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.

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

767

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 nitro-reductase, 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 10^4 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.

771

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A study conducted by Argimon and Caufield, (2011) compared the antibiotic susceptibility profile of *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 *S. viridans* isolates were recovered from the healthy and intellectually disabled children, respectively. The most dominant isolates were *S. salivarius* (27.3 %) in healthy and *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; *S. sanguis* (45%), *S. mitis* (56 %) and *S. oralis* (55 %) were more resistant, compared to the disabled children 40 %, 47 % and 47 %, respectively. *S. mutans* was the least resistant species to all antibiotics in both groups of children. These data revealed that *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 *et al*., (2010).

6. Antibacterial agents against the oral *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 *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, diarrhea 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. *Streptococci*. For example, Hossain *et al*., (2020) revealed that crude aqueous extract of *Mangifera indica* exhibits productive bactericidal activity against *S. mutans* and *S. mitis*. Moreover, with the MIC of 2 mg/ ml, *Isertia laevis* (belongs to the *Rubiaceae* family) effectively inhibits the growth of *S. sorbinus* and *S. mutans*. Correspondingly, the boiled aqueous extract of *Coffea arabica* L. significantly reduces the attachment of *S. mutans* ATCC 35688 to the tooth enamel and the dentine (Bowen and Koo, 2011). A recent study conducted by Bescos *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 *S. mutans* and the incidence of dental caries.

A study of Liu *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 *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 *Candida* spp. at 1 % (v/v) concentration.

Earlier, Giacaman *et al*., (2010) conducted a study to check the effectiveness of rubusoside (non-caloric sweetener) extracted from *Rubrus suavissimus* (which belongs to the *Rosacea*e family), on minimizing the dental caries caused by *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 *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

772

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

773

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 anti-cariogenic 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, anti-helminthic, 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, Merritt 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 1000 µg/ 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.

Acknowledgement

Conflict of interest

All the authors have no conflict of interests.

Funding source

Ethical approval

Non-applicable.

7. References

Abusleme, L.; Dupuy, A.K.; Dutzan, N.; Silva, N.; Burleson, J.A.; Strausbaugh, L.D. and Diaz, P.I. (2013). The subgingival microbiome in health and periodontitis and its relationship with community

biomass and inflammation. The ISME Journal. 7(5): 1016-1025.

Al-Shami, I.Z.; Al-Hamzi, M.A.; Al-Shamahy, H.A. and Abdul, A.L.A. (2019). Efficacy of some Antibiotics against *Streptococcus mutans* Associated with Tooth Decay in Children and their Mothers. Journal of Dentistry and Oral Health. pp. 1-4.

Anil, S. and Anand, P.S. (2017). Early childhood caries: prevalence, risk factors, and prevention. Frontiers in Pediatrics. 5: 157.

Argimón, S. and Caufield, P.W. (2011). Distribution of putative virulence genes in *Streptococcus mutans* strains does not correlate with caries experience. Journal of Clinical Microbiology. 49(3): 984-992.

Arora, B.; Setia, V.; Kaur, A.; Mahajan, M.; Sekhon, H.K. and Singh, H. (2018). Dental caries vaccine: An overview. Indian Journal of Dental Sciences. 10(2): 121.

Assi, R.A.; Darwis, Y.; Abdulbaqi, I.M.; Vuanghao, L. and Laghari, M.H. (2017). *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arabian Journal of Chemistry. 10(5): 691-707.

Banas, J.A. and Drake, D.R. (2018). Are the *mutans streptococci* still considered relevant to understanding the microbial etiology of dental caries?. BMC Oral Health. 18(1): 129.

Bescos, R.; Ashworth, A.; Cutle, C.; Brookes, Z.L. et al. (2020). Effects of Chlorhexidine mouthwash on the oral microbiome. Scientific Reports. 10(1): 1-8. https://doi.org/10.1038/s41598-020-61912-4

Besra, M. and Kumar, V. (2016). Antimicrobial activity of essential oils and herbal extracts against etiological agent of dental caries. Journal of Essential Oil-Bearing Plants. 19(7): 1807-1815.

Bowen, W.H. (2016). Dental caries–not just holes in teeth! A perspective. Molecular oral Microbiology. 31(3): 228-233.

