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Full Length Research Paper

Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different tooth pastes against *Streptococcus* spp

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Dental caries is a microbiologic infection of the tooth which results in localized dissolution and destruction of the calcified tissue and it is one of the most common chronic infectious diseases in humans throughout the world. People spent huge amounts of money and time in treating dental caries. Hence, it is essential to prevent and control dental caries in public health. In our studies, a total of 70 clinical specimens, 37 from male and 33 from female dental caries patient's samples were collected. All the clinical samples showed positive results with 87 bacterial isolates from which 63 were facultative anaerobes and 24 were anaerobe, among facultative anaerobe, *Streptococcus mutants* (22.98%) *S. salivarius* (13.79%) and *Candida* spp (14.97%), in anaerobes, *Prevotella* spp (12.64%) and *Fusobacterium* spp (5.74%). The antibiotic susceptibility test was carried out against *Streptococcus* spp. the results showed all isolates were resistance to penicillin and sensitive to tetracycline. The antibacterial activity of commercially available tooth paste was checked against *Streptococcus* spp., by agar well diffusion method. The results showed that pepsodent and colgate have good antibacterial activity against *Streptococcus* spp.

Key words: Dental caries, *Streptococcus* spp, *Prevotella* spp, antibiotics, tooth pastes.

INTRODUCTION

Dental caries is one of the most common chronic infectious diseases in the world. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by cariogenic bacteria (Botelho et al., 2007). Bacterial plaque accumulated on dental surfaces and composed of native oral flora is the primary etiologic agent of dental caries. Cariogenic bacteria interact by various recognized ways including co-aggregation (Kolenbrander et al., 2006), metabolic exchange, cell-cell communication (Li et al., 2002), and exchange of genetic material (Roberts et al., 2001). These mechanisms benefit bacterial survival and can make dental biofilms difficult therapeutic targets in dental diseases. Dental caries is the destruction of enamel, dentin or cementum of teeth due to bacterial activities, which if left untreated can cause considerable pain, discomfort, and treatment costs are very high. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases with *Streptococcus mutans* being the primary species associated with the early dental caries process. A wide group of microorganisms are identified from

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carious lesions of which *S. mutans*, *Lactobacillus acidophilus*, various proteolytic bacteria, anaerobic organism including *Prevotella* spp., *Peptostreptococcus* spp., *Vellionella* spp., *Fusobacterium* spp., and *Actinomyces viscosus* are the main pathogenic species involved in the initiation and development of dental caries by Krithika et al. (2004), facultative anaerobes especially *S. mutans* is the important organism are commonly found in human caries as secondary invaders, contributing to the progression of the lesion. Dental caries, with prevalence was as high as 60 to 80% in children, is a major public health problem in India Pandve (2009). According to the World Health Organization (WHO), the prevalence of dental caries worldwide among adults is nearly 100% of the population [Decayed Missing Filled Teeth index (DMFT) ≥14 teeth] in most industrialized countries, with a lower prevalence in developing countries (Petersen et al., 2005). The prevalence and incidence of oral diseases, coupled with the resultant social and economic implications reported by Shivakumar et al. (2009). People were using commercially available toothpastes found to posses efficient antimicrobial properties, especially, those that have triclosan as a major ingredient (Colgate total, Colgate, Anchor white and Pepsodent). Triclosan, a chlorophenol derivative, kills germs by interfering with the enzymes required for fatty acid synthesis. Next to triclosan, fluorinated products e.g., Close-up and Kedoos were found to posses marked antibacterial activities. These active compounds were reducing cariogenic microorganisms to strengthen the teeth by reducing demineralization and increasing remineralization of teeth. Professional hygiene care consists of regular dental examinations and the use of fluoride toothpaste is the primary intervention for the prevention of dental caries.

