

STUDY TO ASSESS THE DISINFECTION OF DENTAL PROSTHESES TO CLEAR LOCALLY PREVALENT MICROBIAL STRAINS

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ABSTRACT: The following study was carried out to assess the effectiveness of four disinfectant solutions (Sodium hypochlorite 1%, chlorhexidine gluconate 2%, 100% vinegar and sodium perborate 3.8%) in making acrylic resin specimens free of locally prevalent strains of three different micro-organisms. The organisms tested were *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The study was conducted following a request by the Prosthodontics Department in the college, as part of the annual quality appraisal. One hundred and fifty samples of the standardized acrylic resin specimens were participated in the study, of which, 30 specimens were run as controls. It was concluded that 1% sodium hypochlorite, 2% chlorhexidine, 100% vinegar and 3.8% sodium perborate are all useful as disinfectants of acrylic resin, to make it free from local strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

KEYWORDS: Dental prostheses, acrylic resin, disinfectant, cross infection control.

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INTRODUCTION: Cross infection between the dental office and the laboratory is a major neglected problem that dentists need to regularly address. Control measures are needed to be put in practice to prevent the cross infection. Cotrim et al¹ related that 52% of dentists interviewed, did not believe in the possibility of cross infection between the dental office and the laboratory. Microbial contamination in the laboratories may occur during procedures involving the use of felt disks, pumice and contaminated hands. Prostheses may also be contaminated by micro-organisms from the patient's oral cavity while adjustments and repairs are carried out in dental offices.^{1,2}

Thus, the dental office-prostheses laboratory connection may represent a potential cross-infection pathway². Effective disinfection procedures are to be taken to prevent the cross infection. Many disinfectants have been suggested for the disinfection of prostheses. However, only such a disinfectant can be advised for regular use if it fulfills most of the criteria of the ideal agent but not causing any alteration in the structure of the prosthesis.³

The present study evaluates the disinfection of cold cured acrylic resin. Four different disinfectants viz, 1% sodium hypochlorite, 2% chlorhexidine digluconate, 100% vinegar and 3.8% sodium perborate were tested for their effect on the local strains of *S. aureus*, *E. coli*, *C. albicans*. Their action was measured by enumerating the residual colony forming units (CFUs).

It was necessary, to evaluate their action, on a yearly basis, to check for the development of resistance to the disinfectant action in the local microbial strains.

MATERIAL AND METHODS: The study was conducted using the standardized acrylic resin specimens sent by the Prosthodontics Department. They were prepared in a size of 3x0.7x0.2 cu cm. They were pre sterilized using ethelene oxide gas⁴. One hundred and fifty resin specimens were subjected to the test, 50 for each of the three organisms tested,

The organisms tested included *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* isolated from clinical specimens from local patients. Criteria for including them in the test were the biochemical tests routinely done in our laboratory for identifying the organism. For *Staphylococcus aureus* it included colony characteristics on nutrient agar, β hemolysis on blood agar and positive tube coagulase test.⁵ For *Escherichia coli*, they included colony characteristics on Mac Conkeys agar, IMViC test results and the result of sugar fermentation test.⁵ *Candida albicans* was identified by growth characteristics on SDA, and the Reynolds Braude phenomenon of positive germ tube test after 2 hrs.⁵

The strength of the bacterial suspension used for contaminating the specimens was decided by using the McFarlands standards⁶. As *Candida albicans* is a larger organism, it is a known fact that for a given standard, *C. albicans* numbers would be 1/30 of the bacterial numbers.⁶

Each of the chosen local strain of the organism was inoculated on plates, nutrient agar for *S. aureus* and *E. coli* and SDA for *C. albicans*. Cultures were incubated for 24 hrs at 37°C. Growths from plates were picked and emulsified in peptone water to match the 0.5 standard tube. With a process of doubling dilution, these were further diluted 100 times to arrive at a final concentration of 10⁶ cells/ml (30,000 cells/ml in the case of *C. albicans*).

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The acrylic resin specimens were distributed, one each, in tubes each containing 2ml peptone water. One tenth ml of microbial suspension was added to each tube, suspension of *Staphylococcus aureus* to 50 tubes, of *Escherichia coli* to 50 tubes and of *Candida albicans* to 50 tubes.

The tubes were incubated for 24hrs at 37°C. After incubation, 10 tubes of each of the test organism suspensions were counted as controls and not exposed to any disinfectant action. The remaining forty tubes of each organism were exposed in groups of 10, to each of the 4 disinfectants. The disinfectants were taken in larger tubes, and the smaller tubes containing the organism suspensions were left inside them, one small tube being placed inside one larger tube, containing the disinfectant. The disinfectant was allowed to act for 10 minutes. Then the resin specimen was picked, immersed in sterile DW for 2 second, and then transferred to tubes containing sterile saline.