Bowen, W.H. and Koo, H. (2011). Biology of *Streptococcus mutans*-derived Glucosyl-transferases: role in extracellular matrix formation of cariogenic biofilms. Caries Research. 45(1): 69-86.

Cephas, K.D.; Kim, J.; Mathai, R.A.; Barry, K.A.; Dowd, S.E.; Meline, B.S. and Swanson, K.S. (2011). Comparative analysis of salivary bacterial microbiome diversity in edentulous infants and their mothers or primary care givers using pyrosequencing. PloS one. 6(8). e23503. https://doi.org/10.1371/journal.pone.0023503

Chakraborty, B. and Burne, R.A. (2017). Effects of arginine on *Streptococcus mutans* growth, virulence gene expression, and stress tolerance. Applied and Environmental Microbiology. 83(15): e00496-17.

Chandrabhan, D.; Hemlata, R.; Renu, B. and Pradeep, V. (2012). Isolation of dental caries bacteria from dental plaque and effect of toothpastes on acidogenic bacteria. Journal of Medical Microbiology. 2(3). https://doi.org/10.4236/ojmm.2012.23009

Cheon, K.; Moser, S.A.; Wiener, H.W.; Whiddon, J.; Momeni, S.S.; Ruby, J.D. and Childers, N.K. (2013). Characteristics of *Streptococcus mutans* genotypes and dental caries in children. European Journal of Oral Sciences. 121(3pt1): 148-155.

Childers, N.K.; Momeni, S.S.; Whiddon, J.; Cheon, K.; Cutter, G.R.; Wiener, H.W. and Moser, S.A. (2017). Association between early childhood caries and colonization with *Streptococcus mutans* genotypes from mothers. Pediatric Dentistry. 39(2): 130-135.

Chu, J.; Zhang, T. and He, K. (2016). Cariogenicity features of *Streptococcus mutans* in presence of rubusoside. BMC Oral Health. 16(1): 54.

Chun, S.; Huh, H.J. and Lee, N.Y. (2015). Species-specific difference in antimicrobial susceptibility among *viridans* group *streptococci*. Annals of Laboratory Medicine. 35(2): 205-211.

Colombo, A.P.V. and Tanner, A.C.R. (2019). The role of bacterial biofilms in dental caries and

775

periodontal and peri-implant diseases: a historical perspective. Journal of Dental Research. 98(4): 373-385.

Daboor, S.M.; Masood, F.S.S.; Al-Azab, M.S. and Nori, E.E. (2015). A review on *Streptococcus mutans* with its diseases dental caries, dental plaque and endocarditis. Indian Journal of Microbiology Research. 2(2): 76-82.

ElSalhy, M.; Honkala, S.; Söderling, E.; Varghese, A. and Honkala, E. (2013). Relationship between daily habits, *Streptococcus mutans*, and caries among schoolboys. Journal of Dentistry. 41(11): 1000-1006.

Forssten, S.D.; Björklund, M. and Ouwehand, A.C. (2010). *Streptococcus mutans*, caries and simulation models. Nutrients. 2(3): 290-298.

Giacaman, R.A. (2018). Sugars and beyond. The role of sugars and the other nutrients and their potential impact on caries. Oral Diseases. 24(7): 1185-1197.

Giacaman, R.A.; Araneda, E. and Padilla, C. (2010). Association between biofilm-forming isolates of *mutans streptococci* and caries experience in adults. Archives of Oral Biology. 55(8): 550-554.

Hassan, S.; Danishuddin, M. and Khan, A.U. (2015). Inhibitory effect of *Zingiber officinale* towards *Streptococcus mutans* virulence and caries development: *in vitro* and *in vivo* studies. BMC Microbiology. 15(1): 1.

Hassan, S.; Danishuddin, M.; Adil, M.; Singh, K.; Verma, P.K. and Khan, A.U. (2012). Efficacy of *E. officinalis* on the cariogenic properties of *Streptococcus mutans*: a novel and alternative approach to suppress quorum-sensing mechanism. PLoS One. 7(7). https://doi.org/10.1371/journal.pone.0040319

Hemadi, A.S.; Huang, R.; Zhou, Y. and Zou, J. (2017). Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. International Journal of Oral Science. 9(11): e1-e1.

Hoiby, N.; Ciofu, O.; Johansen, H.K.; Song, Z.J.; Moser, C.; Jensen, P.Ø. and Bjarnsholt, T. (2011). The clinical impact of bacterial biofilms. International Journal of Oral Science. 3(2): 55-65.

