A comparative study on efficacy of various disinfectant systems on dental impression surfaces: In vivo study

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Abstract

Purpose: To evaluate the microbial load on impressions and the efficacy of various disinfectants on reducing microorganisms from the impression surface after disinfection.

Materials and Methods: A total of 50 dentulous impressions were made for dentate patients each of age 20 to 50 years using Alginate and Polyvinyl siloxane. Impression compound and zinc oxide eugenol were used for edentulous patients each of age 50 to 70 years, total of 50 edentulous impressions were made. Samples were collected with the help of an 8mm diameter sterile cork borer from each individual impression. Alginate, ZOE and impression compound had 100 samples each. Polyvinyl siloxane had 125 samples. Out of 100 samples the first group of the sample was assigned as control (n=25), the second group of samples (n=25) were immersed in 2% Glutaraldehyde, the third group of samples (n=25) were sprayed with Dimenol, the fourth group of samples (n=25) were placed in the ultraviolet light chamber. For polyvinyl siloxane, an additional fifth sample group (n=25) was subjected to microwave radiation of 650W. The viability of microorganisms that can persist after rinsing and disinfecting the impression surface was tested by inoculating in nutrient media. The colony forming units were counted and compared with the control group. The data were documented and statistically analyzed.

Results: There was a significant difference in the count among the control group and the disinfectant groups. Microbial load found on control groups was taken as 100%. Alginate samples which were disinfected with 2% Glutaraldehyde, Dimenol spray and UV light showed microbial load 31%, 46%, 44% respectively, Polyvinylsiloxane samples showed microbial load 35%, 53%, 48% and 0% with microwave radiation respectively. Impression compound samples showed 32%, 49%, 49% respectively & Zinc oxide eugenol samples with 2% Gluteraldehyde, dimenol and UV showed a load of 39%, 51%, 51% respectively.

Conclusion: 2% Glutaraldehyde showed higher efficacy in reducing the microflora compared to Dimenol spray and UV radiation. There was complete removal of microorganisms with Microwave radiation.

Keywords: Impression materials, Disinfectants, Microbial load.

Introduction

Oral microflora is inhabited by numerous microorganisms. Dental impressions and casts made from contaminated saliva and blood pose serious risk to dental personnel. Sterilization is the process by which all forms of microorganisms such as viruses, bacteria, fungi, and spores are destroyed.[1] Disinfection is generally a less lethal process when compared to sterilization. It virtually eliminates all recognized pathogenic microorganisms, but not necessarily all microbial forms (bacterial endospores), on inanimate objects.[2] Disinfectants used in dental settings must be registered as a hospital disinfectant by Environmental Protection Agency.[1] Surface disinfection will inactivate the infectious agents and reduce the spread of infection to dental personnel from contaminated impressions. The purpose of this article is to evaluate the microbial load on impression and the efficacy of various disinfectants on the reduction of microorganisms from the impression surface after disinfection.

Materials and Methods

Selection of patient: A total of 50 dentate patients of age group between 20 to 50yrs and 50 edentulous patients of age group between 50 to 70yrs were selected based on medical and dental histories. They had not received antibiotic, antifungal or any form of immunosuppressive or chemotherapy for the past 6 months. Selected patients had not received any oral hygiene measures or specific tooth brushing instructions and were not using any mouth rinse. The materials used in the study have been listed in Table 1.

Table 1: Materials used in the study

Alginate	Algitex, Mumbai
Polyvinyl Siloxane [putty and light body]	Adsil, Mumbai
Impression compound	Y-Dent, New Delhi
Zinc oxide eugenol	DPI, Mumbai
2% glutaraldehyde	Raman and Well Pvt. Ltd, Mumbai
Dimenol spray	SEPTODONT
UV Chamber	STERILIZE model no: 4538
Microwave	Samsung

Sample size

For dentate patients impressions were made using alginate and polyvinyl siloxane and for edentulous patient's impression compound and zinc oxide eugenol paste were used. A total of 25 impressions were made

with each impression material. Four samples were made from each impression of alginate, impression compound and zinc oxide eugenol paste impressions which make 100 samples for each. Five samples were made from each polyvinyl siloxane impressions which make 125 samples.

