J Indian Prosthodont Soc (July-Sept 2010) 10(3):182–189
DOI 10.1007/s13191-010-0040-y

ORIGINAL ARTICLE

Efficacy of Different Disinfectant Systems on Alginate and Addition Silicone Impression Materials of Indian and International Origin: A Comparative Evaluation

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Received: 26 August 2010 / Accepted: 27 December 2010 / Published online: 19 January 2011

Abstract Study was planned to evaluate the efficacy of commonly used disinfectants and to study qualitatively and quantitatively the persistence of microflora on the untreated (control group) and the disinfected impression surface after 24 h. Disinfectant systems used were immersion systems like glutaraldehyde, sodium hypochlorite and the ultraviolet chamber. The effect of disinfectant on most commonly used Indian impression materials was carried out in this study and results compared with the most commonly used foreign brands for irreversible hydrocolloid and addition silicone. Impressions were made of 25 healthy volunteers. These were disinfected and incubated in an incubator for 24 h at 37°C for aerobic organisms. The inoculation in nutrient media was done to test the viability of microorganisms that can persist after rinsing and disinfection of the impression surface. The colony forming units were counted and compared with that of control group. Control group of all the impression material samples showed growth of *Streptococcus viridans*, *Diphtheroids*, *Streptococcus pneumoniae* to a greater extent. The growth of *Candida albicans*, *Pseudomonas aerugenosa* and *Staphyloccus albus* was present in all the groups but to a lesser extent. The persistence of the microflora on the impression surface of both the studied brands was similar but the concentration of organisms in the alginate control group was two folds as compared to addition silicone group. Use of ultraviolet chamber gave better results compared to the studied immersion systems. All the disinfection systems were effective in reducing the microbial load with ultraviolet chamber as the most effective.

Keywords Disinfection · Immersion systems · Ultraviolet chamber

Introduction

Dentistry through centuries has always aimed at providing relief from pain, treating loss of function and correcting unesthetic appearance. Sometimes in the smooth execution of a treatment plan, an unforeseen complication can arise due to infectious status of the patient. This was not an issue of major concern in olden days but with the increased awareness of diseases like hepatitis B and AIDS, it has been accentuated.

Infection control has become an imperative issue in dental practice. The risk of infections transmitted by saliva, blood and plaque is considered a potential occupational hazard as they contain pathogenic microorganisms and viruses which can transmit diseases from simple to highly virulent such as common cold, pneumonia, tuberculosis, viral hepatitis, herpes and acquired immunodeficiency syndrome [1]. In hepatitis B there are over 1000 million viral particles per ml of blood and in AIDS there are up to 100 viruses per ml of blood [2]. Studies show that tuberculosis and hepatitis B microbes can survive up to 7 days or longer at room temperature. Thus increased awareness

Research carried (2004–2007) at Dept of Prosthetic dentistry including crown and Implantology, Bharati Vidyapeeth Dental College & Hospital, Pune.

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about the viability of these organisms is very important to take important steps to prevent cross-contamination.

The impression procedures form the starting point in the treatment plan for the fabrication of any prosthesis. The principal potential route of transmission of infection from a patient to dental personnel is by contaminated impressions, where casts made of dental stone poured against these impressions may be the medium for cross contamination [3]. The use of effective disinfection procedures by dental professionals in the operatory and laboratory is necessary to prevent such cross-contamination.

Immersion method with various disinfection solutions and spray disinfection have been tested and proven to be effective for this purpose. However, the most reliable method is immersion as the disinfectant solution comes in contact with all surfaces of the impression material and tray. In 1996, the American Dental Association Council on Dental Materials recommended immersion procedure for polysulphide and addition silicone whereas for polyether, spraying with chlorine compound was recommended for 2–3 min [4].

New methods to disinfect impressions have been introduced like the ultraviolet chamber and its results are being evaluated [5, 6]. Research is going on for a disinfectant which is easy to use, can disinfect adequately and above all does not affect the dimensional accuracy of the impressions.

However, the efficacy of various disinfectants in achieving the target is still questionable. Therefore, a study was planned to compare the efficacy of three commercially available and most commonly used disinfectants in the laboratory: 2% glutaraldehyde, 5.25% sodium hypochlorite and ultraviolet chamber. The impression materials used were irreversible hydrocolloid and addition silicone. Irreversible hydrocolloid is one of the impression materials used frequently in the making of a fixed as well as a removable prostheses. Its use ranges from the making of diagnostic casts, check impressions and finally making of provisional restorations. The other material evaluated in this study is addition silicone which is one of the most accurate impression materials available. Many studies have been conducted on the international products after disinfection but evaluation of the Indian products has not been carried out extensively. An in vivo study for comparing the efficacy of these disinfectants on impressions with irreversible hydrocolloid and addition silicone of national and international brands was conducted.

