# A comparison between the antibacterial and antifungal effects of chlorhexidine digluconate (An in vitro study)

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## ABSTRACT

Background: The use of antimicrobial agent to control plaque and oral disease has been advocated for a number of years. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. The purpose of this research is to find out and to compare between the antibacterial and antifungal properties of Chlorhexidine digluconate 0.2%.

Materials and methods: Mutans streptococci & *Candida albicans* were isolated from 25 saliva samples from healthy volunteers (age range between 21-23 yrs). These isolates were purified and diagnosed according to morphological characteristics and biochemical tests. Chlorhexidine 2mg/ml (0.2%) was used in the *in vitro*; susceptibility of Mutans streptococci and *Candida albicans* were tested by agar diffusion technique.

Results: Agar diffusion technique showed that Chlorhexidine (0.2%) inhibited the growth of Mutans Streptococci, and *Candida albicans,* but the effect of Chlorhexidine (0.2%) on *Candida albicans* was more patent than on Mutans Streptococci *in vitro*. There was statistically highly significant difference ($p<0.001$) between the antifungal and antibacterial effects of Chlorhexidine on the sensitivity of the isolates,

Conclusion: Chlorhexidine digluconate 0.2% was more potent as an antifungal than an antibacterial agent.

Key words: Chlorhexidine digluconate, mutans streptococci, *Candida albicans*. (J Bagh Coll Dentistry 2012;24(2):88-90).

## INTRODUCTION

The use of antimicrobial agent to control plaque and oral disease has been advocated for a number of years [1]. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. Some chemical agents have proven to be helpful against plaque accumulation and thereby to some extent also against caries [2].

Oral mutans streptococci (MS) are responsible for 50–70% of all cases of bacterial endocarditis. The origins of endocarditis lie in invasion of the vascular system through lesions in the oral mucosa [3]. These streptococci can attach to the proteins covering the tooth enamel, where they then convert sucrose into extra cellular polysaccharides (mutan, dextran, levan) [4]. These sticky substances, in which the original bacterial layers along with secondary bacterial colonizers are embedded, form dental plaque. The final metabolites of the numerous plaque bacteria are organic acids that breach the enamel, allowing the different caries bacteria to begin destroying the dentin [5].

A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of Candida, especially *Candida albicans*) [6]. The first exposure to fungi that most humans experience occurs during birth, when they encounter the yeast *Candida albicans* (*C. albicans*) while passing through the vaginal canal. *C. albicans* accidentally penetrate barriers such as intact mucous membrane linings, or when immunologic defects or other debilitating conditions exist in the host, these conditions favorable for fungal infections [7].

## MATERIALS AND METHODS

Stimulated saliva samples were collected under standard conditions to obtain 25 microbial samples. Volunteers with no medical history aged 21-23 years were selected to participate in this study. Each individual was instructed to chew a piece of Arabic chewing gum (0.4-0.5g) for five minutes to stimulate salivary flow as much as possible then saliva was collected in sterilized screw capped bottles. The collected saliva was homogenized by vortex mixer for two minutes. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected for each microbial type and inoculated on the following culture media which are prepared according to the manufacturer's instructions:

1. Mitis-Salivarius Bacitracin Agar (MSB Agar), the selective media for MS: 0.1ml was withdrawn from dilutions $10^{-1}$ and $10^{-2}$ using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader on the plates of MSB agar, the plates were then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hrs at 37°C followed by aerobic incubation for 24hrs at 37°C.
2. Sabouraud Dextrose Agar (SD Agar), the medium is selective for the cultivation and isolation of *C. albicans*: 0.1ml was withdrawn from dilutions $10^{-1}$ and $10^{-2}$ using adjustable micropipette with disposable tips and then spread

in duplicate by using sterile microbiological glass spreader on the plates of SD agar then the plates were incubated aerobically for 48 hrs at 37° C.

A single colony from MS and *C. albicans* separately were transferred to 10 ml sterile BHI-B and then incubated for 24 hrs aerobically at 37°C to activate the inoculums.

