental system, biofilm bacteria can be up to thousand times more resistant to antimicrobial stress than free-swimming bacteria of the same species (Amy, 2008). Thus, this study has been undertaken to detect the ability of *milleri Streptococcus* in the formation of bacterial cariogenic biofilm, and, furthermore, in terms of MICs and MBECs, to determine antimicrobial activity of ciprofloxacin against study sessile cells.

2. Patients and Methods

Forty swabs obtained from patients admitted to Operative Dental Clinic in the College of Dentistry in Al-Ramadi City were studied during the period from December 2009 to March 2010. The swab was taken from inside each badly carious tooth to specify the tested sample and to confine the causative cariogenic bacteria as much as possible. The patients were distributed into 14 male and 11 female with male to female ratio1: 1.27and the age range between 17-64 years old with mean 37.48 ±12.3. Full informative history had been taken directly from patients and the information was arranged in an informative clearly detailed formula sheet. The study on the colonial morphology of grown bacteria on culture media (Blood agar, Chocolate agar and Nutrient agar) was done. Isolation of *mutans Streptococcus*, pigments and other characteristics and all biochemical tests were done according to Baron *et al.,* 1994).

2.1. Lancefield's Grouping Identification

This test was intended to be used for the identification of Lancefield groups A, B, C, D, F, and G by agglutination of specific antibody-coated latex particles in the presence of enzymatically extracted antigen. The principle of the test was based on streptococci carry group specific carbohydrate antigens in their cell walls. After extraction by especially developed enzyme preparation, these antigens will agglutinate latex particles coated with the corresponding antibody. The latex remains in smooth suspension in the absence of group specific antigen. Any colony which has the following characteristic was submitted to α-hemolysis on blood agar, Gram stain, catalase and oxidase tests.

This test was performed by using a kit of Streptococcal grouping latex test (Plasmatic, UK) as following: Two-Six colonies of streptococci were picked by using a sterile bacteriological loop and were emulsified them in 0.4 ml of extraction enzyme. The mixture was incubated in a water bath at 37°C for 10 minutes and the tubes were shaken vigorously after 5 minutes of incubation. The latex reagents were re-suspended by gently agitation, and one drop was delivered of each latex on to a circle on the test slide. One drop of the extract was added by micropipette tip to each drop of latex on to a circle on the test slide. Finally, the slide was rotated for no longer than 1 minute, and then observed for agglutination. A positive result was indicated by the visible agglutination of the latex particles, while a negative result was indicated by a milky appearance without any visible agglutination of the latex particles (Brooks *et al.,* 2004**).**

2.2. Qualitative assay of biofilm formation: Tube Method (Adhesion assay)

Glycocalyx production was determined, as described by Yassien and Khardori (2001) and Mathur *et al.* (2006) , as follows: Two to three colonies of study isolates were inoculated into 5 ml of brain heart infusion broth supplemented with 2% glucose in plastic conical tubes. After that, cultures were incubated at37° C for 18-24 hours and the contents were aspirated; one tube was examined unstained and other stained with crystal violet. Finally, slime positivity was judged by the presence of visible stained or unstained film lining the wall of the tube.

2.3. Quantitative assay of biofilm formation: Micro titer plate assay

Adhesion and biofilm formation was determined by using a spectrophotometric method, described by Yassien and Khardori (2001) and Stepanovic *et al*. (2003) as follows: working cultures were prepared by inoculation study isolate on Columbia agar supplemented with 5% blood and incubated aerobically at 35°C for 24 hrs. The cultures were used to prepare standardized bacterial suspension in sterile distilled water adjusted to a 0.5 McFarland turbidity standard to reach 10^5CFU/ml and the suspensions obtained were inoculated into a brain heart infusion broth with glucose (glucose supplemented medium) and without glucose.200 µL of standardized cultures were added to each well of sterile polystyrene Microtiter plate and incubated at 37°C for 18 hrs. Following incubation, the content of each well was aspirated, and each well was washed 3-4 times with sterile distilled water and the remaining attached bacteria were fixed with 200 µL of methanol per well. After 15 minutes, the plate wells were emptied and left to air dry. After wards the plates were stained for 5 minutes with 160 µL per well of crystal violet (0.25%) and the excess stain was rinsed off by placing the Micro titer plate under the running tap water. The plates were air-dried, and the dye, which was bound to the adherent cells, was re-solubilized with 160 µL of 33% glacial acetic acid per well. Finally, the optical density (OD) of each well was measured at 570 and 630 nm by using Stat Fax 3200 ELISA Reader. The isolates were classified according to biofilm production depending on the criteria laid down by Christensen *et al.* (1985) as follows: Strong producer more than 0.240; weak producer between 0.120-0.240 while non-producer less than 0.120.