The study was conducted to compare the efficacy of three disinfectant systems on the surfaces of dental impression materials. Since polyvinyl siloxane could tolerate heat, the efficacy of microwave was also evaluated on polyvinyl siloxane impression.

Impression Making

Prior to impression making sterile protocol was established. Maxillary perforated stock trays were used in dentate patients to make maxillary arch impressions with alginate and polyvinyl siloxane. Non-perforated edentulous stock trays were used for making impressions with impression compound. For zinc oxide eugenol special tray was fabricated with autopolymerizing acrylic resins for making impressions. Special trays were disinfected by immersion method in 2% glutaraldehyde for 10 min.

Sample Collection and Disinfection

Four samples for alginate and five samples for polyvinyl siloxane were punched with the help of an 8mm diameter sterile cork borer from each impression. For alginate and polyvinyl siloxane impression the sites selected for removal of the samples are molar and premolar regions. The fifth site of polyvinyl siloxane was collected from central incisor region. This was done due to more bacterial colonization at the sites of tooth region. Palatal surfaces were selected for impression compound and zinc oxide eugenol impressions. For edentulous patients the bacteria are more confined to the mucosal surface, therefore, the palatal surface was selected. (Fig. 1,2)

Twenty five samples in each subgroups are named as a control group, 2% Glutaraldehyde group, dimenol group and UV group for alginate, impression compound and zinc oxide eugenol. The first sample was kept as control, second was immersed in 2% Glutaraldehyde for 10min, third was sprayed with dimenol and left for 15 min, the fourth sample was placed in an ultraviolet light chamber at 254 nm wavelength for 3 min. For polyvinyl siloxane, an additional fifth sample was subjected to a microwave of 650W for 7min. For immersion according to manufacturer's instructions 100ml of 2% Glutaraldehyde was poured into sterilized kidney tray to which 11ml of activator was added. After addition of the activator, the solution turned into green color and was ready for disinfection. For dimenol spray single spray was done evenly onto the impression material. UV chamber used in this study had 254nm wavelength with 2 bulbs of 8watts each and was exposed for 3min.

Disinfection was performed at room temperature. In order to remove any traces of the disinfectant from the impression surface, the specimens were again rinsed with distilled water for 15s. The samples were collected in a sterile container and 5ml of sterile water was added. This sample was sent to the microbiological laboratory within 30 min of disinfection (Fig. 3-6)

Microbiological procedure

The sample containers were sent to the Department of Microbiology, GIMSR. The sample containers were agitated so that the microorganisms get separated from the sample forming the microbial suspension. The spread plate method was done which required a diluted mixture of microorganisms. During inoculation, the cells were spread over the surface of a solid agar medium with a sterile L shaped bent glass rod while the Petri dish was spun on a turntable. Blood agar plates were used for the isolation of Gram positive and Gram negative cocci whereas Mac Conkey agar plates were used for the isolation of Gram negative bacilli. All the plates were incubated aerobically at 37º C for 24 hours. The number of colonies that appeared after incubation were expressed as CFUs (colony-forming units), and then the CFUs were counted. The isolates were stained with Gram´s stain and were identified using standard microbiological methods. Colony characteristics found in culture media and gram staining of the isolated organisms were identified and confirmed the microbial growth. Colony forming units (CFU) were counted and the results were documented. The documented results were sent for statistical analysis. (Fig. 7-13)

Statistical Analysis

Chi-square was calculated, which is the sum of the squared difference between observed (o) and the expected (e) data divided by the expected data in all possible categories.

