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Antimicrobial Effect of Alginate Impression after Treatment with Certain Disinfecting Agents

Hanan A. Khalaf
Department of Prosthodontics, College of dentistry, Uruk University B.D.S., M.S.C., Ph.D

Abstract: **Background:** contaminated dental impressions can cross infect stone casts that have been poured against them, surface disinfection of dental impression done through spraying or immersion in chemical antimicrobial agents.

Objectives: To observe the anti microbial action of different types of disinfecting agents (Hexyl tri ethyl ammonium chloride salt, Tetra butyl ammonium iodide salt, 1.2% sodium phenoxide salt, iodophor solutions, ethanol and 2- propanol) on certain aerobic micro organisms.

Materials and methods: the susceptibility of *staphylococcus aureus,, klebsiella and candida albican*s to the selected antimicrobial disinfectant agents (0.5% of quaternary ammonium salts (Hexyl tri ethyl ammonium chloride salt),0.5% of Tetra butyl ammonium iodide salt, 1.2% sodium phenoxide salt, iodophor solutions, ethanol and 2- propanol) was evaluated by Agar diffusion plate method. The data was analyzed by a computerized statistical program.

Results: The results showed that incorporation of alginate with 0.5% of quaternary ammonium salts, 0.5% of Tetra butyl ammonium iodide salt and 1.2% sodium phenoxide salt was found to be very effective in preventing the growth of *candida albicans*, also treating the alginate with iodophor solutions inhibit the growth of *candida albicans* and *klebsiella* spp.as well as treating alginate with Alcohols (ethanol, 2- propanol) showed great activity against *klebsiella spp*. and *candida albicans*.

Conclusion: tested micro organisms are sensitive to the disinfectant agents [Hexyl tiethyl ammonium chloride salt, sodium phenoxide salt, Iodophor, ethanol and 2-propanol] with certain degree of variation among the species.

Key points: Alginate, disinfection, microbial growth inhibition.

INTRODUCTION

Blood and saliva may carry high concentrations of potentially infectious viruses or bacteria that can produce the common cold, chicken pox, herpes,hepatitis B virus (HBV), infectious mononucleosis, influenza, pneumonia, staphylo coccus infections, strepto coccus infections, tuberculosis and are the suspected mode of transmission of *AID.*(1)

To prevent the transmission of disease, effective control procedures should be exercised by all dentists, in office dental auxillaries and dental technicians. If infection procedures are not practiced, a cycle of cross contamination may occur, there by exposing personnel and patients to infection. (2)

Prosthodontics patients are high risk patients relative to their potential to transmit infectious disease as well as acquire it. The dentists have a potential hazard of acquiring or transmitting infectious diseases during delivary of dental care. The cross contamination and disease transmission from patient to patient also occur. (3)

Therefore it has been recommended to disinfect impressions immediately after removal from the mouth in order to prevent contamination of dental staff and dental technicians during pouring of the impressions and further handling of the stone casts. (4)

Chemical disinfectants can produce high and intermediate levels of biocidal activity depending on the length of exposure and the type of material used. (5)

MATERIALS AND METHODS

The culture media, broths, cotton swabs used in this study were sterilized by using an autoclave at 15 b, 121 °C for 15 min. Glass flasks, cyliders, steel spatula, bronze punctures, steel rings were sterilized by dry heat in hot air oven at 160°C for 1 hr. (2,6,7).

The test micro organisms (*staphylococcus aureus, klebsiella and candida albican*s) were isolated on blood agar, mac conkey and sabouraud's dextrose agar for microbial investigation. The inoculated culture plates were incubated for 24-72 hours at 37 °C. The micro organisms were identified by colony morphology (8), figures (1,2,3), Gram stained films (9) and biochemical tests (10, 11).

Alginate was treated with disinfecting powders : (Quaternary ammonium, Tetra butyl ammonium iodide salt salts) each was mixed with alginate in a concentration of 0.5% (12) : 99.5 g of alginate mixed with 0.5 g of Quaternary ammonium salts.While sodium phenoxide powder used in concentration 1.2 % (13) : 98.8 g of alginate mixed with 1.2 g of sodium phenoxide. (measured with digital sensitive balance) with accuracy of 0.001, (Scaltec,Germany) and stored in sterile clean container tightly covered.

These concentrations obtained by applying the following formula (14)

$$\text{Percent of weight (solute)} = \frac{weight\ of\ solute\ in\ g}{weight\ of\ solute\ in\ g + weight\ of\ solvent\ in\ g} \times 100$$

Alginate was treated with disinfecting solutions : irreversible hydrocolloid (alginate) impression material was immersed for 15 minutes in one of 3 disinfectant solutions (15) :

1. 70% ethanol
2. 70% 2-propanol
3. 0.5% iodophor

Production of microbial inoculum

With a sterile wire loop, the tops of 2-3 isolated colonies of the organisms to be tested were picked from the original culture (slant) and introduced into a test tube containing 4 ml of nutrient broth. The broth was incubated at 37°C for 24 hrs to produce bacterial suspension (turbid broth) and for 72 hrs to produce candida suspension.