2.4. Antimicrobial susceptibility for planktonic cells: Broth macrodilution method (MIC method

The antimicrobial susceptibility test for seventeen strong biofilm isolates was done against ciprofloxacin. The bacterial standardization was performed according to 0.5 McFarland turbidity standards **(**Vandepitte *et. al*., 1996; Al-Ouqaili, 2002).

Antimicrobial agents stock solutions were filter sterilized and prepared at concentration (1000µg/ml). Different antibiotic concentrations (0.5-32µg/ml) were prepared in 5 ml of Mueller-Hinton broth and transferred to sterile capped tubes. At least 4-5 morphologically similar colonies were inoculated into Mueller-Hinton broth

and incubated at 37 ^{o}C until the viable turbidity was equal to the 0.5 McFarland, (about 10^8 CFU/ml). After that, the suspension was diluted 1:100 and certain volumes transferred to the tubes containing antibiotic dilutions, to reach a final cell concentration of (about 10^5 CFU/ml). Controls were represented by two tubes; one of them contained broth only and the other contained broth plus microorganism. Then, the tubes were incubated overnight at 37 ^{o}C. The result of minimal inhibitory concentration (MIC) was interpreted as the lowest concentration of antimicrobial agents which inhibits visible bacterial growth after overnight incubation (Ferraro *et al.*, 2002).

2.5. In vitro *biofilm antimicrobial susceptibility test: Biofilm formation by study isolates on catheter segments*

The method used for bacterial biofilm formation on catheter segments was described by Ishida *et al*.(1998). Briefly, the tested bacteria incubated in brain heart infusion broth overnight at 37 ^{o}C. Then, 10 µl of overnight culture was added to 500 µl of sterile media in which catheter segments ($1cm^2$) were inoculated, and subsequently incubated overnight at 37 ^{o}C. After that washing of the segments was achieved by sterile media (3-4) times to remove weakly attached bacteria. Then, segments were resuspended with sterile media and vortexed vigorously for 2 min which was considered as controls.

2.6. Bactericidal activity of antibacterial against biofilm forming sessile cells

To determine the bactericidal activity of selected antibiotics against the sessile cells, the catheter segments were incubated with the organism as described above, were taken out, washed gently with sterile media or saline and, subsequently, transferred to saline containing a given antibiotic with distinct concentrations (10X, 50X, 100X, 500X and 1000X) in which X represented the minimal inhibitory concentration of mentioned antimicrobial agents against planktonic cells which was previously detected. After that, the tubes were incubated for 24 hr. at 37 ^{o}C**.** After exposure of tested organisms to the desired concentrations of antibiotic, they were transferred to 10 ml of fresh brain heart infusion broth and stirred vigorously with a vortex mixer for 2 min. for dispersion sessile or adherent cells. Then, the suspension was diluted and plated on nutrient agar plates for bacterial colony counting and compared with original bacterial count before exposing to antimicrobial agents (Ishida *et al*., 1998).

