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Evaluation of Antimicrobial Susceptibility Pattern of *Streptococcus Mutans* Isolated from Dental Plaques to Chlorhexidine, Nanosil and Common Antibiotics

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**ABSTRACT**

Streptococcus mutans is one of the most important leading causes of dental diseases worldwide and is considered as one of the main causative agents of dental caries. Increasing resistance of oral pathogens to conventional antibacterial agents has resulted in finding alternative therapies to overcome resistance development problems. The aim of this study was to examine susceptibility of *Streptococcus mutans* isolates to some antibiotics, chlorhexidine and nanosil. The study subjects comprised of caries active individual volunteers attending the outpatient department of different dental college hospitals of Kerman. The saliva sample of 2-5 ml was collected from each individual in sterile capped bottles and were immediately transported to the laboratory and processed for the screening of *S. mutans*. The specific selective media, Blood agar and Tryptone yeast cysteine media agar were used for the screening and isolation of *Streptococcus mutans* and incubated anaerobically at 37°C for 48 hrs. Colonies of *mutans* streptococci were examined under a dissecting microscope and identified by their distinctive colony morphology and biochemical tests. Mutacin production of all isolates were investigated. The isolates were tested for susceptibility to some antibiotics (penicillin, gentamycin, vancomycin, cephalotin), chlorhexidine and nanosil mouthwashes by disc diffusion method. The results showed that the majority of mutacin and non-mutacin-producing isolates were more sensitive to the nanosil than antibiotics and chlorhexidine. More extensive research on the use of mouthwashes containing silver nanoparticles is suggested.

**Key words:** *Streptococcus mutans; Antibiotic; Chlorhexidine; Nanosil*

**INTRODUCTION**

Twenty-five species of streptococci live in the human oral cavity and represent about twenty percent of the total oral bacteria (Nicolas, 2011). Each species has developed specific specialized characteristics for colonizing different oral sites and frequently changing conditions to fight competing bacteria and to survive external trials (Banas, 2004). *Streptococcus mutans* is gram-positive bacteria, facultatively anaerobe and has a thick cell wall and capsule that is composed of polysaccharide, that its structural subunit is dextran glucose. The bacteria was first described by James Kilian Clarke in 1924 (Matsui, 2010). *Streptococcus mutans* and *Streptococcus sobrinus* are contributed to oral disease and sometimes for clinical purposes, they are considered together as a group, called the *mutans* streptococci (Loesche, 1986). *Streptococcus mutans* plays a major role in tooth decay (Kuramitsu et al., 2007). One of the virulence factors of *Streptococcus mutans* in cariogenicity is its ability to attach to the tooth surface and form a biofilm (Marsh, 2000). It is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth (Clarke, 1984). The etiological agent of dental caries is associated with its ability to metabolize several sugars using the enzyme glucan sucrose like dextran sucrose, and produce a sticky, extracellular, dextran-based polysaccharide that allows them to adhere, forming plaque (Roeters, 1995, Wilson, 2008). The combination of acid and plaque leads to dental decay (Topazian, 2002). Practice of good oral hygiene including daily brushing and use of appropriate mouthwashes can significantly reduce the number of oral pathogens.
bacteria and inhibit their proliferation (Darby, 2002). Things that can help combat this organism are products containing xylitol such as gums, products containing baking soda like toothpaste, fluoride and mouth washes (Subramaniam, 2011). Using fluoride products are a must as they remineralize tooth enamel that supports to prevent tooth decay (Greenberg, 2003). Chlorhexidine decreases populations of S. mutans, apparently by interfering with bacterial adherence (Autio-Gold, 2008). The nanosil mouthwash (Sanosil) (Kimiafam pharmaceutical Co., Iran) is a hydrogen peroxide formulation with silver nanoparticles. The secret of the Sanosil disinfectants is the combined 2-phase effect of the main ingredients hydrogen peroxide (H2O2) and silver. The oxygen split off by the hydrogen peroxide attacks the cell walls of the microorganisms upon direct contact. The chemical reaction of the oxygen with molecules in the cell walls denaturises and destroys these. This effect is boosted by silver ions that bind to the disulfide bonds of certain proteins, both of the reproduction complex as well as of the metabolic system of the microorganisms, and deactivate or precipitate these (Ayla-Nunez, 2009). The hydrogen peroxide affects the membrane of the microorganisms, the silver the inside (Chen and Bai, 2007). This combined "hammer and ambos" effect boosts and/or exponentiates the biocide effect of hydrogen peroxide and silver on a large scale. While H2O2 breaks down into water and oxygen afterwards, minute traces of silver remain on the disinfected surface (Nikan, et al, 2013, Rai and Yadav, 2008). These traces are invisible and non-toxic but actively and effectively counteract regermination. The present study was directed to evaluate the efficacy of chlorhexidine mouth rinses on some Streptococcus mutans isolates and compare the anti-microbial effect of chlorhexidine with nanosil. Also microbial resistance pattern of Streptococcus mutans isolates to general antibiotics was determined.

