THE EFFECTS OF CALCIUM HYDROXIDE, SODIUM HYPOCHLORITE AND CHLORHEXIDINE, SOLUTIONS ON THE GROWTH OF *STREPTOCOCCUS MUTANS* ISOLATED FROM DENTAL CARIES

Samira G. Jremich ¹, Dina M.R. Alkhafaf ²

¹basic science branch. dentistry college. University of Al-Qadisiyah. Iraq  
²department of biology. Education. University of Al-Qadisiyah. Iraq  
E.Mail/ samira.alabsi@qu.edu.iq

ABSTRACT

Antibiotic resistance by microbes is an escalating health problem frequently seen all over the world. The need for a new strategy is an urgent goal for researchers by testing different natural or synthetic compounds to overcome this detrimental health issue. The present work was produced to identify the effects of prepared solutions of calcium hydroxide (CH), sodium hypochlorite (SH), and chlorhexidine (CHD), on the bacterial microorganism; *Streptococcus mutans*. For the bacterial isolation, the study samples were collected from 20 tooth-root-canal-based patients (20-60 years old) admitted the dental clinic at Al-Diwaniyah Teaching Hospital, Al-Diwaniyah City, Iraq. For the inhibitory activity substance test (IAST), the bacterial viability was tested with the prepared solutions (50mg/100ml distilled water/each solution) that were applied to the Mueller Hinton Agar (MHA) plates inoculated with *Strept. Mutans* at (50%, 40%, 30%, 20%, and 10%), (50%, 40%, 30%, 20%, and 10%), and (4%, 2%, 1%, 0.5%, and 0.25%), respectively, in which each percentage was inserted into one well. The result outcomes revealed that each compound showed significantly ($p<0.05$) increased inhibition to the growth of *Strept.* *mutans*, which is positively correlated with increased concentration percentage of the applied solution. The current experiment data demonstrate promising effects of the used compounds against the growth of *Strept. Mutans*.

Keywords: Antibiotic resistance, calcium hydroxide, chlorhexidine, sodium hypochlorite.

I. INTRODUCTION

Antibiotic effectiveness is jeopardized due to the increase emergence of resistant bacteria around the globe. Bacterial diseases have reappeared as a critical danger to the health after several generations since the first patients were administered with antibiotic agents. The overuse and abuse of antibiotics, as well as a shortage of innovative drug production by the pharmaceutical sector owing to insufficient economic potentials and difficult regulatory terms have been blamed for the antibiotic resistance problem(1–4).

The Centers for Disease Control and Prevention (CDC) in the United States has recognized a broad range of dangerous bacteria, most of which are now placing a detrimental clinical and financial pressure on the healthcare system, patients, and their relatives. Organized attempts to introduce new regulations, update study plans, and develop crisis-management strategies are critical requirements(5,6).

J. Clarke extracted a microorganism from carious pathological lesions in 1924 and named it *Strept. mutans*, believing the oval-shaped cells he found were mutated streptococci. *Strept. mutans* did not receive broad interest from the scientific world until the late 1950s, though by the mid-1960s, experimental trials had identified *Strept. mutans* as a potential causative pathogen in dental caries. *Strept. mutans* usual environment is the oral cavity, dental plaques, a biofilm on the tooth hard surfaces formed by multiple species(7).

The occurrence of the dental caries of this bacterium can be attributed by: 1) The tendency to produce mass amounts of glucan (an extracellular polymeric (EP) agent) from sugars that assist in the persistent occupation of this bacterium on these surfaces, leading to the production of the EP matrix (EPM), 2) the power to transmit and metabolize a diverse spectrum of carbohydrate sources. Although *Strept. mutans* does not cause dental caries on its own, experiments from multiple experiments have shown that it may change the dental region into EPM-rich
and low pH microenvironment, thus providing a conducive setting for other acidogenic and aciduric organisms to survive. The bacterial pathogenic microorganism, *Strept. mutans* has been attributed to extraoral illnesses including cerebral microbleeds, IgA nephropathy, and atherosclerosis, as well as sub-acute endocarditis of the valves, a life-threatening infection (8).

The present work was produced to identify the effects of CH, SH and CHD on the bacterial growth of *Strept. mutans*.

### II. MATERIALS AND METHODS

#### Patients and sample collection

The study samples were collected from 20 dental caries &root-canal-based patients (20-60 years old) admitted the dental clinic at Al-Diwaniyah Teaching Hospital, Al-Diwaniyah City, Iraq, during April to September, 2010. Data regarding gender, history, findings of the clinical examination, and diagnosis were collected. If a patient had of antibiotic administration history within the previous three months, he/she was excluded from the present study.