2.7. Detection of biofilm inhibitory concentrations (BICs) and minimal biofilm eradication concentration (MBECs)

After incubation, the tubes for 24hr. at 37 ^{o}C, the biofilm inhibitory concentration was detected and defined as the lowest concentration of antimicrobial agents which inhibits bacterial biofilm growth on a surface of catheter. It was represented by the clearance of broth medium consisting (1cm) catheters and the required concentrations of antimicrobial agents. After plating the diluted suspension into agar plates and counting the number of bacterial colonies, minimal biofilm eradication concentration (MBEC) was determined. MBEC was defined as the lowest concentration of antibiotic or biocide capable of killing biofilm producer bacteria. It was represented by disappearing of colonies of biofilm producer organisms on the culture plates (Ceri *et al.*, 2006).

2.8. Statistical analysis

All data were analyzed using the SPSS statistical program (statistical Package for the Social Science) version 14.0 and ANOVA test. Statistical significance was taken with p value < 0.05 and 0.001. The significant differences were detected by using either the goodness fit test within chi-square test or independent sample-test. All the study graphics (bar chart, scatter diagram or dot chart) were done by using Microsoft Excel XP (Simon, 2006).

3. Results

3.1. Bacteriological and Lancefield's grouping Identification

Out of 40 study specimens, 25 (62.5) were culture positive cases while 15 (37.5) were culture negative cases. Among culture positive cases, 33 mixed bacterial infection cases were observed consist of both of *Staphylococcus* and *Streptococcus* while *Streptococcus* were identified as pure single culture in 25 (100%) cases (table 1). All α-haemolytic streptococcal isolates 25 (100%) which were submitted to Lancefield's group classification were identified as group F *Streptococcus*.

Table 1. Culture positive and negative cases among study specimens obtained from patients with dental caries.

No. of specimens	Culture positive cases no. %)	Culture negative cases no. (%)	Microbial isolates *Streptococcus* and *Staphylococcus spp.* no. (%)	Group F. *Streptococcus* no. (%)
40	25 (62.5%)	15 (37.5%)	33 (82.5%)	25 (100%)

Out of 25 bacterial study isolates, 20(80%) were biofilm producers distributed into 17 (68%) as strong biofilm producer (OD more than 0.25) and 3 (12%) as weak producer (OD between 0.125-0.25 while 5 (20%) were non-biofilm producer isolates.

Under the field of biofilm production particularly tube method, our results showed that out of 20 isolates (80%)of *mutans Streptococcus* were biofilm producer on the inner lining of the tubes. On the other hand this phenomenon was not observed in 5 isolates (20%).

Furthermore, in the quantitative biofilm formation assay, our results showed that out of 20 (80%) of biofilm producer isolates, 17 (68%) were strong biofilm producer while 3 (12%) were weak producers. All of these events were under the presence of glucose in the experiment.

