

A Study on Dental Caries and Dental Plaques Caused by Streptococcus Mutans, of Patients at Border Guard Hospital Dhaka, Peelkhana

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Abstract

Background: Dental caries is one of the most common chronic infectious diseases in the world. The acids generated through the fermentation of sugar by the microorganisms in dental plaque can decalcify the superficial layers of tooth enamel and can initiate dental caries. Streptococcus mutans have been proposed as the main etiological agents of dental caries. The aim of this study is to identify the main etiological agents of dental caries and plaque which are supposed to be Streptococcus mutans as correlating microorganism.

Materials and Methods: This cross sectional study was carried out in the Pathology department of Border Guard Hospital, Peelkhana, Dhaka during the period of 1st October, 2018 to 31st December, 2018. Study population was newly enrolled soldiers and their families as male, female and children in BGB. A total of 50 clinical samples were included in this study. The samples were collected from the Dental outdoor patient department and all laboratory tests were performed at Pathology department of aforementioned hospital. After performing all possible microbiological tests for bacterial isolation, results were recorded.

Introduction

Dental caries is one of the most common chronic infectious diseases in the world¹. Dental plaque consists of bacterial cells, is the biofilm adheres to the surfaces of the teeth. Biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface generate extracellular polymeric substances which are a polymeric conglomeration of extracellular polysaccharides, proteins, lipids and DNA². The acids generated through the fermentation of sugar by the microorganisms can decalcify the superficial layers of tooth enamel and can initiate dental caries³. Streptococcus mutans have been proposed as the main etiological agents of dental caries⁴. Over 700 bacterial taxa have been found in the oral

Results: Among 50 samples, 46(92%) samples are considered to be positive for Streptococcus viridans and 27(54%) isolates are identified as Streptococci mutans according to ability of producing special kind of exopolysaccharide, lactic acid formation and high salt tolerance tests. Associated organisms (32%) like candida were present in 15(30%) samples and other bacterial colonies were present in 31(62%) samples, along with Streptococcus viridans.

Conclusion: The finding of this study will help the dentists to treat the patients with chronic caries, dental plaques as they pose the gravest threat when they settle down on damaged heart valves, cause subacute bacterial endocarditis and other infections in various sites including mouth, joints, skin, muscle, and central nervous system after tooth extraction or any other invasive procedure.

Keywords: Biofilms; Dental caries; Glucosyltransferase; Microbial ecology; Streptococcus

(J Bangladesh Coll Phys Surg 2020; 38: 121-125)
DOI: <https://doi.org/10.3329/jbcps.v38i3.47059>

cavity; however they are not all present in the same mouth⁵. Bacterial profiles change with disease states and differ between primary and secondary dentitions. Bacterial species other than *S. mutans*, e.g., species of the genera *Veillonella*, *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, low-pH non-*S. mutans* streptococci, *Actinomyces* spp., likely play important roles in caries progression⁶. A symbiotic relationship with *S. mutans* and *Candida albicans* leads to increased glucan production and help to increase the cariogenic potential of *S. mutans*⁷. Most of these microbes are harmless, but under certain conditions some can cause oral infections like caries or periodontal disease. Oral streptococci, like *Streptococcus mutans*, are associated with pyogenic and other infections in various sites including mouth, heart, joints, skin, muscle, and central nervous system⁸.

Materials and methods

This cross sectional and observational study was carried out in the Pathology department of Border Guard Hospital, Peelkhana, Dhaka during the period of 1st October, 2018 to 31st December, 2018. Study population was soldiers and their families as male, female and children who are

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Received: 17 November, 2019 **Accepted:** 10 March, 2020

