Role of *Streptococci* as etiological agents of dental caries

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**Abstract**

Dental plaques are notorious and lead to dental caries responsible for dental decay. *Streptococci* are the leading microorganisms associated with dental plaques. These are Gram-positive, normal microbial flora, non-motile, non-spore forming, and facultative anaerobes. These include Alpha, Beta, and Gamma hemolytic species. *Streptococcus* sp. produces a high amount of lactic acid through the fermentation of sugars, causes lowering of the pH leading to the plaque formation around teeth, and serves as a biofilm. Microbial biofilm provides certain attachment sites for growth and colonization of other bacteria, and also causes resistance to the antimicrobial agents. These *Streptococci* can be transmitted to the infants through parents or caretakers' kiss. This mode of transmission is the key role for the contribution of *S. mutans* in dental caries. In Pakistan, a national survey was conducted in 1992 on tooth decay, which showed that two teeth per person were decayed, missing, or filled, thus becomes an alarming situation. Minimal knowledge related to tooth decay is observed in the clinical settings; however, no similar studies have been carried out in Pakistan.

**Keywords:** *Streptococci*, Dental plaques, Lactic acid, Sugars, Fermentation

1. **Introduction**

The oral cavity is the initial portion of the Gastrointestinal tract (GIT), and it includes several features such as; adhesion ligands, pH, temperature, and redox potential, which makes the oral cavity a distinct habitat for the oral microflora. Abusleme *et al.*, (2013) demonstrated that a few surfaces present in the oral cavity are continuously washed by saliva (which acts as a buffer), and yet they exhibit ecological habitats to some microbes that ultimately reside there. There are numerous bacteria present in the oral cavity including: Gram (+) bacteria such as *Lactobacillus*, *Actinomyces*, *Eubacterium*,...
Corynebacterium, Staphylococcus, Peptostreptococcus, Streptococcus, and Enterococcus are Gram-positive, and Gram (-) bacteria such as Aggregatibacter (formerly Actinobacillus), Prevotella, Wolinella, Leptotrichia, Prophyromonas, Eikenella, Haemophilus, Capnocytophaga, Bacteroides, Tannerella, Treponema, Fusobacterium, and Campylobacter. Some other potentially pathogenic bacteria exist in the oral cavity include; Streptococcus pneumonia, Streptococcus pyogenes, Neisseria meningitidis, Haemophilus influenza, and some members of Enterobacteriaceae, as reported by Bowen and Koo, (2011).

Streptococci as Gram-positive bacteria are significantly important due to its crucial role in industry and medicine. A previous study conducted by Cephas et al., (2011) highlighted that ecologically, the normal microflora of humans and animals, are non-motile and non-spore formers. They are mostly facultative anaerobes lacking the catalase enzyme, unlike the Staphylococci. Later, Chandrabhan et al., (2012) added that usually complex culture media are required for their growth. Based on their hemolytic properties Streptococci are classified into three groups, i.e. alpha, beta and gamma hemolytic.

Beta-hemolytic Streptococci cause complete hemolysis of blood cells and were further classified by Lancefield grouping into 20 groups. Lancefield grouping is a serotype grouping based on the specific carbohydrates present in the cell wall. Medically, the alpha-hemolytic and beta-hemolytic Lancefield group A and group B Streptococci have important roles in causing many diseases (Chu et al., 2016). Chun et al., (2015) reported that regardless of the commensal and significant properties of the genus Streptococci, certain species are involved in sub-acute, acute to chronic infections including; meningitis, pneumonia, pharyngitis, endocarditis and dental caries. Particular cell-associated and extracellular determinants are also involved in the pathogenesis.