Materials and Method

A study was planned to evaluate the efficacy of different commonly used disinfectants in the laboratory on the commercially available Indian and International brands of irreversible hydrocolloid and addition silicone. The study was conducted in the microbiology department of Agharkar Institute, Pune; IRSHA Research Institute, Pune.

Impression materials used were:

Algin-Gum (Prime Dental Pvt. Ltd., India)

Vignette (Dentsply DeTrey GmbH Pvt. Ltd., Germany)

Ad-Sil (Prime Dental Pvt. Ltd., India)

Aquasil (Dentsply DeTrey GmbH Pvt. Ltd., Germany)

The materials used were divided into four groups (A, B, C & D) according to disinfection system. Group A was the control group. Disinfection systems used were the commonly used immersion systems as: Korsolex [Glutaraldehyde 2%] 1:19 dilution (Group B), Sodium Hypochlorite 5.25% 1:10 dilution [Shree chemicals] (Group C) and Ultraviolet chamber (Group D). These were compared to the control group which was the untreated sample. Impressions were made with each material of the volunteers and rinsed with distilled water for 15 s.

Samples for this study consisted of 25 dentate volunteers (microbiology students).Volunteers chosen were healthy between 18 and 25 years of age. Impressions were made of mandible between 10 am to 12 pm. Volunteers should have had their breakfast but not lunch. They were instructed not to have any non-vegetarian food or anything sweet in their diet 2 days prior to making impressions. Four impressions were made of each volunteer with the four different selected impression materials. Thus twenty five impressions were made for each impression material. Total hundred impressions were made.

The stock tray was loaded with the irreversible hydrocolloid mixed according to the manufacturer's instructions and the impressions made. For addition silicone impression, tray adhesive was applied onto the stock tray, allowed to air dry for 15 min and impressions were made by two step putty wash technique with a spacer.

With the help of sterile blade (No. 22) on a B.P. knife, four samples 1 in. by 1 in. were cut from impression surface of each impression. Two beakers for immersion solutions and one sterile plastic bag for the untreated sample to act as the control group was taken (Fig. 1). The samples were immersed in these solutions for 10 min. The fourth sample was dried after rinsing and placed in ultraviolet light chamber at 254 nm wavelength for 3 min on a rotating table to avoid any shadowing effect (Fig. 2). Total there were hundred samples for one impression material.

Disinfection was performed at room temperature. The specimens were again rinsed with distilled water for 15 s to remove any traces of the disinfectant from the impression surface. These were then wiped with sterile cotton swabs. These swabs were then inoculated in petri dishes with agar media/Mc Conkey's media and blood agar media.

These were incubated in an incubator for 24 h at 37°C for aerobic organisms. The inoculation was done to test the

Fig. 1 Immersion system & control group

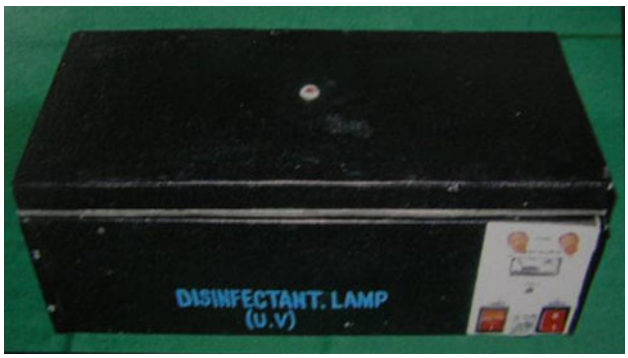

Fig. 2 UV Light disinfection unit

viability of microorganisms that can persist after rinsing and disinfection for that long and can thus spread the infection.

Microbial growth was identified from the colony characters seen in the culture media and was confirmed by biochemical tests and special tests were conducted for the isolated organisms. Colony forming units (CFU) were counted and the results documented.

Results

Microbial Flora

The swabs taken from the tissue surface of impressions were studied for presence of growth. The different microorganisms identified were as follows:

Streptococcus viridans was identified as pin point, semitransparent colonies surrounded by wider zone of β type of hemolysis. Bacitracin test for growth was positive.

Diphtheroids formed small white colonies and sometimes β type of hemolysis. Gram staining showed gram positive bacilli arranged at angles.

Streptococcus pnuemoniae were seen as pin point with α hemolysis. Gram staining showed them as gram positive flame shaped cocci in pairs.