MATERIALS AND METHODS

Collection and processing of clinical samples

Dental caries specimens were collected from 70 infected adults outpatients from K.S.R. Dental and Research Institute, Tiruchengode and Gokul Dental Clinic, Erode, Tamilnadu, India between 20 and 60 years of age. The patient used various toothpastes for more than six months in the different age groups of hospitalized patients. Other factors responsible for caries were (1) not brushing tooth regularly (2) chewing tobacco (3) poor oral hygiene etc. The clinical swab samples were transported in thermocol box containing coolants packs and samples processed in a Microbiology laboratory within an hour. The specimens were inoculated onto Pikes Streptococcus agar, Brain heart infusion agar (Himedia Mumbai) supplemented with 5% defibrinated sheep blood and Mitis-Salivarius agar (Himedia, Mumbai) supplemented with 0.1% (v/v), potassium tellurite solution with and without bacitracin 0.2 U/ml and kept in candle jar incubated at 37°C for 24 to 48 h. Streptococcal colonies were chosen based on their morphology (hemolysis patterns) and were further confirmed by compatible Gram stain and catalase negative tests. These isolates further differentiated by biochemical characteristics were sugars fermentation, glucan production (5% sucrose medium), amino acid and esculin hydrolyses (Isenberg et al., 1991). For the isolation of anaerobes, blood agar base (Himedia, Mumbai) supplemented with gentamicin 75 µg/ml and 5% defibrinated sheep blood. The inoculated plates were kept in anaerobic jars (Himedia Mumbai) in the presence of anaerobic gas generating kit (Himedia, Mumbai) incubated at 37°C for 24 - 48 h and check up to 5 days (Coller et al., 1989).

Antibiotic sensitivity test

For performance of antibacterial susceptibility, testing of *Streptococcus* spp was carried out by disk diffusion procedures were used according to NCCLS (National Committee for Clinical Laboratory Standards) standards and criteria [NCCLS, 2003]. The *Streptococcus* spp were tested against penicillin, vancomycin, clindamycin, bacitracin, chloroamphenicol, tetracycline and streptomycin. *S. pneumonia* ATCC culture was used for assay control.

Toothpastes assesses with antibacterial activity

A total of four tooth pastes were used in the present study. Colgate tooth paste has sodium fluoride and triclosan as the active ingredients. They are anticaries agents that prevent the formation of cavities in teeth. Other uses include lessening mouth odors and polishing the teeth. Pepsodent's active ingredient is fluoride, which makes up 25% of the total ingredients toothpaste has contained fluoride to help prevent cavities. Fluoride works by strengthening the calcium phosphate in teeth enamel. Sorbitol also acts as a sweetener and makes more palatable. Silica is the ingredient in Pepsodent that gives the toothpaste its abrasive quality. Toothpastes must be abrasive to remove plaque, stains and debris. It also doesn't scrape tooth enamel or damage gums. The Close up toothpaste contains Sodium Fluoride, Triclosan, with sorbitol, hydrated silica, water, PEG32 SLS, flavours, cellulose, gum, sodium saccharin, trisodium phosphate, C1 16035 and C1 17200. Dabor babool paste contained herbal extract of different medicinal plants (*Piper nigram, P. langum, Zingiber officinale, Cinnamomum camphora Menthos spicata*) and there is no triclosan and fluorides. Tooth paste stock was prepared in sterile distilled water (1 g/ml). From the stock solution 0.5 ml transferred to first tube mixed well and transferred the same volume to second tube and so on up to sixth tube. The concentration of each tube was containing 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0156 g/ml. For assessing tooth paste antibacterial activity against *Streptococcus* spp, the cultures were matched with McFarland 0.5 turbidity standard (2×10^8 CFU ml^{-1}), then seeded to Muller Hinton agar plate and made 6 mm well with the help sterile well cutter after that loaded different concentration of tooth pastes in a well fixed volume (25 µl) and plates were kept in candle jar and incubated at 37°C for 24 h (Duran et al., 2003).

RESULTS AND DISSCUSION

Prevalence of cariogenic pathogens

Bacteriological examination of caries specimen's results

Table 1. Distribution and percentage of cariogenic pathogens in dental caries specimens.

Dental caries specimens (n=70)					
Facultative anaerobes and aerobes (n=50 and 13)			**Anaerobes(n=24)**		
Name of the organisms	**n**	**%**	**Name of the organisms**	**n**	**%**
Streptococcus mutants	20	22.98	*Prevotella* spp	11	12.64
S. sanguis	08	09.19	*Bacteriod*es spp	02	02.29
S. salivarius	12	13.79	*Lactobacilli* spp	02	02.29
S. mitis	05	05.74	*Fusobacterium* spp	05	05.74
Unidentified *Streptococcus* spp	05	05.74	*Peptostreptococcus* spp	04	04.59
Candida spp	13	14.94			

n- Number.