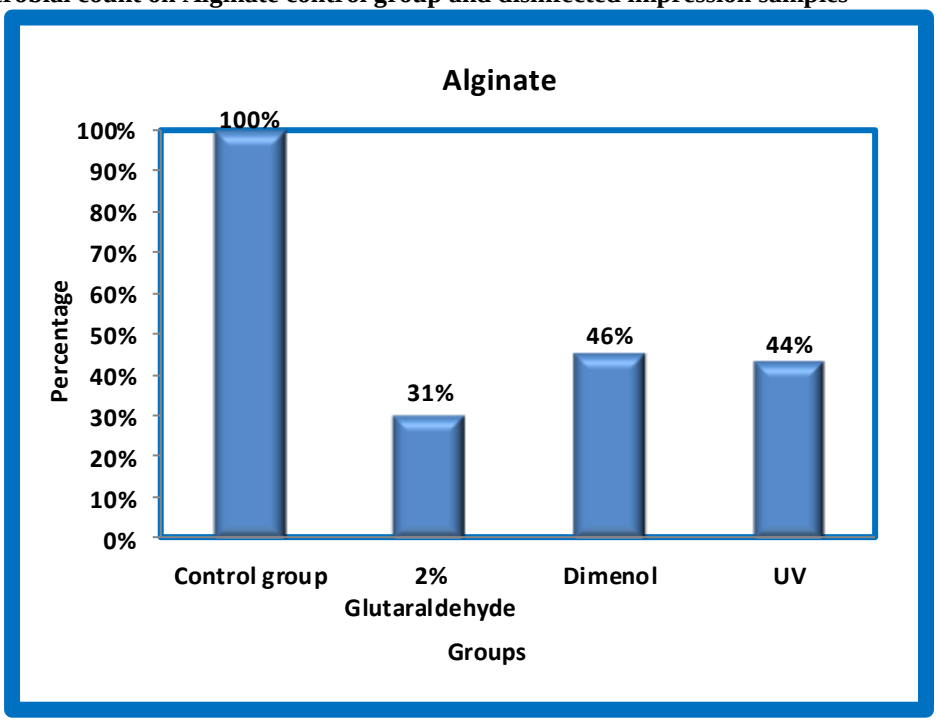
Results

A significant reduction in the microbial growth on different impression material surfaces treated with disinfectants compared to control group was observed.

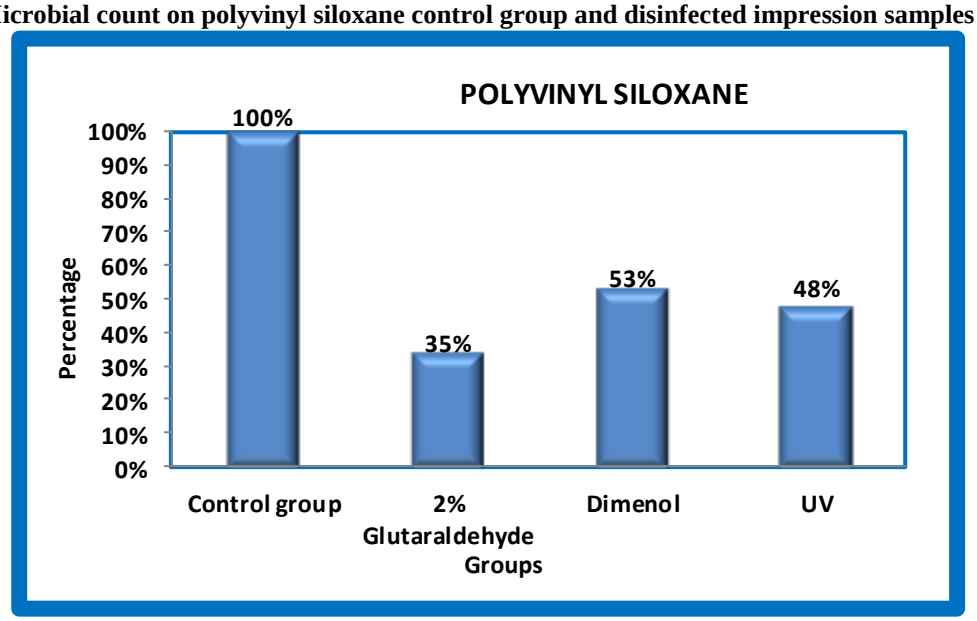
Dimenol [spray] showed more efficacy compared to 2% glutaraldehyde and UV radiation on alginate impression material i.e 46%, 31% and 44% respectively in reducing the microbial load. (Graph 1) Higher efficacy of Dimenol [spray] was also seen compared to 2% glutaraldehyde and UV radiation on polyvinyl siloxane impression material i.e 53%, 35% and 48% respectively. (Graph 2) Dimenol [spray] and UV radiation demonstrated equal efficacy i.e 49% as compared to 42% of glutaraldehyde on impression compound impression material. (Graph 3) Dimenol [spray] and UV radiation had comparable efficacy i.e 51% as compared to 39% of glutaraldehyde on zinc oxide eugenol material. (Graph 4)