Chu et al., (2016) reported that oral Streptococci exhibit distinct features, such as being able to survive in an acidic environment (aciduricity), acid production (acidogenicity), and production of extracellular and intracellular polysaccharides. Hoiby et al., (2011) added that they synthesis and produce endodextranase, which enable these bacteria to be involved in the formation of dental plaque. These bacteria also have an enzyme called Glucosyltransferase (GTF), which uses sugars like sucrose as a substrate to produce the extracellular polysaccharides, due to this characteristic Streptococci can accumulate and attach to the tooth surfaces (Hoiby et al., 2011).

Inside the oral cavity, the first step of the establishment of bacteria as a diverse microbial community is the attachments of these bacteria to oral linings (Hooper et al., 2011). This is followed by attachment of similar bacterial species that afterwards adapt the adjacent conditions, and make them favorable for colonization by other microbes as well. This stable establishment of microbes is called the climax community. Jubair, (2015) revealed that the most important environmental factors (i.e. pH and redox potential) that are essential for breakdown of the microbial homeostasis changes, which results in the development of many diseases mainly, periodontitis and dental caries.

Dental caries and cavities are due to the bacterial activity causing the breakdown of teeth. Symptoms start with the pain in the tooth and difficulty in eating, followed by tissue inflammation around the tooth, which ultimately results in tooth loss (Legenova and Bujdakova, 2015). Several varieties of oral bacteria are present in the mouth, but only a few are involved in causing dental plaque, these include S. mitis, S. mutans and Lactobacillus. A recent study of Matsumoto-Nakano, (2018) reported that Streptococci supersedes all other bacteria in terms of causing dental caries as they produce high amounts of lactic acid through fermentation of sugars, and they resist the effect of low pH resulting in plaque formation around teeth.

Streptococcus mutans is primarily involved in dental plaque formation. Except for the anterior teeth, the molar teeth are profoundly colonized, and the gaps
between them are massively vulnerable to the colonization of microbes, compared to the buccal cavity and the approximal groove of teeth. The use of chlorhexidine (antiseptic) provides more evidence about the attachment of Streptococci to the tooth surfaces, and its long-term use can cause discoloration of the teeth surfaces (Marsh, 2012).

Tellez et al., (2010) demonstrated that co-aggregation is a process of physical interface between the different bacterial species, and this process is not random among the oral microbiota, each bacterium attaches significantly to the others. The distinct biofilm of microbes present on the tooth enamel was initially determined as a colorless adhesive deposit. Still, with the time, it forms tartar, which is brown or pale yellow (not readily cleaned by rinsing water), and is described as a plaque. According to the plaque locality, it is of two types: 1- Supra-gingival (above the gum line), and 2- Sub-gingival (within the gum line) plaques (Tellez et al., 2010). A recent study conducted by Banas and Drake, (2018) highlighted that the process of development of dental plaque mainly takes place in three steps: adhesion of initial bacterial colonizers to the teeth surface, production of extracellular polysaccharides by these colonizers, finally multiplication and maturation of the other bacteria on the teeth surface.

A previous study of Bowen, (2016) reported that antibiotics are considered to be the miracle drugs discovered so far for the health benefits of human beings and animals as well. Due to their excessive use, bacteria started to produce some resistance mechanisms against the antibiotics by specific defence mechanisms.

In case of dental infections, both of the Gram-positive and Gram-negative bacteria are involved; however, certain antibiotics including; beta-lactams, amoxicillin, metronidazole, tetracycline, macrolides and lincosamides are used widely to treat these dental bacteria. Most of these antibiotics used in dentistry are involved either in inhibiting the bacterial cell wall synthesis, or the inhibition of protein and RNA synthesis. Specifically talking about S. mitis and S. mutans, these Streptococci develop resistance against the prescribed antibiotics, through enzymatic inactivation and target site modifications, as reported by Lin et al., (2018).