was a total of 87 isolates from 70 samples which included 72% (facultative anaerobes) and 28% (obligate anaerobes). In facultative anaerobes, viridan streptococcal were identified by Grams staining, alpha hemolysis and catalase negative results. *S. mutans* was manitol positive, *S. salivarius* was positive for glucan production and lactose positive and *S. sanguis* was sorbitol positive. In facultative anaerobes *S. mutants* was the predominant isolates (22.98%) followed by *Candida* spp., (14.94%) and less than 10% in *S. sanguis*, *S. mitis* and unidentified *streptococcus* spp (Table 1). On the other hands Salako et al. (2007) reported that the most common isolates from dental plaque samples were *S. salivarias* (27.3%) in healthy children; *S. sanguis* (22.6%) was predominant among children who were disabled. *S. mutans* was found in 12.1% of the healthy children and in 16.5% of the children who were disabled. Diaz et al. (2006) reported similar findings; viridian streptococci- *S. mutans, S. salivarius S. sanguis* and *S. mitis* are the major pathogens of the dental infection. While others reported oral streptococci pose the significant health risks if they enter into bloodstream via. Wounds, oral infection, dental procedures and can cause endocarditis. Followed by primary invaders, oral cavities are vulnerable for secondary invaders like *Candida albicans* and species of *Actinomyces*, *Bacteroids*, *Spirochetes* and *Lactobacillus* etc. (Dige et al., 2007). There is extensive evidence associating *S. mutans* and *Streptococcus sobrinus* (mutans streptococci) with dental caries. Mutans streptococci use sucrose to produce extracellular glucan, a water insoluble polysaccharide, which enables the bacteria to attach to the tooth surface and also protects them from external factors such as mechanical disruption, salivary clearance, and antimicrobial substances (Tinanoff et al., 2002). Equally important is the ability of mutans streptococci to both produce acid (acidogenic) and survive in an acidic environment (aciduric), properties that enable them to exhibit high pathogenicity (Svensater et al., 1997). On the other hand, several studies have failed to detect *S. mutans* in a significant portion of the children who develop caries (Aas et al., 2008), suggesting that additional species may play a significant role in dental caries development in children.

In our studies obligate anaerobes *Prevotella* spp was predominant (12.64%) and followed by *Fusobacterium* spp (Table 1). *Prevotella* spp. produced black pigment in blood agar plate (Figure 1A) and *Fusobacterium* spp appeared microscopic long slender thin rod with both ends were tapered appearance (Figure 1B). Our results coincide with Shah and Collins (1990) and Tanner et al. (1992) *Prevotella* species of *P. gingivalis*, *P. intermedia/ nigrescens*, and the *P. melaninogenica* (black) group produce pigment when they grow on blood agar. Although pigmentation is largely dependent on the culture media used, pigment production remains a widely used distinguishing character in the early stages of identification. In the present study the *Fusobacterium* spp identification results similarly reported by Morris et al., (1997) they were identified *Fusobacterium* spp by their characteristic cell morphology: long, almost filamentous cell shape with pointed ends, but sub species differentiation can only be achieved by molecular methods, such as multilocus enzyme electrophoresis (Roques et al., 2000). Other “pioneering” genera observed are Gram-positive *Actinomyces, Gemella, Granulicatella,* and *Rothia* species*,* as well as Gram-negative *Neisseria, Prevotella,* and *Veillonella* species. *Lactobacillus* species’ numbers in saliva seem to reflect the amount of simple carbohydrate consumption by the host (Tanzer et al., 2001).

Antibiotics sensitivity results

Antibiotic susceptibility results show that *S. mutants* were resistant to penicillin, streptomycin and clindamycin

Table 2. Distribution of antibiotic susceptible pattern of *Streptococcus* spp S- Sensitive; R-resistance; I- intermediate.