The purity of the isolates was checked by reinoculation of 0.1 ml of the isolates from BHI-B suspensions on their selective media by spreader as mentioned before, then selective colony from each isolate was transferred to 10 ml of sterile BHI-B and incubated for 24 hrs aerobically at 37°C. One ml from this broth was transferred to 10 ml sterile BHI-B and then 1 ml sterile glycerol was added to the inoculated broth; the tubes were labeled (the type of inoculum and the date of inoculation) and freezed until use. This procedure was repeated twice monthly. A colony was picked up from MSB agar and SD agar plates separately under sterilized conditions and subjected to gram's stain; all the isolates were gram positive. The motility of all types of microbial cells was examined under microscope by direct smear and without staining; the isolates were non- motile. Catalase production test was performed; a small amount of pure isolates of MS cultures was transferred using a sterile loop to the surface of clean dry glass slide. Drops of hydrogen peroxide 3% immediately placed onto a portion of bacterial culture on the slide, absence of gas bubbles indicates the absence of catalase enzyme.

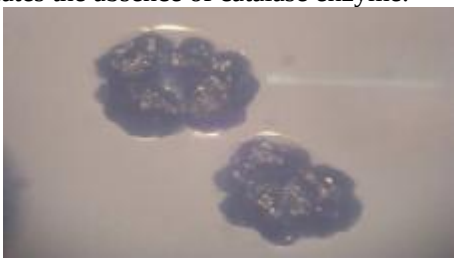

**Figure 1: MS colonies on MSB agar (20 x magnifications).**

Cystine Trypticase-mannitol media had been used to test the ability of MS to ferment the mannitol which was added in a concentration of 1% to the Cystine Trypticase Agar media (which was prepared according to the manufacturer instructions biomerieux Company), then distributed into screw capped bottles (10ml in each bottle) and autoclaved, each bottle was inoculated with 0.1ml of pure MS isolates and incubated aerobically at 37°C for 48 hrs. Changing in color from red to yellow indicated a positive reaction in comparison to the positive control (agar and bacteria without mannitol) and negative control (agar and mannitol without bacteria) because of pH reduction as a result of acid production from the fermentation reaction (Fig. 2).

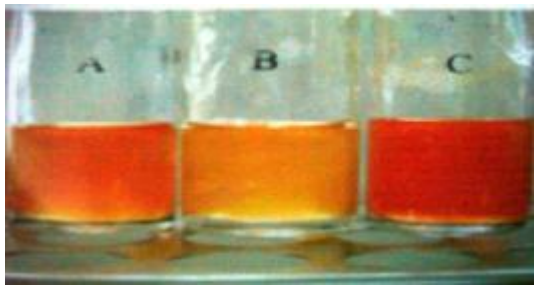


**Figure 2: Biochemical identification of Mutans streptococci.**
**A: Positive control tube (agar and bacteria without mannitol).**
**B: Study tube (agar and mannitol inoculated with MS).**
**C: Negative control tube (agar and mannitol without bacteria).**

*C. albicans* diagnosed according to morphological properties using Gram's stain (Fig. 3) and germ tube formation in human plasma.

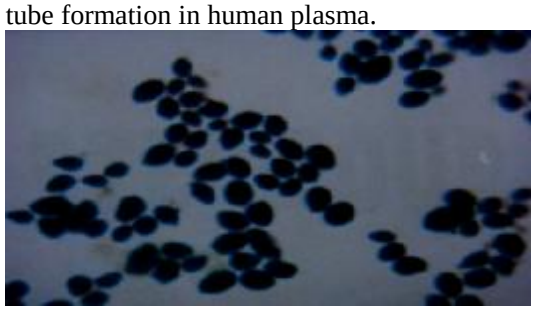

**Figure 3: Gram's stain of C albicans showing gram positive stains (1000x magnification).**

All culture media and normal saline were sterilized by autoclave at 121°C and pressure of 15 pound/inch$^2$ for 15 minutes except for the CTA medium which was sterilized by autoclaving for 10 minutes. Bacitracin solution was filtered using millipore filter size 0.20 µm. Sterilization of all cleaned glass wares was conducted by hot air oven at 180°C for 1 hr. Benches and floor of the laboratory were disinfected by bleaching antiseptic solution (Fas).