Ten irreversible hydrocolloid (nutrient agar) plates for each type of disinfecting agents were seeded with the test bacterium (*staphylococcus aureus, klebsiella)* and test candida (*candida albican*s) by streaking surface of the nutrient agar with a sterile cotton wool swabs that immersed in the microbial suspension and lightly squeezed against the walls of the test tube. (11, 15)

The disposable plates contain a standardized amount of nutrient agar (20 ml) which give a depth of 5 mm in each plate. after seeding the surface of the agar, then left several minutes till it become dry.

Wells measuring 1 cm in diameter by 5 mm in depth were created in the agar (14,16,17) with a sterile bronze cylinders having the same diameter and depth. Alginate plugs the have the same dimensions as the wells were placed in them.

Agar plates were incubated in the aerobic environment for 24 hrs at 37°C. Clear zones of inhibited growth were observed. The zones of inhibition were visually measured a cross the diameter of the wells. the measurements were recorded in millimeters.

Analysis of data was done by using one way analysis of variance (ANOVA)

RESULTS

Identification of *staphylococci* on blood agar culture media shows abundant growth, the colonies are fairly large, smooth, round and glistening after 24-48 hrs incubation period,fig.(1).

while slide coagulase test (clumping factor) identify coagulase positive *staphylococci*.

Identification of *klebsiella* on differential medium such as Mac conkey agar shows lactose and non lactose fermenting colonies, fig. (2).

Tripple sugar Iron agar test detect the ability of bacterium to ferment sugars which are glucose (0.1%), lactose and sucrose (1%).

Identification of *candida albican*s appears as small, creamy or white colonies that are somewhat extend out from the margins into the surrounding agar, fig. (3).

Sugar fermentation test (glucose,lactose, maltose and sucrose) shows changes in the color of the sugar broth with or without appearance of the air bubbles in the test tube.

Agar diffusion plate method regarding disinfecting powders reveals that Tetra butyl ammonium iodide salt was more effective than sodium phenoxide and hexyl tri ethyl ammonium chloride against *staphylo coccus aureus*. table (1,2), fig.(4)

For *klebsiella*, sodium phenoxide was more effective than the other disinfecting powders, table (1,2), fig.(5)

For *candida albicans*, hexyl tri ethyl ammonium chloride produce more inhibition zone than tetra butyl ammonium iodide and sodium phenoxide salts, table (1,2), fig.(6)

Agar diffusion plate method regarding disinfecting solutions reveals : 0.5 % iodophor was more effective against *candida albicans* and *klebsiella spp.,* table (1,3).

2- propanol (70%) was more effective against *klebsiella spp., candida albicans* and *staphylo coccus aureus.* table (1,3).

Ethanol (70%) shows more activity against *klebsiella spp.* in relation to other micro organisms table (1,3).

Discussion

The microbial colonization of impression materials by the oral micro flora is an indication that impression needs disinfection.(18)

This in vitro investigation primarily identifies the ability of antimicrobial agents to limit the growth of micro organisms by diffusion from the test material. The results of this study demonstrate a clear difference between the disinfectant agents tested.

Hexyl tri ethyl ammonium chloride salt (Quaternary ammonium salts) was more active against *Candida albicans* which is considered the most sensitive micro organisms tested followed by other bacterial species. This is in agreement with several studies (19,20).

The results demonstrate 0.5% of Tetra butyl ammonium iodide salt that was more effective against *Candida albicans* than other bacterial species, this is in agreement with other studies (21,22) who found that the antimicrobial activity of Tetra butyl ammonium iodide salt against gram positive and gram negative bacteria could be due to respective dissociation constants of cationic detergents and the acidic and basic groups of bacterial protein.

Sodium phenoxide salt was more active against *Candida albicans* and *Klebsiella spp.* followed by *staphylococcus aureu.* No studies were conducted to find out the effect of such type of material on the micro organisms investigated.

Iodophor immersion exhibited great antimicrobial activity against *Candida albicans* and *Klebsiella spp.*, least antimicrobial activity was observed against *staphylococcus aureus,* this resut was in

agreement with other studies (19, 23,24) which revealed that iodophor was effective against viruses, spores and bacterial micro organisms.

Ethanol 70% was more active against *Klebsiella spp.*, the remaining micro organisms listed in order of response were *Candida albicans* and *staphylococcus aureus,* while 2-propanol 70% exhibited activity against *Klebsiella spp.* and *Candida albicans*, less activity was observed against *staphylococcus aureus*. The results were in agreement with (24,25).