**MATERIAL AND METHODS**

Saliva samples were collected from caries active individual volunteers attending the outpatient department of different dental college hospitals of Kerman and were immediately transported to the laboratory. 2-5 milliters of whole saliva was inoculated into a tube containing 1 ml of trypticase soy broth (Merck) and incubated in 37°C for 48 hours in anaerobic condition using anaerobic jar (Jensen, 1988). After incubation period, samples were cultured on blood agar (Merck) and TYC (Tryptone yeast cysteine) agar (Merck),

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**Figure 1.** Granular colony of *S. mutans*

**Figure 2.** Heaped colony of *S. mutans*

**Figure 3.** Comparison of inhibition zone between chlorhexidine (down) and nanosil (above)

**Figure 4.** Antibiotic susceptibility test to 4 antibiotics
with 0.1 unit of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml. From each cultured saliva sample, four different S. mutans-like colonies were chosen for further diagnostic studies (Jensen, 1988). Isolates were subcultured in plate count agar (Difco) and identified by gram staining, catalase production, fermentation and biochemical tests. (Baron and Finegold, 1990). A total of 40 Streptococcus mutans isolates were identified. The isolates were screened for mutacin production (Novak et al, 1994). Standard suspension of each isolate (mutacin and non-mutacin producing bacteria) inoculated to sterile normal saline and compared to 0.5 McFarland solution to obtain 1.5 x 10^8 bacteria/ml and cultured on plate count agar and the susceptibility of them to the following agents were tested: gentamycin, vancomycin, penicillin, cephalothin (Sigma), chlorhexidine and nanosil (Wayne,1979, Arzu and Erdem, 2012). The plates were incubated at 37°C in a 5% CO2 atmosphere and anaerobic condition for 24 hours. Level of susceptibility were measured by zone of inhibition in mm.

RESULTS

At first, growth colonies on blood agar media with alpha hemolysis were selected. Then alpha hemolysis isolates showing granular and heaped colony on the TYC agar were considered as Streptococcus mutans and based on results of sugar fermentation, biochemical tests and ability to glucon production, were confirmed respectively .Glucon production of Streptococcus mutans isolates resulted in highly refractile, adherent or white dry, adherent growth. Glucon production was also showed when gelatinous, adherent deposits formed on the bottom and walls of the growth tube, typical of Streptococcus mutans (Fig 1 and 2). Catalase, urease and esculin hydrolysis test about Streptococcus mutans are negative and Sucrose, glucose, fructose and mannitol fermentation are positive. Most of isolates produced mutacin .The susceptibility to used antimicrobial agents about mutacin and non-mutacin producing isolates were the same. But nanosil was more effective against all the Streptococcus mutans isolates than chlorhexidine because of increasing of inhibition zone (Fig 3). All the isolates were sensitive to used antibiotics, penicillin, gentamycin, vancomycin, cephalothin (Figure 4).

DISCUSSION

Mouthwashes can be used as adjunct to restoration for short duration as temporary measure in reduction of Streptococcus mutans count and restorations provide longer effect. The goal of this study was to compare antibacterial properties of chlorhexidine and nanosil on 40 Streptococcus mutans isolates. The findings of the present investigation, indicated the susceptibility of isolated Streptococcus mutans to both mouthwashes but nanosil showed more effect than chlorhexidine. This finding is related to nano silver particles in nanosil. Silver ions have combined as silver nanoparticles in nanosil. A silver nanoparticle consists of many silver atoms or ions clustered together to form a particle 1-100 nm in size. Due to their small size, these nanoparticles are able to invade bacteria and other microorganisms and kill them. Although there is an increasing and continuing of microbial resistance to usual antibiotics, we found the all Streptococcus mutans isolates were sensitive to penicillin, gentamycin, vancomycin and cephalothin. Jarvinen studied the susceptibility of Streptococcus mutans to chlorhexidine and six commonly antibiotics (amoxicillin, cefuroxime, penicillin, sulfamethoxazole-trimethoprim, tetracycline, and erythromycin) for 424 clinical isolates and there was no resistance to the antimicrobial agents (Jarvinen et al, 1993). Meurman and co-workers studied susceptibility of 128 bacterial strains to amine fluoride-stannous fluoride (AmF + SnF) and chlorhexidine (CHX) solutions and found both solutions tested exerted a definite inhibitory action on the dental plaque pathogens (Meurman, et al, 1989). In another study, comparison between the antibacterial effect of Nanosil and chlorhexidine mouthwash was done and their finding showed that Nanosil mouthwash had a significant statistical superiority in comparison with the placebo in both aerobic and anaerobic environments. Nanosil had a significant effect on anaerobic environment, meanwhile, the placebo indicated a superior effect in anaerobic environment (Esfahanian, et al, 2012). Salehi and Momeni Danaie compared the antibacterial effects of persica mouthwash with chlorhexidine on Streptococcus mutans in orthodontic patients. They showed significant reduction of Streptococcus mutans colonies by persica (Salehi and Momeni Danaie, 2006).

CONCLUSION

In conclusion, although there is an increasing and continuing selection pressure on Streptococcus mutans by commonly used antimicrobial agents, especially chlorhexidine, oral Streptococcus mutans has remained susceptible in Iran. The efficacy of chlorhexidine has demonstrated in several studies, therefore it can introduced as a positive control in the next studies. Nanosil mouthwash also can be applied as an effective antibacterial substance though chlorhexidine as a standard mouthwash has still the strongest effect in this field.

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