2. Natural resistance of oral microflora

Natural resistance of bacteria also called intrinsic resistance could be attributed to the absence of particular structures and/or lack of specific metabolic pathways that are crucial for the activity of the antibacterial agents. In oral microflora, natural resistance is attributable to the absence of specific metabolic processes. For instance, the enzyme nitroreductase, which is essential for the conversion of metronidazole to its metabolic products, is not present in Actinomyces spp., Streptococcus spp. and Aggregatibacter sp. Besra and Kumar, (2016) added that the activity of metronidazole does not harm these microorganisms at regular therapeutic meditations.

3. Acquired resistance of oral microflora

Compared to the mechanism of natural resistance, acquired resistance is present in certain bacterial species due to genetic variation, and can be attained by two primary mechanisms mainly:

-Chromosomal alteration in the established bacterial genome

-Horizontal gene transfer between the bacterial species

According to Daboor et al., (2015), the horizontal gene transfer mechanism (most frequent pathway) permits the bacterial inhabitants to develop acquired resistance against the antibiotics at a higher level, which is considerably greater than that would be afforded by the chromosomal alterations. A recent study of Childers et al., (2017) revealed that due to the low frequency of side effects, low-cost effectiveness and appropriate antibacterial spectrum, penicillin group of antibiotics have long been used as the first line of defence against the dental diseases. These are conventional antibiotics that can interfere with dental
biofilm development. Additionally, penicillin antibiotics are not suitable for prolonged use, due to the development of resistance and ecological disparity favoring the adaptable dental diseases.

A study carried out by Daboor et al., (2015) highlighted that resistance to penicillin is not collective in the *Streptococcus* spp. In the case of dental caries; however, certain Gram-negative bacteria show an increased level of resistance against penicillin as compared to the Streptococci. According to Hassan et al., (2015), *Streptococci* were reported in 1989, after the first report of the production of beta-lactamases. The consideration started that *S. mutans* is also a significant contributor in dental caries, due to its ability to metabolize carbohydrates such as sucrose and glucose, with the production of high level of acids and enhancing biofilm production. Microbial biofilm provides specific attachment sites for bacterial colonization, growth and resistance to the antibacterial agents, as reported by Zeng and Burne, (2016).

Resistance to tetracycline and this family is also notable in case of *S. mitis* and *S. mutans*. Children are more susceptible to infection by tetracycline-resistant bacteria, compared to other age individuals. A recent study conducted by Colombo and Tanner, (2019) highlighted that almost 27 of the tetracycline-resistant genes have been discovered so far. Previously, Hemadi et al., (2017) reported that although the use of tetracycline is limited in dentistry due to its side effects related to the change in tooth color; however, the combination of antibacterial drugs like tetracycline with penicillin and erythromycin are making an alarming situation, due to the emergence of tetracycline resistance.

Erythromycin as a member of the macrolide’s family is widely used in dentistry, and its resistance in *S. mitis* and *S. mutans* is also increasing in an alarming level. Resistance patterns of erythromycin and clarithromycin are almost similar in the recent reported studies (Lemos et al., 2019). The resistance mechanism of macrolides is attributed to the methylation of adenine bases in 23S rRNA. Chu et al., (2016) demonstrated that the co-transfer resistance of three classes of antibiotics named as tetracycline, erythromycin and beta-lactam are mostly reported, because of the horizontal transfer of the resistance genes.

In Pakistan, a national survey was conducted in 1992 on tooth decay which showed that two teeth per person were decayed, missing or filled that was an alarming situation. This may become more lethal due to excessive usage of sugars and sweets after effective preventive treatments. Since then, unfortunately, the surveillance of tooth decay in Pakistan has not been considered; it is a neglected field in Pakistan.

4. Epidemiology of the oral *Streptococci*

The ecosystem of the oral cavity is diverse, which constitutes a unique niche for the existence and survival of microorganisms (Marsh, 2012). The composition of this oral microflora is very complex, so that an average of 700 cultivable and non-cultivable species exists as normal inhabitants (Salman et al., 2017).