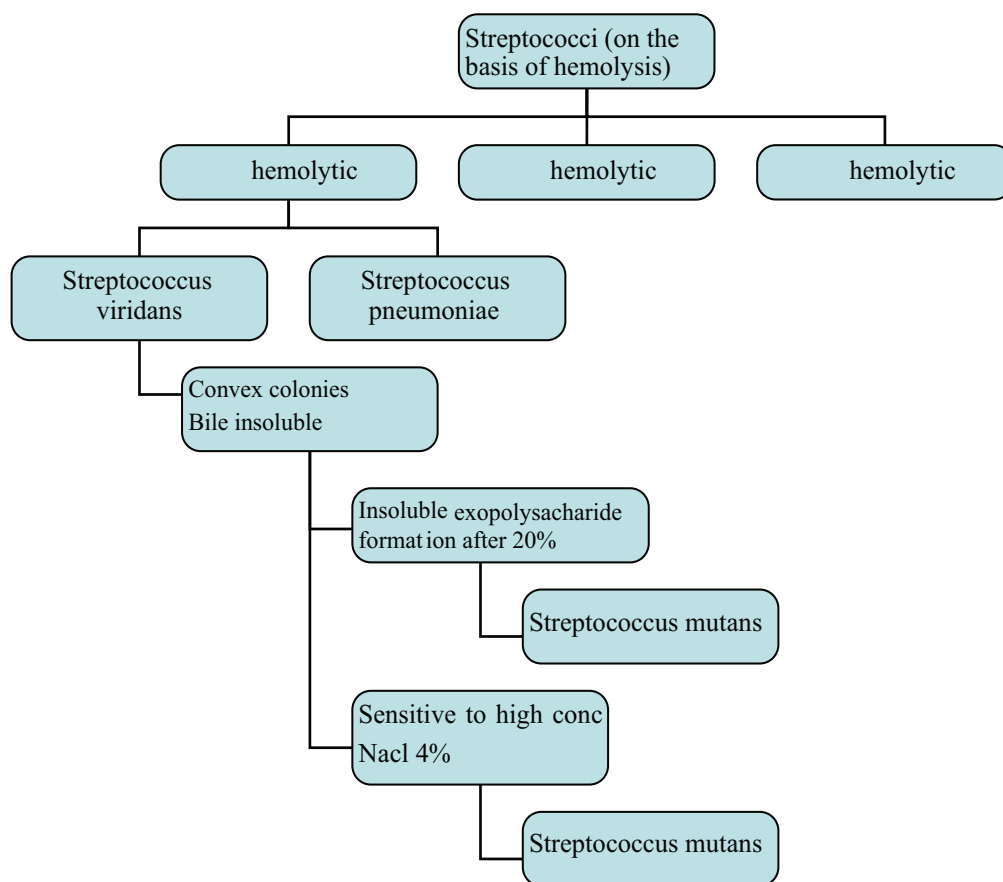
entitled to get dental treatment in this hospital. The clinical samples were collected only from those patients who reported with dental caries and dental plaques. The patients those reported for other treatment like tooth extraction, root canal or other dental procedures except dental caries and plaques were excluded in this study. A total of 50 clinical samples as it is a convenient size, were included in the study. The samples were collected from the Dental outdoor patient department and all laboratory tests were performed in the Pathology department of aforementioned hospital. With all aseptic methods by the dental surgeons after the verbal consent from the patients the samples were collected. After performing all possible microbiological tests for bacterial isolation at the laboratory of BGH, Peelkhana, results were recorded.

Isolation and identification of *Streptococcus mutans*

Streptococcus mutans is a facultative anaerobic, gram-positive coccus commonly found in the human oral cavity and is a significant contributor to tooth decay. Caries-associated bacteria traditionally have been identified by

using culture-based methods, which exclude not-yet-cultivated species⁷. All specimens were Gram stained and cultured on chocolate agar, blood agar media and also in BHI broth in anaerobic condition at 35°C for 48 hrs. As oral commensal present in the mouth they also grew on those medias (Fig-2), so typical colonies were selected, identified and examined further. Alpha hemolysis was detected by culture on chocolate agar media. Gram positive cocci arranged in pairs and short chains were identified by Gram stain (Fig-1), Optochin disc was used for resistance test and bile salt insolubility tests were done by Sodium taurocholate. These tests were performed to differentiate *S. viridans* to *S. pneumoneae*. Then TSI media was used to identify the lactic acid production from glucose, sucrose and lactose. Production of insoluble long chain exopolysaccharide was identified by 20% sucrose solution due to glucosyle transferase enzyme. High salt tolerance test was done by NaCl 4% solution. The isolates are considered to be belonging to the group *Streptococcus mutans* after performing these biochemical confirmatory tests.