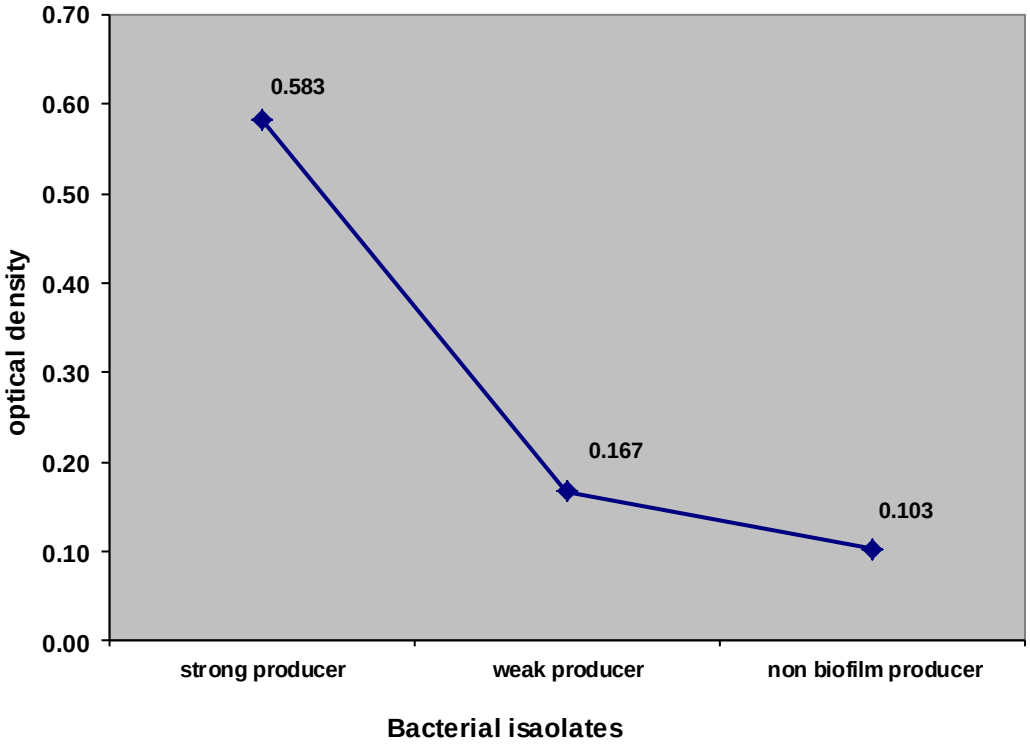

Figure 1. Biofilm production phenomenon regarding isolates potency.

Our results showed that the strong biofilm producer isolates have the highest mean of 0.583in comparison with the non-biofilm producer isolates which have the lowest mean of 0.103 (see Fig. 1). The study revealed that there is a highly significant difference between strong, weak and non-biofilm producer isolates of *mutans Streptococcus* (F-value was 74.882). Further, the age effect on bacterial isolates reveal highly significant difference between them and the F-value was 6.941 (Fig. 2).

Regarding sex differences between volunteers and its relation with biofilm phenomenon, our results showed that male isolates (14 isolates) had the highest mean in biofilm production which was 0.46829 ±0.187714 and for female the mean was 0.40027 ±0.283325.

The result of ciprofloxacin, against sessile cell of *milleri Streptococcus* obtained from patients with dental caries, the BIC of ciprofloxacin against strong biofilm study isolates S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, and S17 were 50 (50X MIC), 800 (50X MIC), 80 (10X MIC), 100 (100X MIC), 160 (10X MIC), 800 (50X MIC), 200 (50X MIC), 100 (100X MIC), 800 (50X MIC), 400 (50X MIC), 400 (50X MIC), 200 (50X MIC), 50 (50X MIC), 800 (100X MIC), 100 (50X MIC), 1600 (100X MIC), and 1600 (50X MIC) µg/ml, respectively. These isolates have shown clearance of broth (inhibition of biofilm) and reduced the viable count of bacterial biofilm from 20 $\times 10^{5,}$ 68×10^5, 75×10^5, 62×10^5, 45×10^5, 37×10^6, 81×10^5, 45×10^5, 43×10^6, 45×10^5, 50×10^5, 61×10^5, 53×10^5, 77×10^5, 120×10^4, 53×10^5, 88×10^5, respectively. On the other hand, the MBEC of 500 (500X MIC), 8000 (500X MIC), 800 (100X MIC), 1000 (1000X MIC), 16000 (1000X MIC), 8000 (500X MIC), 2000 (500X MIC), 1000 (1000X MIC), 8000 (500X MIC), 5000 (500X MIC), 4000(500X MIC), 4000 (1000X MIC), 100 (100X MIC), 4000 (500X MIC),1000 (500X MIC), 16000 (1000X MIC), 32000(1000X MIC) µg/ml were enough to eradicate biofilm of above mentioned isolates respectively (Table 2).