#### Inhibitory activity substance test

The IAST was done by using MHA with a method described by (9), by which at least IAST 3-5 bacterial pure colonies were inoculated in 4-5 ml of brain heart infusion broth (BHIB). Then, the inoculated BHIB was set up for an incubation period of up to eight hours at 37°C. The BHIB growth turbidity was optimized to the 0.5 McFarland standards tube (growth equivalent to 1.5 X10^8 cell/ml). The prepared solutions (50mg/100 ml distilled water/each compound) out of CH, SH, CHD, and CS were applied to the MHA plates inoculated with *Strept. Mutans* at (50%, 40%, 30%, 20%, and 10%), (50%, 40%, 30%, 20%, and 10%), (4%, 2%, 1%, 0.5%, and 0.25%), respectively, in which each percentage was inserted into one well. MHA was incubated for an over-night period at 37°C. Later, the zones of inhibitions were measured. The findings were compared to the minimum inhibition diameter of the National Committee for Clinical Laboratories Standards (NCCLS) (2007). The concentrations and methods used in the present work were followed from (10–13).

### III. RESULTS

The result outcomes revealed that each compound showed significantly ($p<0.05$) increased inhibition to the growth of *Strept. mutans*, which is positively correlated with increased concentration percentage of the applied solution. This is demonstrated in the figures (1A, B, C, and D and 2 A, B, and C) for CH, SH, and CHD, respectively.
Figure 1: Inhibition zones of A) calcium hydroxide, B) sodium hypochlorite, and C) chlorhexidine, against the growth of *Streptococcus mutans*.
IV. DISCUSSION

The most frequently occurring infectious disorder in the oral cavity is dental caries, also known as "tooth decay." In children and young adults, dental caries are the leading trigger of tooth damage and loss, and in the elderly, it is the leading trigger of tooth damage. The World Health Organization (WHO) indicates that the incidence of dental caries is 60–80 percent in children and about 100 percent in adults. Microbes find a special physiological niche in the oral cavity, with the most accumulating on dental surfaces to shape dental biofilm (plaques). The main cariogenic factor concerned in the formation of dental caries is *Strept. mutans*. Demineralization occurs quicker than remineralization as a consequence of ecological changes, such as the rise in the numbers of these pathogenic flora in dental biofilm(14).

The current work findings revealed successful inhibition of the growth of *Strept. mutans* as it was demonstrated by the IAST. Endodontics has long utilized CH as an intracanal medication to destroy any residual microbes following chemical and mechanical preparations. CH has been studied for antimicrobial characteristics tissue dissolving capacity, tooth resorption suppression, and hard tissue dissolution capacity against the growth of *Streptococcus mutans*.

Figure 2: Inhibition zones of A) calcium hydroxide, B) sodium hypochlorite, and C) chlorhexidine, against the growth of *Streptococcus mutans*.
construction, and its widespread usage in root canal care has been consistent with periradicular recovery and few side effects. CH is the most used root canal dressing substance.(15). The present study data of the CH agree with the above mentioned fact.CH is a white and odorless powder. It is highly water insoluble and eventually emits calcium and hydroxyl ions. This ion-release can explain the antimicrobial activities of CH. This leads to the characteristic that CH takes a long time to get dissolved in tissue liquids while in close interaction with essential tissues, which makes it a positive clinical feature.(15).

Poggiøet al (16) have used SH-based irrigation solutions and found that, at 5.25%, the solution was significantly effective against Strept. mutans, Enterococcus faecalis, and Staphylococcus aureus. Evans et al (17) have used SH in an agar diffusion test and detected that SH, at 0.5%, was highly active in inhibiting the growth of Strept. mutans, Strept. sanguinis, and Lactobacillus acidophilus. This potentially agrees with the current findings regarding SH as a strong growth inhibitor of Strept. mutans. SH is widely utilized to disinfect infant bottles and pacifiers and could have significance as a disinfectant to reduce cariogenic bacterial colonization in infants(18,19).

For the CHD, the present findings declared that the compound was strong in decreasing the growth rate of Strept. mutans. Evans et al (17) have also used CHD and reported that CHD, at 0.01%, was strongly defective to the growth of Strept. mutans, Strept. sanguinis, and L. acidophilus. CHD is a strong antiseptic with a hydrophilic bisguanide structure. It prevents the development of Gram-positive cariogenic microbes, such as Strept. mutans and certain Lactobacillus bacteria, and thus decreases plaque production. It has a strong substantivity since it attaches to negatively charged components such as microbial cell wall, salivary pellicle, and mucosa. CHD is bacteriostatic at tiny amounts, interacting with the transportation of the cell wall and causing intracellular material leakage. It is bactericidal at elevated amounts (>1%), allowing the intracellular cytoplasm to precipitate. It also prevents bacteria from adhering to the tooth surface by inhibiting the activity of glycosyltransferase(17,20,21).

V. CONCLUSION

The current experiment data demonstrate promising effects of calcium hydroxide, sodium hypochlorite, and chlorhexidine, against the growth of Strept. Mutans. These effects were especially potent at the highest concentrations.

REFERENCES