Agar diffusion technique was applied to study the antimicrobial effects of CHX against the isolates spread on Brain Heart Infusion Agar (BHI-A); wells of equal sizes and depths were prepared in the agar using Kork porer for the evaluation of CHX. Each well was filled with 50µl of 0.2% CHX. Plates left for 15 minutes in the room temperature and then incubated aerobically for 24 hrs at 37°C. Inhibition zones' diameters were measured using a scientific ruler.

# RESULTS

Diameters of inhibition zones for CHX were found to be greater in the plates inoculated with *C. albicans* than those formed in the plates of MS (Fig. 4). Student's t-test showed highly significant differences among different CHX inhibitions' zones (Table 1).

Results of agar diffusion experiments are affected by many factors like the molecular weights and concentrations of the antimicrobial agent, the types of the isolates and the fluidity and/or stickiness of the solutions. The thickness of the agar was well controlled through out the experiment by measuring the volume of the agar while it was liquid before poring it into the same sized petridishes in order to avoid the variation of the results which will appear as a result of agar thickness variations. The size of inoculums was controlled by using adjustable micropipettes with disposable tips to ensure that equal volumes of the isolates' suspensions were dispensed into all the plates and the same precaution was carried out for the volumes of the extracts and CHX which were dispensed into the wells made in the agar plates.

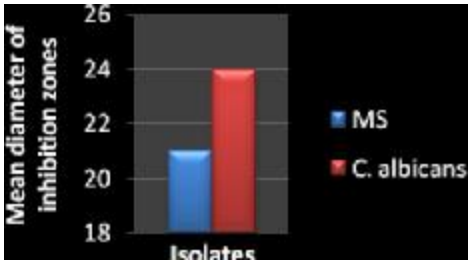


**Figure 4: Comparison between the mean diameters of inhibition zones of CHX in relation to Mutans streptococci and *C. albicans*.**

**Table1: Statistical analysis for the sensitivity of Mutans streptococci, lactobacilli and *C albicans* to different concentrations of aqueous extract of eucalyptus using Student's t- test.**

| Isolates | CHX 0.2% | |
|---|---|---|
| | t-value | t-value |
| MS & *C. albicans* | 16.43 | 0.000 **** |

*****P<0.001 High significant**

Results showed that there were some differences in the sensitivity of the isolates to CHX; it can be explained by the differences between eukaryotic cells (fungi) and prokaryotic cells (bacteria) especially of the cell wall. The cell walls of fungi consist of nearly 90% carbohydrate (chitin, glucans, mannans) and fungal membranes are rich in sterol types not found in other biological membranes like ergosterol [8]. The sensitivity of MS and *C. albicans* to the aqueous extract of eucalyptus could be due to the hereditary contents or attraction ability or the permeability of the cell wall of the microorganisms. CHX disrupts cell membrane and cell wall permeability of many Gram- positive and Gram-negative bacteria and interferes with the adherence of plaque-forming bacteria, thus reducing the rate of plaque accumulation [9], it can inhibit the adenosine triphosphatase (ATPase) which is an important enzyme that is linked to cytoplasmic membrane and thus can inhibit the process of returning potassium ions into cells in exchange for sodium and hydrogen ions, also inhibits metabolic enzymes [10].

Differences in the microbial susceptibility to CHX could also be due to the hereditary contents of the isolates which may alter the susceptibility of the organisms by modifying the targets to be attacked by the active constituents like the proteins and lipids of the microbial membrane or inhibiting the constituents of the leaves' extract or modifying the structures of these constituents by some enzymes rendering them to less effective compounds.

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