Microwave disinfection showed more antimicrobial efficacy compared to 2% Glutaraldehyde, Dimenol [spray] and UV radiation. Zero values were obtained for the samples subjected to a microwave so has not been included in the statistical analysis

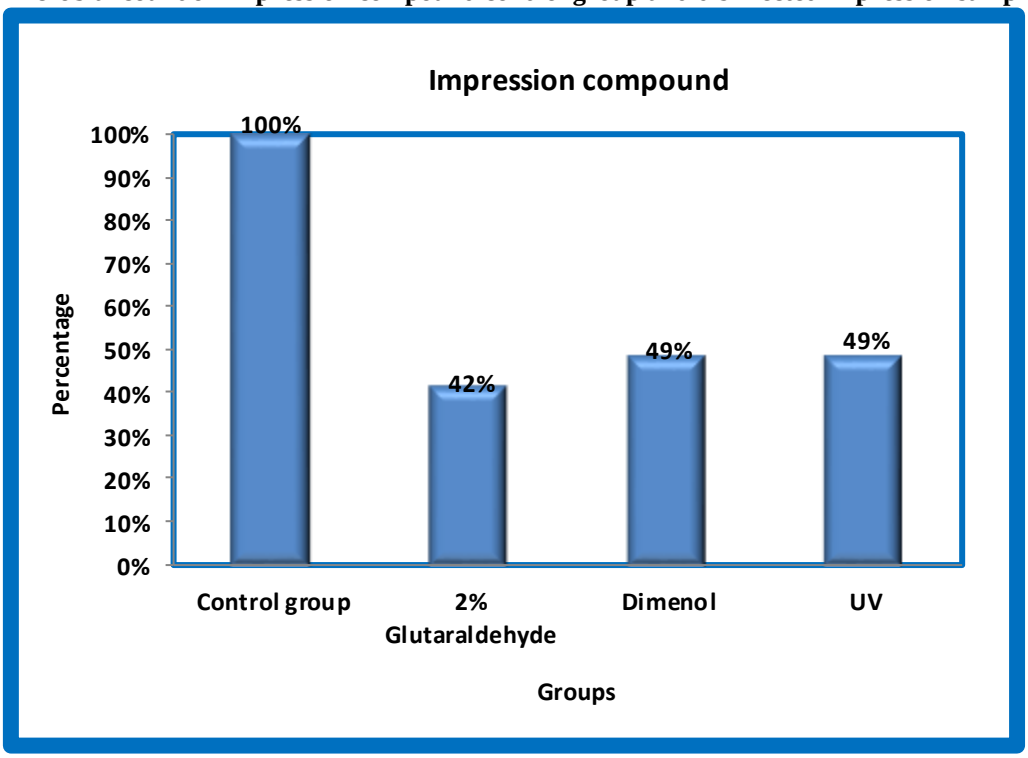
Graph 1: Microbial count on Alginate control group and disinfected impression samples



Graph 2: Microbial count on polyvinyl siloxane control group and disinfected impression samples



Graph 3: Microbial count on impression compound control group and disinfected impression samples



Graph 4: Microbial count on zinc oxide eugenol control group and disinfected impression samples