A flow chart to isolate the *Streptococcus mutans* among *Streptococci virridans*



Results

The colonies produced well-marked greenish discolouration on chocolate agar media and small semi-transparent colonies about 1 mm in diameter with alpha hemolysis on blood agar media. They were Gram positive and arranged in pairs and short chains (Fig-1), catalase negative, optochin resistant and bile insoluble. Forty

six (92%) isolates are considered belonging to the group *Streptococci viridans*, among these 27(54%) isolates are expected to be *Streptococcus mutans* according to ability of producing special kind of exopolysaccharides and high salt tolerance test with 4% NaCl. Among fifty (50) samples, four(4) of them showed no growth on any of the medias. All findings are recorded in tabulated forms thereafter.

Table-I

Primary culture on BHI, BA and CA (n=50)				
Gram stain	Culture in BHI	Culture on CA	Culture on BA	No growth
Gram positive cocci in pairs and short chains-46	Opaque, yellowish orange colour with deposits- 46	Pin point, convex colonies, greenish zone of hemolysis - 46	Pin point, convex colonies with Alpha hemolysis- 46	4

Table-II

Isolation by biochemical tests (n=50)					
Bile salt test	Lactic acid production	20% sucrose utilization	4% NaCl tolerance test	Optochin sensitivity test	Catalase test
46	46	27 produced exopolysaccharide	27-tolerant	46-resistant	46-Negative
Total sample	50				

Table-III

Number of organisms found after laboratory tests			
No of Streptococcus spp. (%)	No of <i>S.mutans</i> (%)	No of other organism (%)	No growth (%)
46(92%)	27(54%)	40(candida-15+ other organisms 31) 80%	4(8%)

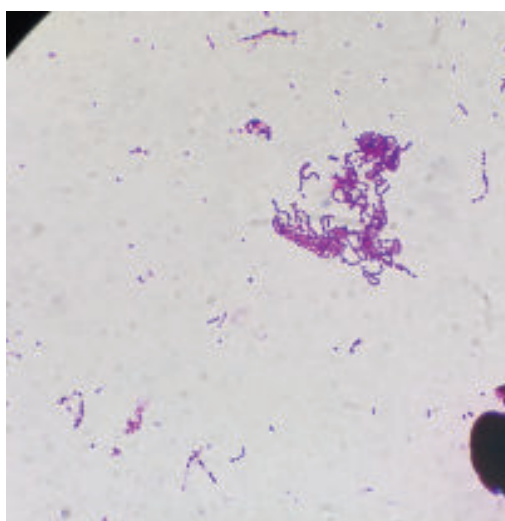


Fig-1: Gram stained smear from cultured colonies shows Gram positive cocci arranged in pairs and short chains

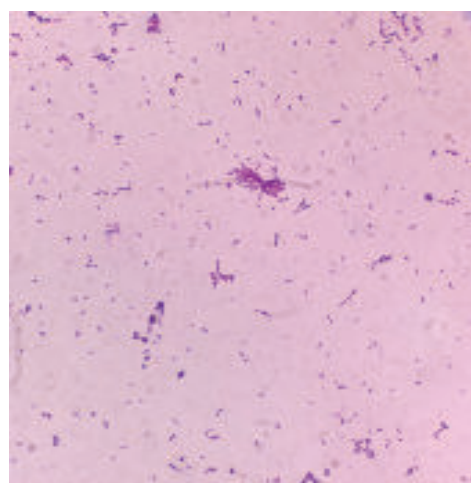


Fig-2: Gram stained smear from cultured colonies shows Gram positive cocci arranged in pairs along with Gram negative bacilli and few budding yeast

Discussion

Worldwide, approximately 2.43 billion people (36% of the population) have dental caries in their permanent teeth⁶. In the United States, dental caries is the most common chronic childhood disease. It is the primary pathological cause of tooth loss in children. *Streptococcus mutans* is the main cause of dental decay⁴.