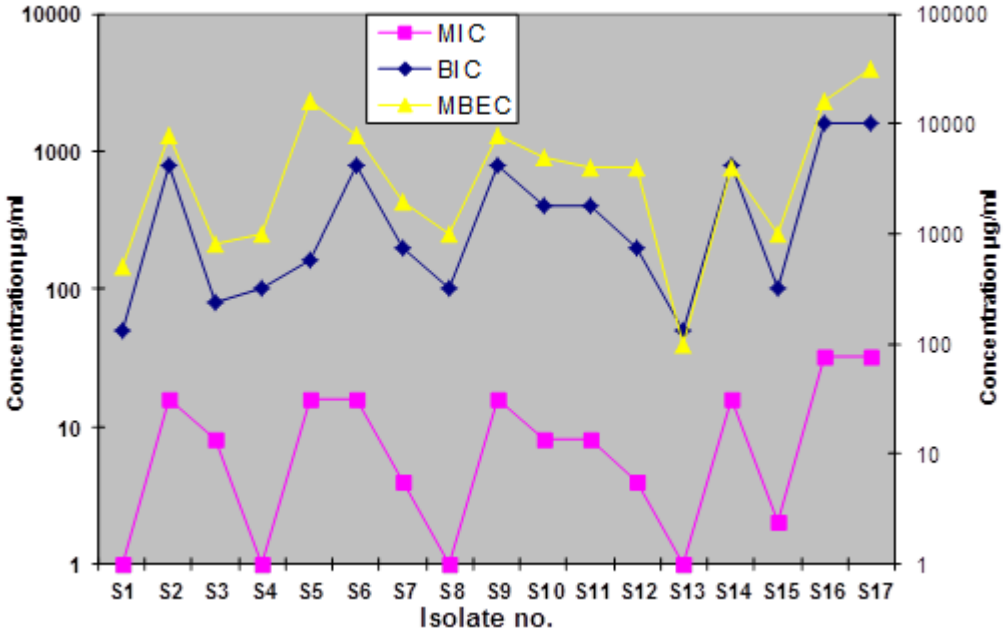

Figure 2. Relationship between of Minimal inhibitory concentration, biofilm inhibitory concentration and minimal biofilm eradication concentrations (µg/ml)

Table 2. The minimal inhibitory concentration (MIC), the biofilm inhibitory concentration (BICs) and minimal biofilm eradication concentration (MBECs) for ciprofloxacin against biofilm producing isolates among study specimens.

Isolate no.	MIC µg/ml	Colony count for control (CFU/ml)	Biofilm Inhibitory Concentration (BIC)			Minimal Biofilm Eradication Concentration (MBEC)		
			No. of folds higher than MIC	Conc. mg/ml	Colony count CFU/ml	No. of folds higher than MIC	Conc. mg/ml	Colony count CFU/ml
S1	1	20×10^5	50 x	50	45	500x	500	zero
S2	16	68×10^5	50 x	800	29×10^2	500x	8000	zero
S3	8	75×10^5	10 x	80	25×10^2	100x	800	zero
S4	1	62×10^5	100x	100	30×10^2	1000x	1000	2
S5	16	45×10^5	10x	160	75	1000x	16000	1
S6	16	37×10^6	50x	800	45×10^2	500x	8000	1
S7	4	81×10^5	50x	200	30×10^2	500x	2000	3
S8	1	45×10^5	100x	100	43	1000x	1000	1
S9	16	43×10^6	50x	800	43×10^2	500x	8000	2
S10	8	45×10^5	50x	400	33	500x	5000	zero
S11	8	50×10^5	50x	400	49×10^2	500x	4000	2
S12	4	61×10^5	50x	200	48	1000x	4000	zero
S13	1	53×10^5	50x	50	33×10^2	100x	100	zero

4. Discussion

As a biofilm, dental plaque exhibits an open architecture much like that of other biofilms. The open architecture, which consists of channels and voids, helps to achieve the flow of nutrients waste products metabolites enzymes and oxygen through the biofilm (Overman, 2000). Because of this structure a variety of microbial organisms can make up biofilms including both aerobic and anaerobic bacteria. Dental plaque biofilms are responsible for many of diseases common to the oral cavity including dental caries, periodontitis, gingivitis and the less common peri-implantitis (similar to periodontitis but with dental implants); however biofilms are present on healthy teeth as well (Sbordone and Bortolaia, 2003).

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased 1000 fold (Stewart and Costerton, 2001). The concept that biofilms are more resistant to antimicrobials is not completely accurate. For instance, the biofilm form of *Pseudomonas aeruginosa* has no greater resistance to antimicrobials, when compared to stationary phase planktonic cells. However, when the biofilm is compared to logarithmic phase planktonic cells, the biofilm does have greater resistance to antimicrobials. This resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells (Spoering and Lewis, 2001).