Conclusion

The incorporation of alginate with 0.5% Quaternary ammonium salts, 0.5% of Tetra butyl ammonium iodide salt,1.2% Sodium phenoxide salt was found to be very effective in preventing the growth of the tested micro organisms specially *Candida albicans.* Treating alginate with iodophor solutions inhibit the growth of all the micro organisms investigated specially *Candida albicans* and *Klebsiella spp.*

Treating alginate with Alcohols (Ethanol, 2-propanol) showed a great activity against *Klebsiella spp.* and *Candida albicans*.

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Table 1: The mean zones of inhibition together with the standard deviations for the effect of each specimen on the three species of micro organisms.

Disinfectants	Micro organisms			
	S.aureus	*Klebsiella spp.*	*Candida albicans*	Results
Hex. powder	16.25±0.13	16.25±0.21	18.1±0.18	**(p<0.05)**
Na. powder	16.75±0.29	17±0.19	17±0.28	**(p<0.05)**
Tetra. Powder	16.8±0.2	15.5±0.21	17.8±0.41	**(p<0.05)**
Iod. Imm. 0.5%	15±0.58	15.7±0.71	25.35±2.13	**(p<0.05)**
Eth. (70%)	14.05±0.55	17.05±1.32	14.55±0.76	**(p<0.05)**
2- prop. (70%)	14.5±0.41	18.3±1.42	16.1±0.84	**(p<0.05)**

Table 2: The mean zones (mm) together with the standard deviations for the effect of disinfecting powders on each micro organism.

Disinfecting materials	Micro organisms		
	S.aureus	*Klebsiella spp.*	*Candida albicans*
Hex. powder	16.25±0.13	16.25±0.21	18.1±0.18
Na. powder	16.75±0.29	17±0.19	17±0.28
Tetra. Powder	16.8±0.2	15.5±0.21	17.8±0.41
Results	**(p=0.16)**	**(p=0.05)**	**(p<0.05)**

Table 3: The mean zones (mm) together with the standard deviations for the effect of disinfecting solutions on each micro organism

Disinfecting materials	Micro organisms		
	S.aureus	*Klebsiella spp.*	*Candida albicans*
solutions			
Iod. Imm. 0.5%	15±0.58	15.7±0.71	25.35±0.675
Eth. (70%)	14.05±0.55	17.05±1.32	14.55±0.762
2- prop. (70%)	14.5±0.41	18.3±1.42	16.1±0.843
Results	**(p=0.05)**	**(p=0.05)**	**(p<0.05)**

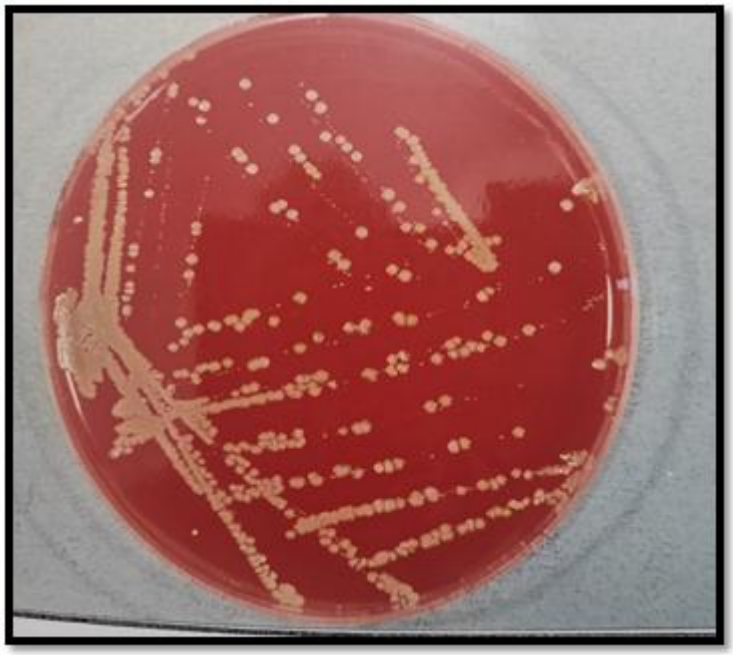

Figure 1. *staphylococcus aureus* grown in blood agar medium after 24 hours Incubation