The resin specimens were tested for any residual colony forming units (CFUs) by plating the saline, using a standard 4mm diameter nichrome wire loop, on NA for *S. aureus* and *E. coli*, and on SDA for *C. albicans*. The control tubes were not placed in any disinfectant tubes, but the

resin specimen inside was picked up and successively passed in DW tubes and saline tubes, and these saline specimens were also plated for CFUs. Incubation was continued for 48 hrs at 37°C; numbers of CFUs were counted from the plates, using a colony counter. Values of the CFUs on the plates were determined by the semiquantitative method used for determining bacteriuria.⁷

The controls of *Staphylococcus aureus*, and *Escherichia coli* consistently yielded more than 100,000 CFU/ml. The controls of *C. albicans* yielded CFU values between 3000-10,000/ml.

Subcultures on NA from the saline specimens, in which the resin specimens were last rinsed after disinfectant action, yielded very low and insignificant CFU values. These low values were used to determine the scale of probability measurement by the multiplication rule.⁸ Baye's rule may be applied here to evaluate the microbial strengths of the saline specimens just as it is used to evaluate other diagnostic tests.

RESULTS:

Subculture Under Conditions	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Controls	All 10 show >100,000 cfu/ml	All 10 show >100,000 cfu/ml	All 10 show >3000 cfu/ml
1% Sodium Hypochlorite	All sterile	All sterile	All sterile
2% chlorhexidine digluconate	1000 cfu/ml in 1 of 10 subcultures	2000 cfu/ml in 1 of 10 subcultures	All sterile
100% Vinegar	1000 & 2000 cfu/ml in 2 of 10s/cs.	All sterile	1000 cfu/ml in 1 of 10s/cs.
3.8% Sodium perborate	1000 cfu/ml in 1 of 10 s/cs	1000 & 2000 cfu/ml in 2 of 10 s/cs	All Sterile

DISCUSSION: Microbial adherence capacity is influenced by the microbial agent factors like fimbriae,⁹ which are considered as virulence factors. Additionally, host factors may influence in a normal scenario.¹⁰ But in Prosthodontics, the differences in the surfaces of the prostheses play a part.^{11,12} Microtraumas in oral tissues caused by the roughness in prostheses' surfaces was implicated by Davenport.¹³ Williams and Lewis¹⁴ suggested that surface roughness favors colonization by micro-organisms which indirectly contribute to tissue injury.

Sodium hypochlorite is endowed with many advantages like being inexpensive, having a broad spectrum of activity, and requiring a short period of disinfection.^{1,2} It was suggested by Rodrigues et al¹⁵ as the most effective method for the disinfection of acrylic resin prostheses, provided it contains 2% active chlorine and immersion duration is 30 minutes. Chau et al¹⁶ confirmed that immersion more than 10 minutes ensured disinfection of

the inner surface of the material. However, its disadvantages include corrosive activity on metal surfaces, irritant effect on the skin and other cells, and the destruction of cloth, including cotton.¹⁷

Use of glutaraldehyde based disinfectants was first suggested in 1962 following studies by Pepper and Lieberman.³ They are one of the most commonly used disinfectants in dentistry.¹⁸ Their advantages include not being inactivated when in contact with organic material, not being corrosive and not degrading plastics or rubber materials.¹⁹ Due to their toxicity, they must be manipulated with care. The effectiveness of these disinfectants is related to the period of exposure. Angelilo et al²⁰ demonstrated its better effectiveness against *Staphylococcus aureus* and *Candida albicans*, and more time being needed for elimination of *Bacillus subtilis* spore elimination. Silva et al¹⁹ implicated immersion time to be 10 minutes to eliminate *Streptococcus mutans*, *Escherichia*

coli, and *Candida albicans*, and 20 minutes for *Bacillus subtilis* spores. However, Gluteraldehyde based disinfectants were not included in the present study because of their potential toxicity if removal after treatment is not thorough.

In recent years, chlorhexidine has gained the interest of research scholars, as it is the best antiseptic for dental biofilm control, for prevention of dental caries, gingivitis and stomatitis. It is also useful for hand antisepsis.²¹ Its antibacterial activity is mainly for Gram positive bacteria.³

Acetic acid is a component of vinegar. It has been cited both in the medical and food-engineering literature as a disinfectant with a good potential.²² It has been suggested for disinfection of semi-critical articles, control of oral and throat inflammatory processes, and for antisepsis of sores.²³ Acetic acid has been used in diluted form as an antifungal and antiprotozoal solution.¹⁹ Nascimento et al²² found white vinegar to be effective against *E. coli* and *S. aureus*. Vinegar and other solutions of acetic acid have gained popularity because of the toxicity of chlorine and other disinfectants.^{22,24}

Tabs of sodium perborate based denture cleanser can be used as chemical disinfectant only complementary to mechanical cleaning as the two together only result in the effective removal of the biofilm.^{25,26}

CONCLUSION: From the table, it is evident that the probability of the three given organisms surviving the disinfectants' action and causing biofilm formation on the dentures, and morbidity in the patient, is negligible.

SUMMARY: Thus, any of the above tested disinfectant may be advised for regular use to clean dentures of acrylic resin. Also, compared to the conclusion drawn during the previous year's study, the present study continues to advocate the use of the same disinfectants for acrylic resin prostheses used in prosthodontics.

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