Technological progress in microscopy, molecular genetics and genome analysis has significantly advanced our understanding of the structural and molecular aspects of biofilms, especially of extensively studied model organisms such as *Pseudomonas aeruginosa*. Biofilm development can be divided into several key steps including attachment, microcolony formation, biofilm maturation and dispersion; and in each step bacteria may recruit different components and molecules including flagellae, type IV Pili, DNA and exopolysaccharide (Jarrell, 2009; Ullrich, 2009). The rapid progress in biofilm research has also unveiled several genetic regulation mechanisms implicated in biofilm regulation such as quorum sensing and the novel secondary messenger cyclic-di-GMP. Understanding the molecular mechanisms of biofilm formation has facilitated the exploration of novel strategies to control bacterial biofilms (An, 2010).

Biofilms, by their nature, are very resistant to change, and when they do change, it usually takes time for evolution of the bacterial species to occur. Modifying pressures can cause a change from constant overload of pathogenic organisms, external risk factors and risk behavior. These can all lead to environmental changes within the biofilm, which favor the proliferation of aciduric and acidogenic pathogenic species like *mutans Streptococcus* and *Lactobacilli*, that help them to take over the biofilm (Busscher and Evans, 1998). A cariogenic biofilm may consist of over 96% acidogenic/aciduric, pathological bacteria, compared to less than 1% in a healthy biofilm. When all the factors that may contribute to a biofilm evolution are examined, it appears the primary driver is an acidic pH shift that can be either extrinsic or intrinsic to the dental biofilm or both (Marsh, 2006).

It is remarkable that the biofilm (adherence) mode of growth of bacteria on the tooth surfaces in the oral cavity has been well documented as one of the important causes of dental caries. Numbers of tests are available to detect slime production by bacteria, including tissue culture plates or spectrophotometric assay tube method (Mathur *et al*., 2006). Under the field of biofilm production, particularly qualitative adhesion assay, tube method, our results showed that out of 20 isolates (80%) of *mutans Streptococcus* were biofilm producer in the inner lining of the tubes; this phenomenon was not observed in 5 isolates (20%). These results were in agreement with Zezhang and Robert (2002) who found the genes required for biofilm development by *S. mutans* isolated from the oral cavity. In the quantitative biofilm formation assay, spectrophotometric method with ELISA reader was achieved under the presence of glucose and the crystal violet stain was dependent in this technique. Because crystal violet uniformly stains bacterial cells regardless of the presence or absence of slimy materials, properly speaking, the optical densities of bacterial films stained with crystal violet indicate the concentration of bacteria on the surface of the plates, not the presence of the slime. Thus, researchers considered these readings as index of the adherence of an organism to a surface and not a measure of slime production. Also the same researchers used weight of bacterial biofilm through counting the chromosomes by measuring the DNA content as an index to the number of bacterial cells per gram of film. They concluded that the weight of bacterial cells in biofilm was relatively constant among study isolates and eventually indicated that measuring of optical densities by spectrophotometric assay is a reasonable method for comparing the adherence phenomenon to plastic surfaces.

In order to enable easier study of bacterial attachment and colonization, a variety of experimental, direct and indirect observation methods have been developed. Microtiter plate assay (spectrophotometric assay) is the most frequently used techniques for quantifying biofilm formation (Stepanovic *et al.*, 2003). Microtiter plate assay is an indirect method for estimation of bacteria in situ, it has the advantage of enabling researcher to rapidly analyze adhesion of multiple bacterial strains or growth conditions within each experiment, easy technique and used widely for antimicrobial agents susceptibility of biofilm. In this technique our results showed that among17 isolates (68%) of bacterial isolates were strong biofilm producer and 3 (12%) were weak biofilm producer isolates and highly significant difference was observed between groups of isolates in their ability of biofilm production. Our results were in coincidence with the observation laid down by Christensen and co-workers (1985) that bacterial adherence and glycocalyx formation was enhanced with the supplementation of glucose in the culture media.