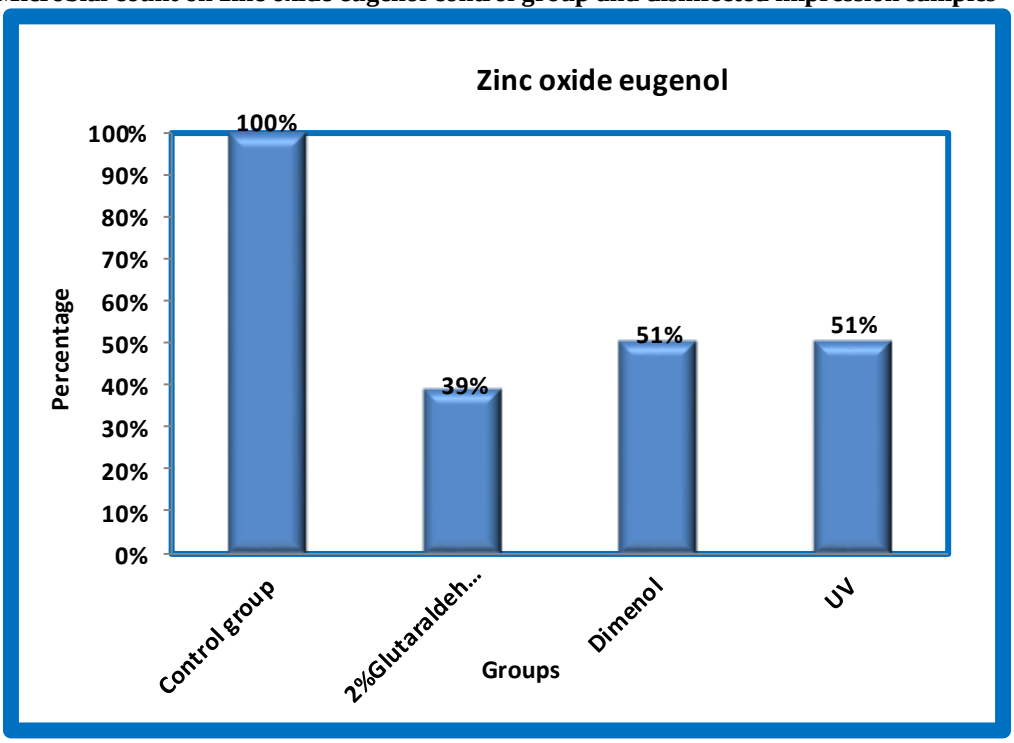


Fig. 1: Cork borer

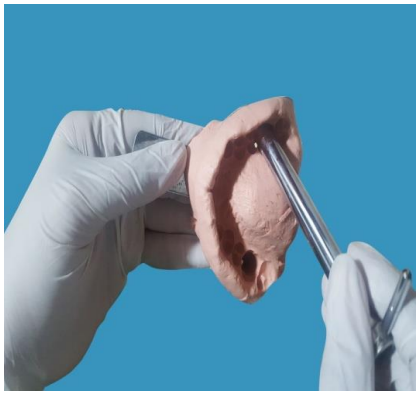


Fig. 2: Sample punching

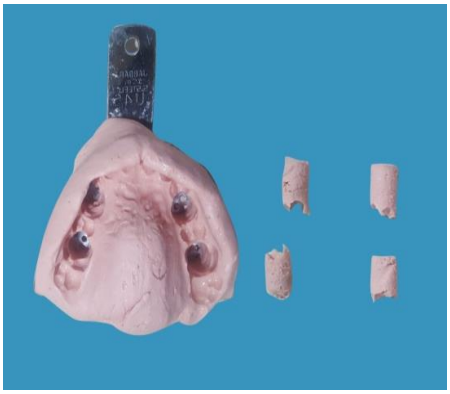


Fig. 3: Alginate samples

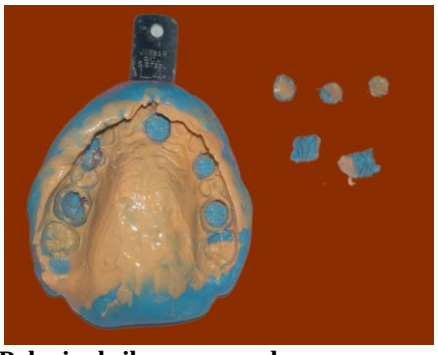


Fig. 4: Polyvinyl siloxane samples

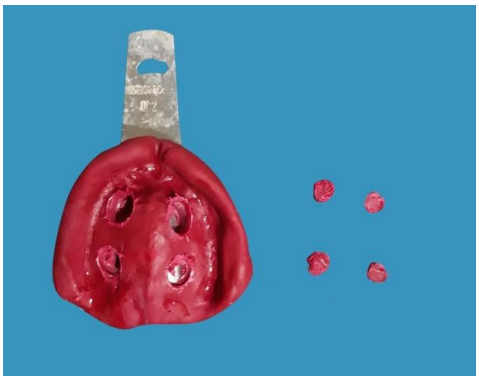


Fig. 5: Impression compound samples

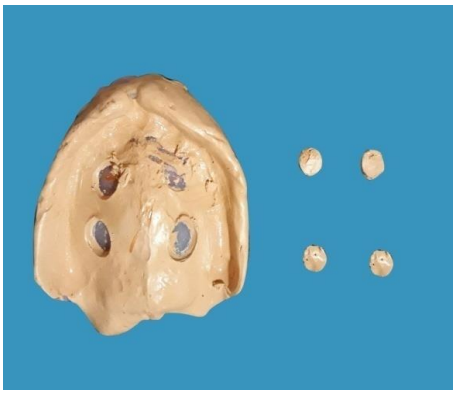


Fig. 6: Zinc oxide eugenol samples

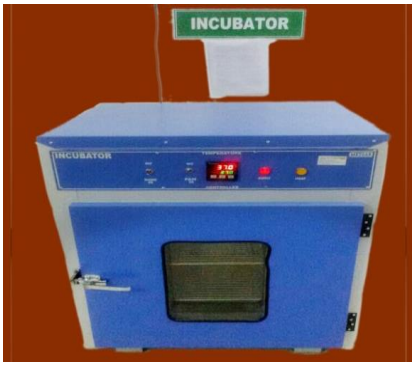


Fig. 7: Incubator

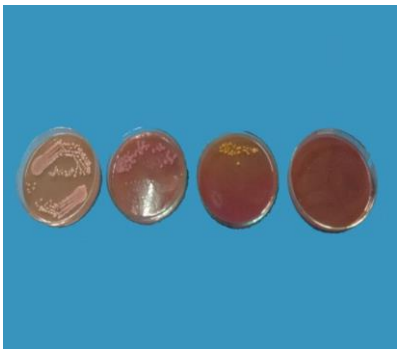


Fig. 8: Microbial growth

Fig. 9: Binocular microscope

Fig. 10: Micrococci clusters

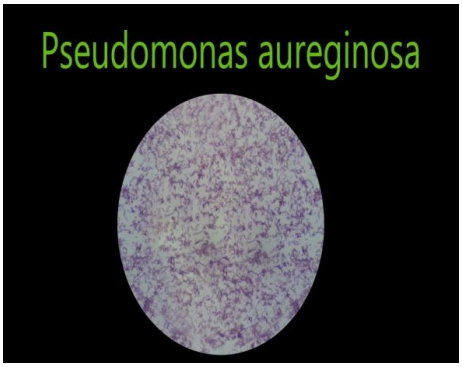


Fig. 11: Pseudomonas aureginosa