Streptococcus faecalis were seen as tiny deep pink colored colonies. Heat resistance test showed that they survived at 60°C for 30 min. On Gram staining they appeared as large oval cocci arranged at an angle to each other.

Staphylococcus aureus were seen as golden yellow colonies surrounded by β zone of hemolysis, 1–3 mm in size showing complete solubility in normal saline. Growth was coagulase positive.

Candida albicans showed chalky white, non hemolytic easily emulsifiable colonies. Growth was confirmed by germ tube formation and chlamydospore formation in corn meal agar medium.

Pseudomonas aeruginosa showed non lactose fermenting colonies with pigmentation and fruity odour with metallic sheen.

Streptococcus hemolyticus showed small pin point colonies with β haemolysis. Gram staining showed gram positive cocci in chains. Biochemical test and catalase test was negative.

Streptococcus albus showed chalky white non hemolytic colonies which were coagulase test negative.

Escherichia coli were found as thick, large, pinkish colonies which were smooth, moist and opaque. Indol test and methyl red tests were positive. Voges–proskauar tests and citrate utilizing tests were negative. Gram staining test showed gram negative bacilli.

An evaluation of the microbial flora was carried for the different groups. The results are graphically represented for each group. (Figs. 3, 4, 5, 6). Percentage was on y-axis. x-axis represents types of microbial flora.

All the disinfectants were effective in significantly lowering the microbial growth. But significant differences were found in the efficacy of the disinfection achieved by glutaraldehyde and sodium hypochlorite immersion systems and the ultraviolet chamber. Sodium hypochlorite and ultraviolet chamber produced better results than glutaraldehyde immersion. Average effectiveness of glutaraldehyde ranged from 64 to 72% while that of sodium hypochlorite ranged from 92 to 96% for almost all the groups. Ultraviolet chamber showed the maximum efficacy of 96% for all the groups. Persistence of microbial load was twice in alginate impressions as compared to addition silicone batch. This could be explained due to hydrophilic nature of alginates (Tables 1, 2, 3, 4). Comparison of microbial flora in the different disinfectant systems was not significant as compared to that of control group which gave significant p value (Table 5). Comparison of efficacy of different disinfectant systems gave highly significant p value compared to control group (Tables 6, 7, 8). Comparison of disinfectants with

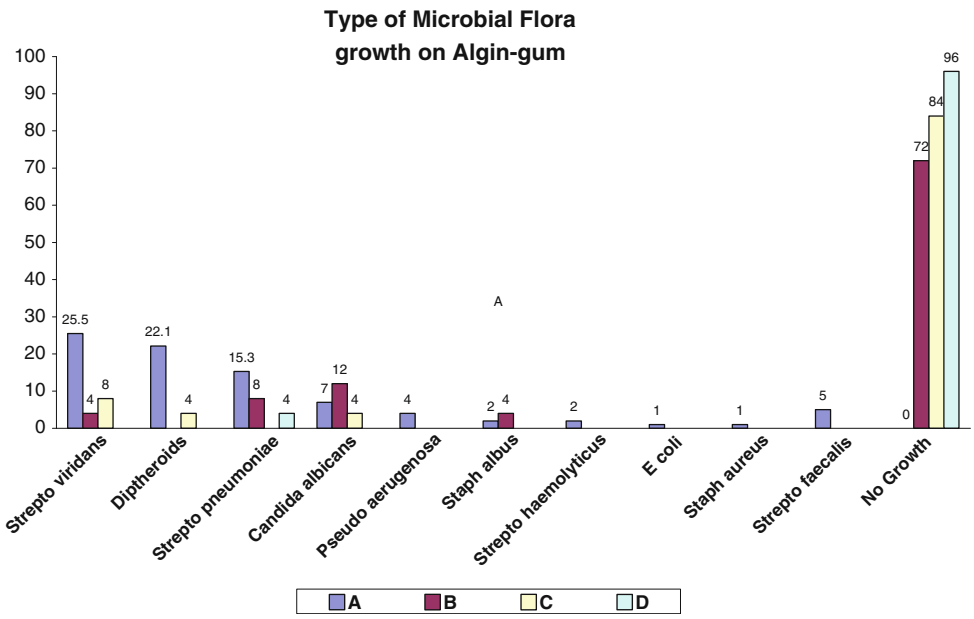

Fig. 3 Comparison of microbial flora with different disinfectant systems on Algin-Gum alginate sample