It is well authorized that biofilms are considered to be highly resistant to antimicrobial agents. Generally speaking, this is not the case – biofilms that grow in the presence of antimicrobials better than planktonic cells do.

Biofilm is indeed highly resistant to killing by bactericidal antimicrobials, compared to logarithmic-phase planktonic cells. This should properly be referred to as phenotypic tolerance or tolerance. Several factors have been suggested to account for biofilm tolerance, slow growth, the presence of an exopolysaccharide matrix that can slow the diffusion of antimicrobials; multiple resistance pumps represent a generalized resistance mechanism and have been considered as candidates for a biofilm resistance mechanism (Spoering and Lewis, 2001). Antimicrobial susceptibility test for sessile cells of strong biofilm study bacterial isolates was achieved by detecting minimal biofilm eradication concentration (MBEC) was based on minimal inhibitory concentrations obtained by broth macrodilution technique achieved logarithmic phase planktonic cells of these bacteria. Regarding to ciprofloxacin our result revealed that the biofilm cells were required 10-100 times the MIC values for inhibition of bacterial biofilm while 100-1000 times the MIC values were needed to remove bacterial biofilm.

Virtually all antimicrobials are more effective in killing rapidly growing cells. Some antibiotics have an absolute requirement for cell growth in order to kill some of the more advanced β-lactams, flouroquinolones can kill non growing cells, but they are distinctly more effective in killing rapidly dividing cells. Slow growth undoubtedly contributes to biofilm resistance to killing (Al-Doori, 2009).

The study concluded that group F *milleri Streptococcus* isolated from patients with dental caries produced cariogenic biofilm qualitatively and quantitatively. Also, in terms of BICs and MBECs, the biofilm producer isolates were required 10-50 X MICs of ciprofloxacin to inhibit bacterial biofilm and 100-1000 X MICs to remove of bacterial biofilm in patients with dental caries.

References

Al-Doori,R.A.H.2009.Microbiological and clinical parameters of microbial biofilm in patients with upper and lower urinary tract infections. (M.Sc. thesis). College of Medicine University of Al-Anbar, Iraq.

Al-Ouqaili, M. T. S. 2002. In terms of MIC_{50} and MIC_{90}, the role of extended and non-extended spectrum beta-lactamase in the resistance of enteropathogenic *Escherichia coli* to beta-lactams. Al-Anbar.Med. J. **4**:16-20.

Amy P. 2000. **Understanding Biofilms**; Marshal protocol.

An S. 2010. **The Impact and Molecular Genetics of Bacterial Biofilms. Environmental Molecular Microbiology**. Caister Academic Press.

Baron EJ, Peterson L R. and Finegold S. M. 1994. **Bailey and Scott's Diagnostic Microbiology**. Toronto:C.V. Mosby Company .9th edition.

Brooks G F, Butel JS. and Morse S A. 2004. **Jawetz, Melnick and Adelbergs: Medical Microbiology**. McGraw-Hall. 23rdedition.

Burne R A. 1998a. Oral *Streptococci*: products of their environment. J. Dent. Res., **77**:445-452.

Burne R A. 1998b. Regulation of gene expression in adherent population of oral *Streptococci*. In: **Microbial Pathogenesis: Current and Emerging Issues.** D J LeBlanc, M S Lantz, and L M Switalski (Eds.), Proceedings of the 2nd Annual Indiana Conference. Indiana University Press.

Busscher HJ. and Evans LV. 1998. **Oral Biofilms and Plaque Control**. Gordonand Breach Publishing.

Ceri H, Olson M E, Morock D W. and Storey D. G. 2006. Minimal Biofilm Eradication Concentration (MBEC) Assay: Susceptibility Testing for biofilms. In: **Biofilms Infection and Antimicrobial Therapy.** Pace, J.L., Rupp, M. E., Finch, R. G. (Eds), London. Taylor and Francis group.