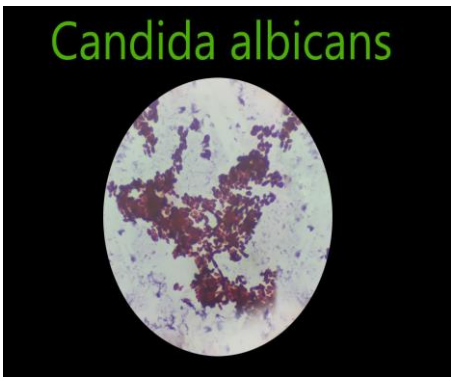


Fig. 12: Candida albicans

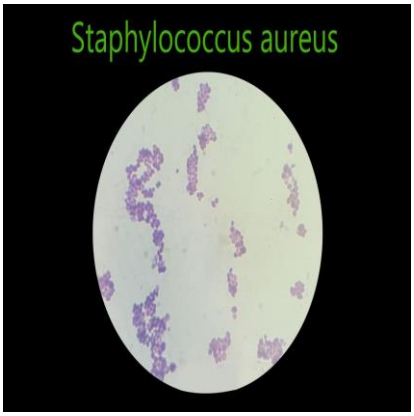


Fig. 13: Staphylococcus aureus

Discussion

The process which destroys many pathogenic microorganisms except bacterial spores is known as disinfection. Sterilization is a process that destroys or eliminates all forms of microbial life including bacterial spores in health-care facilities by means of physical or chemical methods. Earle H. Spaulding believed that the nature of disinfection could be readily understood if instruments and items for patient care were categorized as critical, semicritical, and noncritical according to the degree of risk of infection involved in the use of the items.[3,4]