Current studies demonstrated that, by using 16SrRNA amplification process; an average of 40 % of the bacterial species of unique phylotypes can be characterized. Most probably, many of these bacterial cannot be isolated and purified by using routine culture techniques. The alpha-hemolytic *Streptococci* are among those bacteria that are recovered by usual cultural methods (Marsh, 2016).

Chakraborty and Burne, (2017) revealed that Streptococci are natural inhabitants of the oral cavity. Naturally, they are commensals but are involved in the formation of biofilm on the tooth, and lead to tooth decay known as dental caries. Both, *S. mutans* and *S. mitis* are notorious in this situation causing deleterious effects that range from simple change in color of the teeth, to acute and even chronic tooth decay, eventually lead to tooth loss.

Several epidemiological studies revealed that *S. mutans* is the primary etiological agent of dental caries
in humans (Lemos et al., 2019; Hossain et al., 2020; Li et al., 2020). These studies demonstrate that the bacterial populations comprise a relatively high ratio of S. mutans with a high incidence of dental caries, compared to those populations that have low incidence. Another study of Moayad et al., (2015) demonstrated that reduction in dental caries was analogous to the decrease in the salivary counts of S. mutans in Swedish children. Moreover, reduction in the rate of S. mutans in pregnant females led to minimizing the colonization and development of caries in their children (Hossain et al., 2020). According to Marsh, (2016), many studies revealed that these Streptococci are commonly isolated from well-developed carious lesions, but less frequently from sound tooth surfaces.

A recent study of Ramadugu et al., (2020) demonstrated that transformation of bacteria from the mother to child is an initial route for their primary acquirement of oral microflora. After birth, the first time when the mother feeds her baby, several bacteria enter and colonize the oral cavity of an infant as normal flora. A recent study demonstrated that high complexity of bacteria was observed in the saliva of newborns and adults (Saputo et al., 2018). Many bacterial genera including Fusobacterium, Rothia, Gemella, Neisseria, Haemophilus, Granulicatella, Veillonella and Leptotrichia were present in newborn babies, but the genus Streptococci was the most dominant. However, in adults, Fusobacterium, Actinomyces, Rothia, Treponema, Neisseria, Oribacterium, Veillonella and Haemophilus were the most prominent rather than Streptococci (Ranganathan and Akhila, 2019). Several immunological studies also revealed evidences about the dominant role of S. mutans in the etiology of dental caries in humans. Generally, populations with a low incidence of dental caries have high titers of serum and salivary antibodies to S. mutans antigens; conversely, the frequency of dental caries is relatively high in the immunocompromised patients, as reported by Al-Shami et al., (2019). Another study of Karikalan and Mohankumar, (2016) showed that the existence ratio of S. mutans in dental plaque rose in a period of 6 to 24 months, before the clinical appearance of dental caries/cavities.

5. Oral Streptococci infection and antibiotic resistance

Due to the destructive potential of the oral microflora (S. mutans, Lactobacilli and Actinomyces), they can attach and colonize on the hard surfaces of teeth. Nobbs, (2017) added that the increased level of dental plaque is associated with excessive usage of sucrose-containing diets that leads to the formation of organic acids, such as lactic acid. A recent study of Giacaman, (2018) revealed that as a result of acid production, the pH of mouth decrease (becomes acidic) that favor the survival of some microbes in such an acidic environment. These organic acids cause decalcification of the tooth enamel by dissolving calcium phosphate. Tahir and Nazir, (2018) added that the habitat of the oral microbiome may be disturbed due to several factors occurring in the mouth including; the use of sugar-containing diet, prolonged use of antibiotics, variability in salivary flow, tooth extraction, caries, cavities and dentures.