Christensen GD, Simpson W A, Younger J A, Baddour LM, Barrett FF. and Melton DM. 1985. Adherence of coagulase negative *Staphylococci* to plastic tissue cultures: a quantitative model for the adherence of *Staphylococci* to medical devices. J. Clin. Microbiol., **22**:996-1006.

Featherstone JD. 2003. Caries management by risk assessment: consensus statement. J Calif Dent Assoc., **31**: 257-269.

Ferraro M J, Craig W A, Dudly M N, Ehopoulos G M. and Hecht D W. 2000. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved Standard, 5th edition. NCCLS. **20**:1-24.

Florio FM, Klein MI, Pereira AC. and Goncalves BR.2004. Time of initial acquisition of *mutans Streptococci* by human infants. J Clin Pediatr Dent., **28**: 303-308.

Gauduchon V , Benito Y, Célard M, Mouren C, Delorme V, Philippe-Bert J, Etienne J. and Vandenesch F. 2000. Molecular diagnosis of recurrent *Streptococcus mutans* endocarditis by PCR amplification and sequencing. Clin Microbiol Infect J., **7**: 36–37.

Hall-Stoodley L, Costerton JW. and Stoodley P. 2004. Bacterial biofilms: from the natural world to infectious disease. Nat. Rev. Microbiol.,**2 (2):** 95–108.

Ishida H, Ishida Y, Kurosaka Y, Otani T, Sato K. and Kobayashi, H. 1998. *In vitro* and *in vivo* activities of levofloxacin against biofilm-producing *Pseudomonas aeruginosa*. J Antimicrob Agents Chemother., **42**:1641-1645.

Jarrell K. 2009. **Pili and Flagella: Current Research and Future Trends**. Caister Academic Press.

Marsh P. 2006. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health **6**: S14.

Mathur T, Singhal S, Khan S, Upadhyay D J, Fatma T. and Rattan A. 2006. Detection of biofilm formation among the clinical isolates of *Staphylococcus:* An evaluation of three different screening methods. Ind J Med Microbiol., **24**:25-29.

Overman PR. 2000. Biofilm: A new view of plaque. J. Contemporary Dental Practice. **1(3):** 18.

PaesLeme AF, Koo H, Bellato CM, Bedi G. and Cury JA. 2006. The role of sucrose in cariogenic dental biofilm formation: new insight. J Dent Res., **85**:878-887.

Paul Stoodley, James W, Armin G, Dirk D. and Christiane O. 2008. Biofilm plaque and hydrodynamic effects on mass transfer, fluoride delivery and caries. J Am Dent Assoc. **139**: 1182-1190.

Rogers A H. 2008. **Molecular Oral Microbiology**. Caister Academic Press.

Sbordone L. and Bortolaia C. 2003. Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. Clin Oral Invest., **7**: 181-188.

Simon S.D. 2006. **Statistical Evidence in Medical Trials. What do the Data Realy Tell us**? Oxford University Press.

Spoering A. and Lewis K. 2001. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. J. Bacteriol., **183** : 6746–6751.

Stepanovic S, Djkic V, Djordjevic V. and Djukic S. 2003. Influence of the incubation atmosphere on the production of biofilm by *Staphylococci*. Clin. Microbiol. Infect., **9**: 955-959.

Stewart P. and Costerton J. 2001. Antibiotic resistance of bacteria in biofilms. Lancet **358** (9276): 135–138.

Ullrich M. 2009. **Bacterial Polysaccharides: Current Innovations and Future Trends**. Caister Academic Press.

Vandepitte J, El-Nageh M, Tikhomiros E. and Stelling JM.1996. Guidelines for Antimicrobial Resistance Surveillance. World Health Organization. Alexandria, Egypt.

Yassien M. and Khardori, N.2001. Interaction between biofilms formed by *Staphylococcus epidermidis* and quinolones. J Diag Microbiol Infect. Dis. **40**: 79-89.

Zezhang T W. and Robert A. B. 2002. Analysis of cis-and trans-Acting factors involved in regulation of the *Streptococcus mutans* fructanase gene (fru A). J Bacteriol., **184**:126-133.