Name of the antibiotics	*Streptococcus* spp			
	***S. mutants* (n=20)**	***S. salivarius* (n=12)**	***S. sanguis* (n=08)**	***S. mitis* (n=05)**
Bacitracin	S	R	S	R
Penicillin	R	R	R	R
Streptomycin	R	R	I	I
Clindamycin	R	S	S	I
Chloroamphenicol	I	I	S	S
Vancomycin	I	R	S	S
Tetracyclin	S	S	S	I

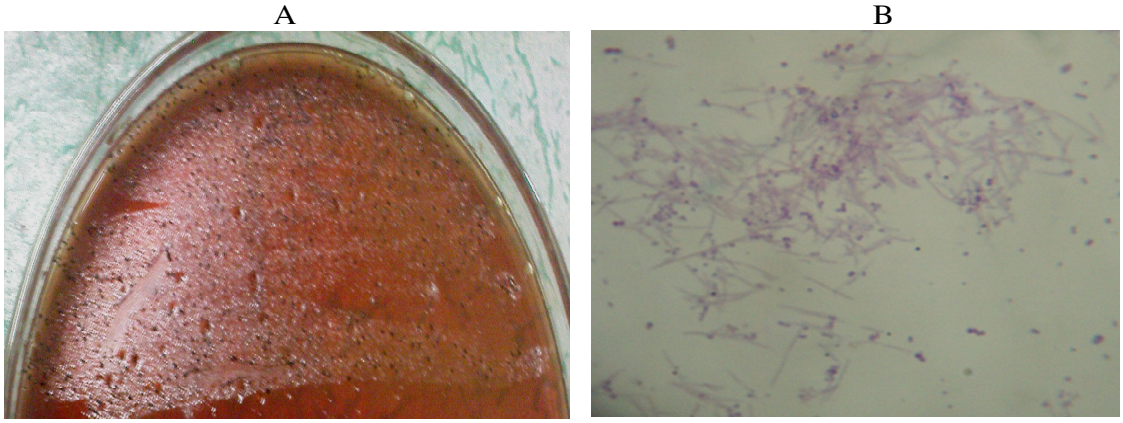

Figure 1. A- *Prevotella* spp on blood agar plate with black pigments; B- Cell morphology of *Fusobacterium* spp by simple staining appeared thin slender rod with tapered ends.

whereas *S. salivarius* was sensitive to clindamycin and tetracycline. In *S. sanguis* was resistant to penicillin and all other drugs were sensitive except streptomycin it was intermediate results. *S. mitis* were sensitive to vancomycin and chloroamphenicol and rest of the drugs were observed resistant and intermediate results (Table 2). Our results were closely coincide with Salako et al. (2007) they reported antibiotic resistance studies from healthy and intellectually disabled children dental plaque samples, streptococcal isolates the percentage of resistant strains was found to be highest with amoxicillin (43%) and lowest with vancomycin (12%). *S. sanguis, S. mitis* and *S. oralis* were more resistant in healthy children (45, 56 and 55% respectively) than in children with disabilities (40, 47 and 47% respectively). *S. mutans* was the least resistant species to all antibiotics in both groups of children. About 56% of streptococci isolated from both groups were resistant to at least one of the antibiotics tested. Dental caries is an infectious disease of bacterial origin; antimicrobial agents constitute a reasonable approach toward controlling bacterial bio-film in *situ*. Present-day chemotherapeutic approaches seem well reasoned approach for the control of caries. It should be aimed not toward elimination of all the plaque micro-organisms but toward affecting an ecological shift from a cariogenic to a non-cariogenic biofilm (Bradshaw et al., 1990; Bader et al., 2000).

Assessment of antimicrobial activity of tooth pastes

The distilled water extracts of the toothpaste were found to have marked antimicrobial properties. The optimum concentration of the tooth paste for antimicrobial activity assessment against *S. mutans, S. salivarius, S. sanguis* and *S. mitis* were determined using different tooth pastes. There were no differences observed between the *Streptococcus* spp, the data shown only on *S. mutans* (Figure 1). The zones of inhibition against the *Streptococcus* spp results by the toothpastes pepsodent was more effective followed by the Colgate and so on (Figure 2). Dental problems are the most frequent cases