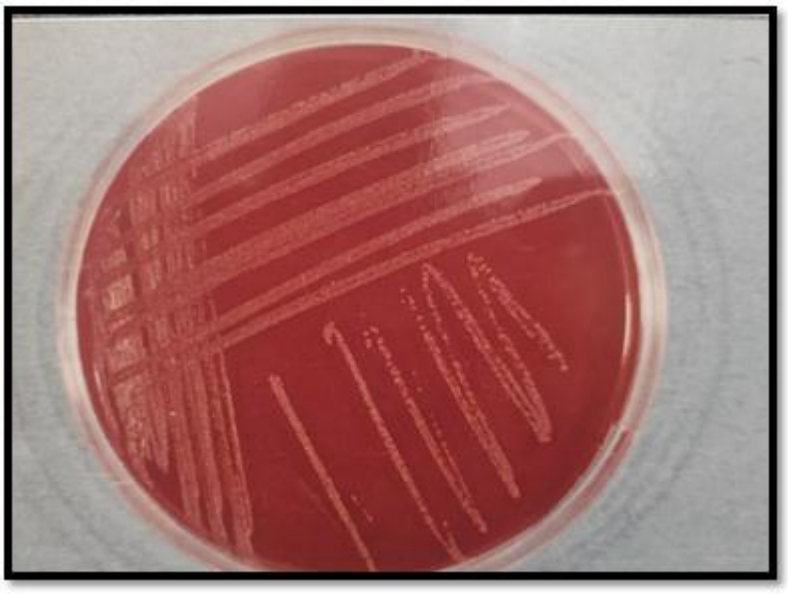

Figure 2. *klebsiella* spp. grown in Macconkey agar medium after 24 hours incubation

Figure 3. *candida albican*s grown in dextrose agar medium after 72 hours incubation

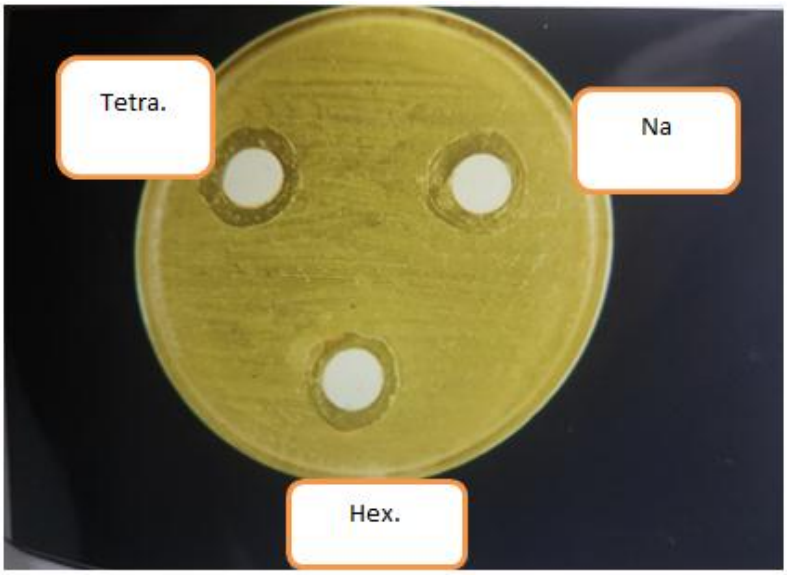

Figure 4. Inhibitory activity of disinfectant powders on *staphylococcus aureus*

Figure 5. Inhibitory activity of disinfectant powders on *klebsiella* spp.

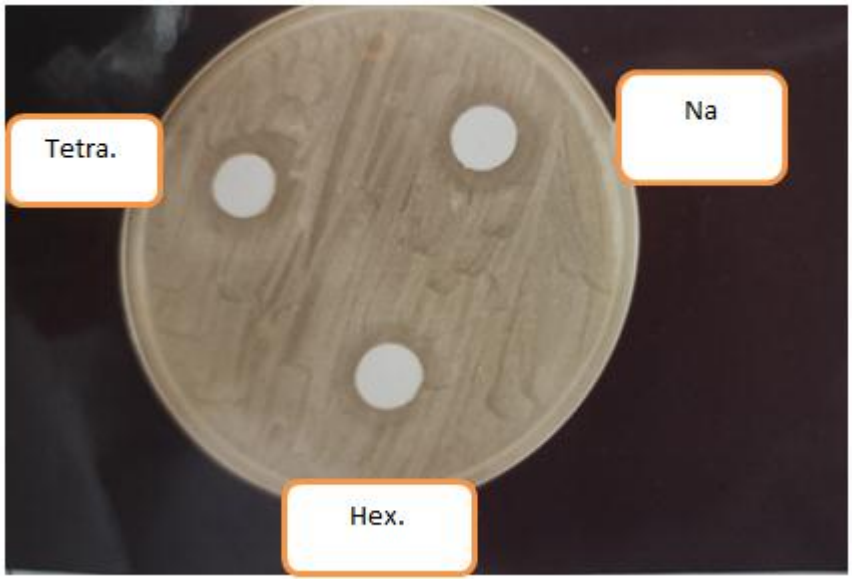

Figure 6. Inhibitory activity of disinfectant powders on *candida albicans*.