It has been reported that 17.2% of prosthodontists have a positive HBV serologic blood marker, which is 6 to 7 times greater than in the general population. In addition, dental laboratory personnel may be relatively at a high risk of infectious diseases, and 14.2% of dental technicians showed a positive blood marker for HBV.[4]

Bergman[5] reported that the entire dental staff is routinely exposed to numerous viral and bacterial pathogens that have the potential to cause serious illness. Schiff et al.[6] showed that dental technicians have a significantly higher prevalence of HBV than the general population. Dental technicians may be at risk of HBV and other infections from laboratory material that have been in contact with a patient's blood and saliva although the degree of hazard of infection varies.

Powell et al.[7] reported the contamination of 67% of all dental materials sent to the dental laboratories with the bacteria of varying degrees of pathogenicity. Therefore to prevent possible cross-contamination, sterilization is mandatory. Mycobacterium tuberculosis, hepatitis B virus (HBV), herpes simplex virus (HSV), and other pathogenic microorganisms can be transmitted by impressions. Other normal oral microflora, may become pathogenic and can cause opportunistic infections, especially in immune-compromised individuals.

Gross contamination of the impressions with saliva and blood can be eliminated by rinsing the impressions under running tap water immediately after removal from the mouth. However, Bergman[5] (1989), McNeill[8]

(1992) and Beyerle[9] (1994) reported that rinsing the impression materials with water alone is regarded as gross decontamination because it removes only 40-60% of bacteria. Rowe and Forrest[10] suggested that dental impressions must always be disinfected to prevent infection because the interference of salivary mucins and the adhesive salivary proteins make it difficult to clear away all blood and saliva from impression surface with a simple washing.

Four methods of disinfection followed in this study are immersion method, a spray method, UV radiation and microwave irradiation. Disinfectants are 2% Glutaraldehyde for 10 min immersion[11,12], Isopropyl alcohol spray [Dimenol] for 15 min[13] and UV radiation for 3 min[14,15] based on previous studies. The fourth method is microwave for polyvinyl siloxane at 650W for 7min.[16] The present study showed polymicrobial growth in control groups of all impression materials.

In the control group of this study, polymicrobial growth was observed. Microorganisms isolated were alpha-hemolytic streptococci, Candida albicans, Diphtheroid, E.coli, Enterococci feacalis, Klebsiella pneumonia, Micrococci, Pseudomonas aureginosa, Staphylococci aureus, Staphylococci albus, Streptococci viridians. The result of the study are near to the study carried out by Eugosa et al.[12] Didem Atabek et al.[17] and Samra et al.[18] More microbial growth was observed on alginate as compared to the other three materials, the reason would be hydrophilic nature of the material. Similar findings were observed in previous studies. [19,20]

By the results, it was observed that the 2% Glutaraldehyde showed higher antimicrobial efficacy for disinfecting alginate, impression compound and zinc oxide eugenol. But for polyvinyl siloxane, Microwave radiation had higher efficacy compared to 2% glutaraldehyde. There was a marked reduction of microbial growth after disinfection with a disinfectant agent. 2% glutaraldehyde was more efficient in reducing the microbial load when compared with UV radiation and Dimenol [spray]. The UV radiation and Dimenol [spray] showed equal potential in reducing the microbial count but less efficient when compared to 2% Glutaraldehyde. But in polyvinyl siloxane microwave was more effective which had removed the microorganism completely compared to Glutaraldehyde. Similar results were obtained by Bhasin et al.[16] and Yu-Ri Choi et al.[21] in their studies.