Hooper, S.J.; Lewis, M.A.O.; Wilson, M.J. and Williams, D.W. (2011). Antimicrobial activity of Citrox® bioflavonoid preparations against oral microorganisms. British Dental Journal. 210(1): E22. https://doi.org/10.1038/sj.bdj.2010.1224

Hossain, M.S.; Hossain, M.S.; Alam, S.; Nibir, Y.M.; Tusty, T.A.; Bulbul, S.M. and Islam, S.S. (2020). Genotypic and phenotypic characterization of *Streptococcus mutans* isolated from dental caries. BioRxiv.

Hosseini, A.; Nikfar, S. and Abdollahi, M. (2012). Probiotics use to treat irritable bowel syndrome. Expert Opinion on Biological Therapy. 12(10): 1323-1334.

Ito, K.; Ito, S.; Shimamura, T.; Weyand, S.; Kawarasaki, Y.; Misaka, T. and`Iwata, S. (2011). Crystal structure of glucansucrase from the dental caries pathogen *Streptococcus mutans*. Journal of Molecular Biology. 408(2): 177-186.

Jubair, H.H. (2015). The Relationship Between Biofilm Forming and Antibiotics Resistance of *Streptococcus mutans* Isolated from Dental Caries. International Journal of Current Microbiology and Applied Science. 4(5): 568-574.

Juneja, A. and Kakade, A. (2012). Evaluating the effect of probiotic containing milk on salivary *mutans streptococci* levels. Journal of Clinical Pediatric Dentistry. 37(1): 9-14.

Karikalan, S. and Mohankumar, A. (2016). Antibiogram of *Streptococcus mutans* isolated from dental caries patients. International Journal of Medical and Health Research. 2(3): 79-83.

Krzysciak, W.; Jurczak, A.; Kościelniak, D.; Bystrowska, B. and Skalniak, A. (2014). The virulence of *Streptococcus mutans* and the ability to

form biofilms. European Journal of Clinical Microbiology and Infectious Diseases. 33(4): 499-515.

Kuramitsu, H.K. and Wang, B.Y. (2011). The whole is greater than the sum of its parts: dental plaque bacterial interactions can affect the virulence properties of cariogenic *Streptococcus mutans*. American Journal of Dentistry. 24(3): 153-4.

Lamont, R.J.; Koo, H. and Hajishengallis, G. (2018). The oral microbiota: dynamic communities and host interactions. Nature Reviews Microbiology. 16(12): 745-759.

Legenova, K. and Bujdakova, H. (2015). The role of *Streptococcus mutans* in the oral biofilm. Epidemiol Mikrobiol Imunol. 64(4): 179-187.

Lemos, J.A.; Palmer, S.R.; Zeng, L.; Wen, Z.T.; Kajfasz, J.K.; Freires, I.A. and Brady, L.J. (2019). The biology of *Streptococcus mutans*. Microbiology Spectrum. 7(1): 1-26.

Li, J., Wu, T., Peng, W. and Zhu, Y. (2020). Effects of resveratrol on cariogenic virulence properties of *Streptococcus mutans*. BMC Microbiology. 20(1): 1-11.

Lin, T.H.; Lin, C.H. and Pan, T.M. (2018). The implication of probiotics in the prevention of dental caries. Applied Microbiology and Biotechnology. 102(2): 577-586.

Liu, Y.; Wang, L.; Zhou, X.; Hu, S.; Zhang, S. and Wu, H. (2011). Effect of the antimicrobial decapeptide KSL on the growth of oral pathogens and *Streptococcus mutans* biofilm. International Journal of Antimicrobial Agents. 37(1): 33-38.

Manji, F.; Dahlen, G. and Fejerskov, O. (2018). Caries and periodontitis: contesting the conventional wisdom on their etiology. Caries Research. 52(6): 548-564.

Marsh, P.D. (2016). Dental biofilms in health and disease. Understanding Dental Caries. pp. 41-52. https://doi.org/10.1007/978-3-319-30552-3_5

Marsh, P.D. (2012). Contemporary perspective on plaque control. British Dental Journal. 212(12): 601.