A study was conducted by Matsumoto-Nakano, (2018) on the relationship between biofilm formation of different Streptococci isolated from the oral cavity and their antibiotic sensitivity. Results of this study showed that 71% of the isolates belong to S. viridans related to the Streptococci group. However, S. mutans has a better ability to produce the biofilm. Antibiotic resistance profiling of the Streptococci showed more resistance to amoxicillin and erythromycin than the other antibiotics. Most of the isolates were sensitive to the cefotaxime and ciprofloxacin. Recently, Manji et al., (2018) study indicated that entrance and establishment of S. mutans and other biofilm-forming bacteria in children, occur via direct or indirect exposure to the closest persons such as mothers and caregivers, who have particular dental plaque or dental infections in their oral cavities. Age factor is also involved in dental caries; children are sensitive to dental caries than other age groups. A study conducted
in Japan by Li et al., (2020) on infective endocarditis with children and adults concerning amoxicillin resistance, where nine bacterial strains were recorded as resistant to this antibiotic. In addition, 16S rRNA sequencing of each of the isolated strains showed that all belong to the oral Streptococci.

S. mutans is the major contributor to human dental plaque formation. A study conducted by Salman et al., (2017) on the isolation and biochemical characterization of S. mutans showed that almost all the samples were positive with bacterial growth on selective Mitis Salivarius (MS) medium, recording 10⁴ cells/ml. About 60% of the isolates were considered to be related to Streptococci and especially to the mutans group including; S. mitis (40%), S. mutans (30%) and S. rattus (21%), detected using sero-grouping systems. Antibiotic sensitivity was checked with optochin, vancomycin and bacitracin antibiotics, which showed that more than 50% of the isolates were resistant towards two antibiotics only, whereas 10% of the isolates were resistant to the three antibiotics.

Several susceptibility tests were carried out by Lemos et al., (2019) on 207 isolates of oral Streptococci including; S. mutans, S. mitis, S. salivarius and S. oralis. Results presented that only S. mutans was significantly susceptible to penicillin antibiotic. A study conducted by Potgieter et al., (1992) stated that S. mitis with 16-32 mg/ml MIC value was resistant to penicillin and other antibiotics including; aminoglycosides, kanamycin, gentamycin and tobramycin. In addition, a recent study of Arora et al., (2018) also demonstrated the susceptibility of S. mutans to penicillin and many other antibacterial agents.

A study conducted by Lamont et al., (2018) on oral Streptococci in healthy Greek kids demonstrated that out of 200 isolates, 77(38.5%) of isolates were resistant to erythromycin, while 67(33.5%) of the isolates were resistant to clarithromycin, although the MIC value of erythromycin was double that of clarithromycin. About 53% of the S. oralis isolates exhibited a maximum resistance to erythromycin.

Moreover, 44% of S. sanguis isolates and 48% of S. salivarius isolates were also resistant to this antibiotic.

A study was conducted by Xin et al., (2016) to demonstrate the resistance pattern of oral Streptococci against amoxicillin and erythromycin antibiotics, in patients that were at higher risk from infective endocarditis (a problem of heart valves). About 54 samples of the gingival crevicular flora (at the place of any dental surgery) were collected from those patients who received prophylaxis of amoxicillin, and 11 samples from those who received prophylaxis of erythromycin. Meanwhile, 65 samples were also collected from those patients who did not receive any antibiotic prophylaxis, and were not at risk from infective endocarditis. Results indicated that the MIC of amoxicillin (>24 mg/l) was not recorded by any isolate. While, MIC of > 3.5 mg/l was recorded by erythromycin-resistant oral Streptococci including S. sanguis biotype I and II, S. salivarius and S. mitis. These isolates were recovered from those patients who were receiving prophylaxis of amoxicillin (22%), erythromycin (9%), and from those who were not receiving any prophylaxis (9%), respectively.

A comparative study was conducted on young children investigating the effect of amoxicillin on oral microbiota. About 40 children were selected for screening that was divided into two groups; 15 of them used amoxicillin, and 25 did not use amoxicillin at least 3 months before sampling. Amoxicillin resistant Streptococci were detected in 10% of the children who used amoxicillin, while 2.4% were recorded in those children who did not use amoxicillin. The main isolated genera were Streptococcus and Haemophilus (Krzysciak et al., 2014).