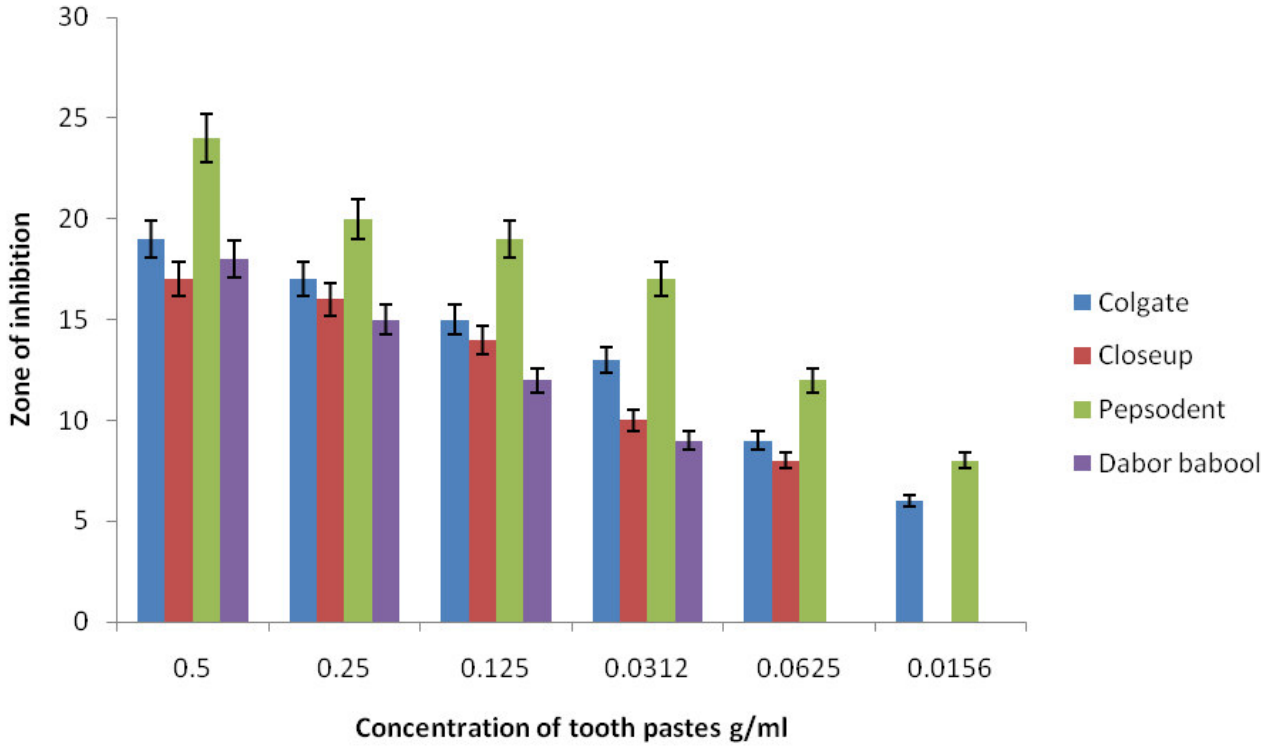

Figure 2. Distribution of different commercially used tooth pastes concentrations effect on *Streptococcus* spp zone of inhibition, error bars for selected chart with 5% value.

in the general population associated mainly with dental hygiene practices. Furthermore, the efficacies of the toothpastes regarding their chemical composition is not less important especially in developing countries like India where low grade products can be found in local markets and consumers are forced unknowingly to choose the products. The present finding is similar to those of Tiwari et al. (2007) who observed the anti-bacterial effect of different commercially available tooth pastes Colgate and pepsodent has good antibacterial effect against *Streptococcus* spp than the dabor babool and neem paste. Ayurvedic contents of Dabur babool the laung helps to prevent toothache, pudina helps to prevent bad breath and tomar kills harmful germs. Statistical analysis showed that the zones-of-inhibition of toothpastes against the test organism were not differed significantly on repeated attempts ($P > 0.05$). However Walsh et al. (2010) similarly reported that the benefits of using fluoride toothpaste in preventing caries in children and adolescents when compared to placebo, but only significantly for fluoride concentrations of 1000 ppm and above. The relative caries preventive effects of fluoride toothpastes of different concentrations increase with higher fluoride concentration. The present studies concluded that the prevalence of cariogenic bacterial pathogens were found facultative anaerobe, *S. mutants* (22.98%) *S. salivarius* (13.79) and *Candida* spp (14.97), in anaerobes, *Prevotella* spp (12.64) and *Fusobacterium* spp (5.74). The antibiotic susceptibility test results of *Streptococcus* spp all isolates were resistance to penicillin and sensitive to tetracycline and toothpastes-Pepsodont and Colgate were found to be highly effective against the pathogens. It showed that the toothpastes containing Triclosan as a major chemical ingredient posses significant antibacterial activities.

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