Matsumoto-Nakano, M. (2018). Role of *Streptococcus mutans* surface proteins for biofilm formation. Japanese Dental Science Review. 54(1): 22-29.

Merritt, J. and Qi, F. (2012). The mutacins of *Streptococcus mutans*: regulation and ecology. Molecular Oral Microbiology. 27(2): 57-69.

Moayad, N.M.; Bnyan, I.A.; Hayam, K.A. and Kezar, M.Y. (2015). The role of some Virulence factors of *Streptococcus mutans* bacteria isolated of patients with dental diseases in Hilla city. World Journal of Pharmaceutical Research. 4(12): 357-376.

Nobbs, A. (2017). Getting to the heart of the matter: Role of *Streptococcus mutans* adhesin Cnm in systemic disease. Virulence. 8(1): 1-4.

Nurelhuda, N.M.; Al-Haroni, M.; Trovik, T.A. and Bakken, V. (2010). Caries experience and quantification of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Sudanese schoolchildren. Caries Research. 44(4): 402-407.

Philip, J.M.; Rebecca, L.J.; Abraham, H.M.; Venkatakrishnan, C.J. and Chandran, C.R. (2018). Plant extracts inhibiting the adhesion of oral bacteria. Drug Invention Today. 10(7): 100-1101.

Potgieter, E.; Carmichael, M.; Koornhof, H.J. and Chalkley, L.J. (1992). *In vitro* antimicrobial susceptibility of *viridans streptococci* isolated from blood cultures. European Journal of Clinical Microbiology and Infectious Diseases. 11(6): 543-546.

Ramadugu, K.; Blostein, F.; Bhaumik, D.; Jiang, W.; Davis, E.; Salzman, E. and Marazita, M.L. (2020). Co-occurrence of yeast, *streptococci*, dental decay, and gingivitis in the post-partum period: results of a longitudinal study. Journal of Oral Microbiology. 12(1): 1746494.

Ranganathan, V. and Akhila, C.H. (2019). *Streptococcus mutans* has it become prime perpetrator for oral manifestations. Journal of Microbiology and Experimentation, 7(7): 19-166.

Raul, B.; Ashworth, A.; Cutler, C.; Brookes, Z.L.; Belfield, L.; Ana, R. and White, D. (2020). Effects of Chlorhexidine mouthwash on the oral microbiome. Scientific Reports. 10(1). https://doi.org/10.1038/s41598-020-61912-4

Tahir, L. and Nazir, R. (2018). Dental Caries, Etiology, and Remedy through Natural Resources. Dental Caries: Diagnosis, Prevention and Management. pp. 19-33.

Tellez, N.; Téllez, M.; Perdomo, M.; Alvarado, A. and Gamboa, F. (2010). Anticariogenic activity of the active fraction from *Isertia laevis* against *S. mutans* and *S. sobrinus*: Comparison of two extraction methods. Acta Odontologica Latinoamericana. 23(3): 188-195.

Salman, H.A.; Senthilkumar, R.; Imran, K. and Selvam, K.P. (2017). Isolation and typing of *Streptococcus mutans* and *Streptococcus sobrinus* from caries-active subjects. Contemporary Clinical Dentistry. 8(4): 587.

Saputo, S.; Faustoferri, R.C. and Quivey, R.G. (2018). Vitamin D compounds are bactericidal against *Streptococcus mutans* and target the bacitracin-associated efflux system. Antimicrobial Agents and Chemotherapy. 62(1): e01675-17.

Simon-Soro, A.; Belda-Ferre, P.; Cabrera-Rubio, R.; Alcaraz, L.D. and Mira, A. (2013). A Tissue-dependent hypothesis of dental caries. Caries Research. 47(6): 591-600.

Xin, X.; Yuan, Z.; Wenyuan, S.; Yaling, L. and Xuedong, Z. (2016). Biofilm and Dental Caries. Dental Caries. Springer, Berlin, Heidelberg. pp. 27-58. https://doi.org/10.1007/978-3-662-47450-1_2

You, Y.O. (2019). Virulence genes of *Streptococcus mutans* and dental caries. International Journal of Oral Biology. 44(2): 31-36.

Zeng, L. and Burne, R.A. (2016). Sucrose-and fructose-specific effects on the transcriptome of *Streptococcus mutans*, as determined by RNA sequencing. Applied and Environmental Microbiology. 82(1): 146-156.