Glutaraldehyde has been classified as a higher level disinfectant, that destroys microorganisms, in particular, tubercle bacilli, HIV and HBV, by acting as a fixative reagent against proteins. The biocidal activity of glutaraldehyde results from the alkylation of sulfhydryl, hydroxyl, carboxyl and amino groups of microorganisms, which in turn alters synthesis of protein, DNA, and RNA.[22] Li et al.[23] examined the germicidal power of 2% Glutaraldehyde which destroyed 99.99% of staphylococcus aureus. Doddamani et al.[24] observed that 2% Glutaraldehyde destroyed S. aureus and S. viridans effectively. Similar findings were found in this study.

Giammanco et al. [25] reported 0.5% glutaraldehyde to be more effective in three tested conditions irrespective of the material used. Demajo et al.[26] concluded that glutaraldehyde [MD520] is more effective when compared with alcohol-based chemical disinfectants when both are used in spray form, particularly when alginate is used as an impression material.

Osama Al Jabrah et al. [20] determined the antimicrobial effect of four disinfectants namely dimenol, perform ID, MD520 and has tabs on impression materials such as alginate, polyether, and polyvinyl siloxane. They demonstrated that elimination of microorganisms from the surface of the impressions were 100% successful with all the four disinfectants. Dahar et al.[27] in 2017 evaluated the antimicrobial efficacy of 0.5% sodium hypochlorite and 2% glutaraldehyde disinfection solutions and concluded that both disinfectant agents effectively disinfected alginate impressions.

Some studies reported that 2% Glutaraldehyde in combination with other disinfectants showed higher efficacy than individually used. Eugosa et al[12] evaluated combined use of 0.25% benzalkonium chloride with 2% Glutaraldehyde or 1% sodium hypochlorite are recommended for clinical and laboratory use.

Boylan et al.[28] reported that disinfection by ultraviolet chamber had shown higher efficacy. They had recommended the use of this disinfection method as it reduced the surface contamination and did not produce any irritating vapors. Samra et al.[18] evaluated that ultraviolet chamber is the most effective in reducing the microbial count when compared 2% glutaraldehyde and sodium hypochlorite disinfectants.

Munagapati et al.[29] 2011 evaluated the efficacy of 2% glutaraldehyde and U.V. light as disinfectants and concluded that the immersion method of disinfection in 2% glutaraldehyde and U.V. light disinfection did not show any statistically significant dimensional change on polyvinyl siloxane impressions, but 2% glutaraldehyde showed comparatively more dimensional change than U.V.light.

Anand et al.[30] in 2013 compared the efficacy of ultraviolet light (UV light) with direct current glow discharge in disinfecting Candida albicans coated elastomeric impression material and found that U-V light exposure had more efficiently decreased the colony counts of C. albicans on samples when compared with direct current glow discharge exposure. Zhang et al.[31], stated that UV radiation combined with 2% glutaraldehyde immersion exerts the highest effect upon the disinfection of HIV and HBV infected dental impressions compared with that of individual disinfection.

Himanshu et al.[32] compared the usage of UV chamber for disinfection of various dental impressions

at different time interval with 2% glutaraldehyde and stated that disinfection achieved with 2% glutaraldehyde for 10 min and UV radiation for10 min was an equal amount.

In the present study, the efficacy of ultraviolet rays to disinfect the dental impression materials at 254nm for 3min was determined and was compared with 2% glutaraldehyde. In this study, UV radiation was found to be less effective when compared to 2% glutaraldehyde. This might be because of inadequate time or inadequate penetration of UV light onto the impression. 8W bulb which was used might not be sufficient to kill the bacteria.

Dimenol [spray] showed less efficacy when compared to 2% Glutaraldehyde immersion and almost equal efficacy with UV radiation. The most likely explanation for the antimicrobial action of alcohol is denaturation of proteins.

Because of the different brands of impression material and different time duration of disinfection process used, it is not possible to review the results of this study with the others. This study showed the efficacy of the disinfecting procedures under clinical conditions against oral pathogenic bacteria and fungi. Further research is needed to investigate the efficacy of disinfectants, especially for viruses and resistant bacteria species.

Conclusion

Within the limitations of this study, it is concluded that all the three disinfecting agents were effective in minimizing the microbial load with 2% Glutaraldehyde being the most effective. Microwave showed complete removal of microorganism on polyvinyl siloxane impression surfaces at 650W for 7min.

Conflict of Interest: None.

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