Another study of Kuramitsu and Wang, (2011) was conducted in Japan with amoxicillin resistant adults infected with endocarditis and dental plaque. Seven strains of Streptococci were isolated from the endocarditis patients, which were resistant with amoxicillin. All of the isolates were also resistant with other commonly used drugs in clinical settings, except the quinolones.
A study conducted by Argimon and Caufield, (2011) compared the antibiotic susceptibility profile of \emph{S. viridans} isolated from the oral cavity of healthy children and those children with disabilities in Kuwait. A total of 204 dental plaque samples were collected from the teeth and tongue surfaces of healthy and disabled institutionalized children. The resistance to seven antibiotics including: amoxicillin, vancomycin, erythromycin, tetracycline, cephalothin, penicillin G and clindamycin were tested. A total of 330 (44.5%) and 411 (55.5%) of \emph{S. viridans} isolates were recovered from the healthy and intellectually disabled children, respectively. The most dominant isolates were \emph{S. salivarius} (27.3%) in healthy and \emph{S. sanguis} (22.6%) in the disabled children. The combined rate of resistant strains (from healthy and disabled children) was found to be highest with amoxicillin (43%) and lowest with vancomycin (12%). In healthy children; \emph{S. sanguis} (45%), \emph{S. mitis} (56%) and \emph{S. oralis} (55%) were more resistant, compared to the disabled children 40%, 47% and 47%, respectively. \emph{S. mutans} was the least resistant species to all antibiotics in both groups of children. These data revealed that \emph{S. viridans} isolated from the oral cavity of healthy and disabled children showed a difference in their resistance level against commonly used antibiotics in dentistry, in accordance with the previous study of Nurelhuda \emph{et al.}, (2010).

6. Antibacterial agents against the oral \emph{Streptococci}

According to Juneja and Kakade, (2012), several epidemiological studies revealed that notable reduction in the prevalence of dental caries occurred in the developing countries, due to the advancement of oral health by water fluoridation, usage of fluoride toothpaste, healthful nutrition comprising sucrose alternatives and oral health training.

Raul \emph{et al.}, (2020) reported that many antimicrobials such as chlorhexidine and several types of mouthwash having fluoride are available in the market, which can change the overall profile of the oral microorganisms; however, they cause several adverse effects including; vomiting, diarrhoea and yellowing of teeth. Since the earliest era, various medicinal plants/herbs have been used for oral hygienic purposes against the initial plaque colonizers, i.e. \emph{Streptococci}. For example, Hossain \emph{et al.}, (2020) revealed that crude aqueous extract of \emph{Mangifera indica} exhibits productive bactericidal activity against \emph{S. mutans} and \emph{S. mitis}. Moreover, with the MIC of 2 mg/l ml, \emph{Isertia laevis} (belongs to the Rubiaceae family) effectively inhibits the growth of \emph{S. sorbinus} and \emph{S. mutans}. Correspondingly, the boiled aqueous extract of \emph{Coffea arabica} L. significantly reduces the attachment of \emph{S. mutans} ATCC 35688 to the tooth enamel and the dentine (Bowen and Koo, 2011). A recent study conducted by Bescos \emph{et al.}, (2020) also proved that the regular use of chlorhexidine (a broadly active plaque-control agent) is helpful in causing noticeable reduction in the ratio of \emph{S. mutans} and the incidence of dental caries.

A study of Liu \emph{et al.}, (2011) was conducted to demonstrate the antibacterial effect of Citrox®; prepared from bioflavonoids that are acquired from citrus fruits, on the oral microflora. Two forms of Citrox® (1- BC30 and 2- MDC30) were investigated with various combinations of bioflavonoids against 20 oral microbes (14 bacterial spp. and 6 \emph{Candida} spp.), by using the serial dilution method. Both formulations exhibited bactericidal potential, but the BC30 formulation was significantly active against all the bacterial spp. and some \emph{Candida} spp. at 1% (v/v) concentration.