In this study, it is identified that the main etiological agents of dental caries are supposed to be *Streptococcus mutans* as correlating microorganism. Sofia, Marika, and Arthur stated that although *Streptococcus mutans* has been implicated as a major etiological agent of dental caries, a cross-sectional preliminary study indicated that 10% of subjects with rampant caries in permanent teeth do not have detectable levels of *S. mutans*⁵. *Streptococcus mutans* are major cariogenic organisms—the result of their ability to produce large quantities of glucans as well as acid, exceeding the salivary buffering capacities, which gives the bacteria an advantage to outcompete noncariogenic commensal species at low pH environments⁶. H. Koo, J. Xiao, and J.G. Jeon found the presence of EPS within and surrounding microcolonies throughout the biofilm may create chemical gradients due to the differential diffusion (more cross-linked glucans may limit diffusion) of nutrients and metabolic products throughout the matrix, which could affect the microenvironment pH in the biofilms⁷. *Streptococcus mutans* is a key contributor to the formation of biofilms associated with dental caries disease, although other microorganisms may also be involved; *S. mutans* (i) effectively utilizes dietary sucrose (and possibly starch) to rapidly synthesize exopolysaccharides using glucosyltransferases and a fructosyltransferase that adsorb to surfaces, (ii) adheres tenaciously to glucan-coated surfaces, and (iii) is acidogenic and acid tolerant. Furthermore, Gtf B binds to other oral microorganisms, including non-mutans streptococci, *Lactobacillus* species, and *Actinomyces* species, in an active form which could provide additional binding sites for *S. mutans*⁸.

Data presented in this study indicates that out of 50 samples, collected from dental carries, 46 samples (92%), were positive for *Streptococcus viridians*. They were Gram positive cocci arranged in short chains and pairs, showed alpha hemolysis on chocolate agar, Optochin resistant and bile insoluble. Associated organisms like

Candida were present in 15(30%) samples and other bacterial colonies were present in 31(62%) samples along with *Streptococcus viridans*, 54% of clinical samples were positive for the growth of *Streptococcus mutans*, which are supposed to be the major causative agents of dental caries and plaques. This study shows different Gram positive and Gram negative bacteria along with *Candida* were simultaneously present with the streptococcus species.

Globally, 36% population has dental caries in their permanent teeth and 9% population has in baby teeth⁹. The occurrence of this disease is most frequent in Latin American countries, countries in the Middle East, South Asia, and least rampant in China¹⁰. According to the study carried out in Faculty of Dentistry of Piracicaba, University of Campinas, Brazil revealed that among the isolated and identified species, *S. mutans* showed a significantly greater frequency of isolation (59.2%) which almost correlates with this study¹¹. Further, the most frequently detected streptococcus species in patients with streptococcal valvular diseases is known to be *Streptococcus sanguinis*, while *S. mutans* was reported to be recovered from approximately 15%¹². According to a review of 848 Infective endocarditic cases in Japan performed recently, the most common type of microorganisms isolated from patients was streptococcus species (49.5%), followed by staphylococcus species (31.7%). Another study held in the United States that reported the presence of *S. mutans* in cardiovascular specimens, though the frequency was relatively low at approximately 20%. This study differs with aforementioned studies might be due to the food habits and environmental influences.

The limitation of this study is that no molecular diagnostic equipments were used to identify the organisms as confirmatory diagnosis. The results were established on only manual conventional methods at the laboratory in a secondary care hospital.

Conclusion

This study showed, prevalence of *S. mutans* in dental caries and dental plaque is quite high as 27(54%) among 46(92%) *Streptococcus spp* out of 50 samples. Practice of good oral hygiene including daily brushing, flossing and the use of appropriate mouthwash can significantly reduce the number of oral bacteria, including *S. mutans* and inhibit their proliferation. Control of caries also

involves limitation of sucrose intake, good nutrition with adequate protein intake and reduction of acid production in the mouth. Children should be considered for more intensive care at home. Thus, strategies to prevent and reduce the risk for dental inflammation and caries are important. The finding of this study will help the dentists to treat the patients with chronic caries, dental plaques as they pose the gravest threat when they settle down on damaged heart valves, cause subacute bacterial endocarditis and other infections in various sites including mouth, joints, skin, muscle, and central nervous system after tooth extraction or any other invasive procedure.¹²

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