Earlier, Giacaman \emph{et al.}, (2010) conducted a study to check the effectiveness of rubusoside (non-caloric sweetener) extracted from \emph{Rubrus suavissimus} (which belongs to the Rosaceae family), on minimizing the dental caries caused by \emph{S. mutans}. The effectiveness of rubusoside was examined by quantifying the optical density of the bacterial culture using a spectrophotometer at 450 nm. In addition, the influence on acidogenicity of \emph{S. mutans} was investigated by calculating the pH of the culture. Many other alternating sugars like glucose, sucrose, maltose, fructose and xylitol were also used to compare the activity of rubusoside. Results indicated that the group
treated with rubusoside showed minimum growth of S. mutans, compared to the other sugars. Moreover, acid production and attachment to glass surface were also notably minimized by rubusoside.

Natural compounds of individual medicinal plants are used as modulators of bacterial biofilm production by the oral bacteria. A clinical study was carried out by Hassan et al., (2012), to test the antibacterial effect of miswak in toothpaste on the dental caries patients, with particular interest of acid production by S. mutans and Lactobacilli. A total of 45 patients between the ages of 20-45 were selected for this study, and were divided into four groups. Approximately, 50 % of the miswak extract was used with the toothpaste. Significant decrease in the population of S. mutans was observed after miswak treatment; however, non-significant reduction in Lactobacilli was observed. Accordingly, this study concluded that the antibacterial effect of miswak in toothpaste and/or miswak without toothpaste has an immediate antibacterial potential.

Recently, Philip et al., (2018) reported that methanolic extract of Pediomelum cuspidatum has shown a bactericidal and inhibitory potential on the virulence factors of S. sorbinus and S. mutans. Another earlier study of Ito et al., (2011) focused on evaluating the bactericidal effect of root fractions of P. cuspidatum on the dental caries related factors of S. sorbinus and S. mutans. At MIC levels, F1 (an ethyl acetate fraction) showed inhibitory effect on S. sorbinus and S. mutans biofilm formation. The activity of F1 was attributed to the presence of terpenoids, phenolic compounds, cardiac glycosides and anthraquinones. Elsalhy et al., (2013) study demonstrated that an ethyl acetate fraction F1 separated from P. cuspidatum primarily composed of; physcion (2.07%), resveratrol (16.2%) and emodin (18.9%) of the weight of F1, showed an inhibitory efficacy on the acidogenicity and F-ATPase activity of S. mutans in the biofilms.

Eugenia caryophyllata (clove) plant belongs to Myrtaceae family, is extensively used around the world and well recognized for its antiseptic properties, as reported by Simon-soro et al., (2013). Conventionally, clove essential oil is widely used in dental care as an analgesic and antiseptic agent against the oral bacteria involved in dental caries, and periodontal disease. In addition, this plant is also effective against many other pathogenic bacteria. Due to effective analgesic, fungicidal and bactericidal potentialities of eugenol (the primary phenolic constituent of clove oil), it is broadly used in medical and dental practices. Currently, expression of biofilm- and Quorum sensing (QS)-related genes: gtfB, gtfC, comDE, smu630, ftf, brpA,relA, vicR, gbpB and spaP checked by quantitative real-time PCR revealed that eugenol at a subminimum inhibitory concentration significantly down regulated these genes in S. mutans (You, 2019). Therefore, these results suggested that eugenol exhibits its potent therapeutic potential against oral biofilm, and it can effectively inhibit the formation of plaque-associated dental caries. The previous study of Forssten et al., (2010) reported the antibacterial efficacy of aqueous extract of Artocarpus lakoocha Roxb. (belongs to Moraceae family) against several Gram-positive (S. sorbinus, S. mutans) and Gram-negative bacteria (P. gingivalis), with MIC ranging from 0.10- 0.39 mg/ ml. This aqueous extract of A. lakoocha comprises several active compounds with potent antibacterial characteristics that might be used for medication and prevention of oral infections.

Previously, Hosseini et al., (2012) conducted a study to determine the antibacterial potential of diethyl ether mastic gum extracts of Pistacia atlantica subsp. kurdica (Iranian specie, belongs to Anacardiaceae family) against S. mutans biofilm formation. Results showed that the P. atlantica resin extracts decreased the total viable S. mutans biofilms. At 90% concentration, the extract showed an immediate antibacterial activity against bacterial biofilm formation. This effect was also associated with inhibition of adherence to the polystyrene surfaces. Accordingly, this extract could effectively inhibit the formation of plaque on the tooth surfaces. The inhibition of S. mutans adherence by sub-MIC
concentrations of the *P. atlantica* extract was attributed to the presence of flavonoids and tannins in this extract. In another study of Cheon *et al.*, (2013), a flavonoid-rich extract of *P. atlantica* was also tested for the inhibition of growth and acidogenicity of: *S. sorbinus*, *S. salivarius*, *S. mutans*, *S. sanguis* and *A. viscosus*. The anti-adherence and anti-acidogenic effect of *P. atlantica* suggested that this extract might be helpful for the development of promising anticariogenic agents.

*Morinda citrifolia* is thought to be a miracle plant that grows naturally in terrestrial environments with no care, even though it has several promising properties including; antibacterial, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects (Assi *et al.*, 2017). Later, Anil and Anand, (2017) reported that the antimicrobial activity of *M. citrifolia* much depends upon its ripeness, as its activity is high when this fruit is fully ripe and not dry. Recently, Meritt and Qi, (2012) studies indicated that about 160 of biologically active phytochemicals have been extracted from the *M. citrifolia* plant. Thus, this plant can be considered as an essential medical plant that can be a source of development of many therapeutic drugs.

An earlier study conducted by Juneja and Kakade, (2012) documented that drinking of *M. citrifolia* juice and maintaining healthy oral hygienic conditions showed a prominent role in the reduction of bleeding, and reduced the gingival swellings as well. The antibacterial effect of *M. citrifolia* fruits against *S. mutans* and *S. mitis* was tested by Bowen and Koo, (2011). Crude aqueous extract of these fruits was tested in different concentrations such as 1000-100 µg/ml to check its antibacterial potential, using the Kirby-Bauer method. Only the concentration of 1000µg/ml showed notable activity against oral Streptococci. For *S. mutans*, the recorded inhibition zone diameter was 19±0.5 mm, while it was 18.6±0.3 mm for *S. mitis*, in contrast to streptomycin antibiotic used as positive control and recorded inhibition diameter of 21.6±0.3 mm. Results of this study indicated that phytochemicals including; organic acids, phenolic compounds and alkaloids that are naturally synthesized by *M. citrifolia* fruits, have bactericidal potency against the oral *Streptococci*.

**Conclusion**

In Pakistan (an overpopulated country), a national survey was conducted in 1992 on tooth decay which showed that two teeth per person were decayed, missing, or filled, which was an alarming situation. This may become more lethal due to the excessive usage of sugars and sweets after effective preventive treatments. Since then, unfortunately, the surveillance of tooth decay in Pakistan has not been addressed; it is a neglected field in Pakistan. The current study concludes that *S. mutans* is highly prevalent in dental caries and dental plaque, followed by *S. pyogenes* and *S. mitis*. Clarithromycin, streptomycin, and ciprofloxacin can be the drugs of choice to treat the dental infections. However, there is an urgent need for excessive evaluation of results using in-vivo analysis, to ensure drug availability and monitor dental carries.

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**Conflict of interest**

All the authors have no conflict of interests.

**7. References**


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