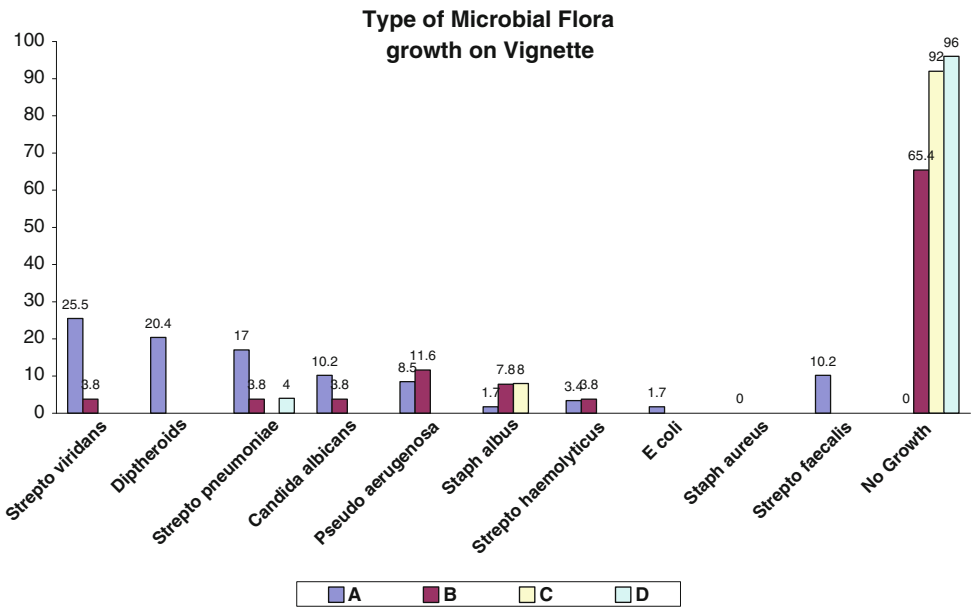

Fig. 4 Comparison of microbial flora with different disinfectant systems on Vignette alginate sample

each other showed p value for the ultraviolet chamber to be significant compared to that of glutaraldehyde while no significance was found with that of sodium hypochlorite (Tables 9, 10, 11).

Discussion

The impression material can act as a vehicle for the transfer of bacteria and fungi. Pathogenic microbes associated with local and systemic diseases have been isolated and cultured from dental impressions as blood and saliva have been proved to carry high concentration of potentially infective viruses and bacteria.

In a healthy patient, the chances of cross contamination are minimal but in the diseased and debilitated patients, chances of cross infection to the dental personnel are high and can pose a serious threat if proper precautions are not taken [7]. Thus there is a need for an effective system for prevention of cross contamination.

Rowe and Forrest [8] suggested that rinsing under water did not clear away all the blood and saliva from the impression surface because the salivary mucins and the adhesive salivary proteins interfered with simple washing.

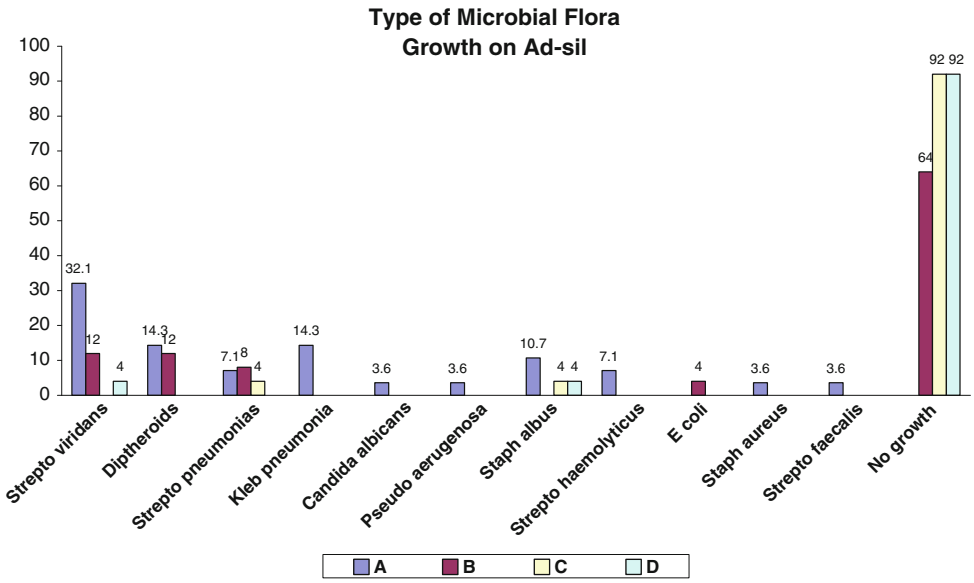

Fig. 5 Comparison of microbial flora with different disinfectant systems on Ad-Sil Addition silicone sample

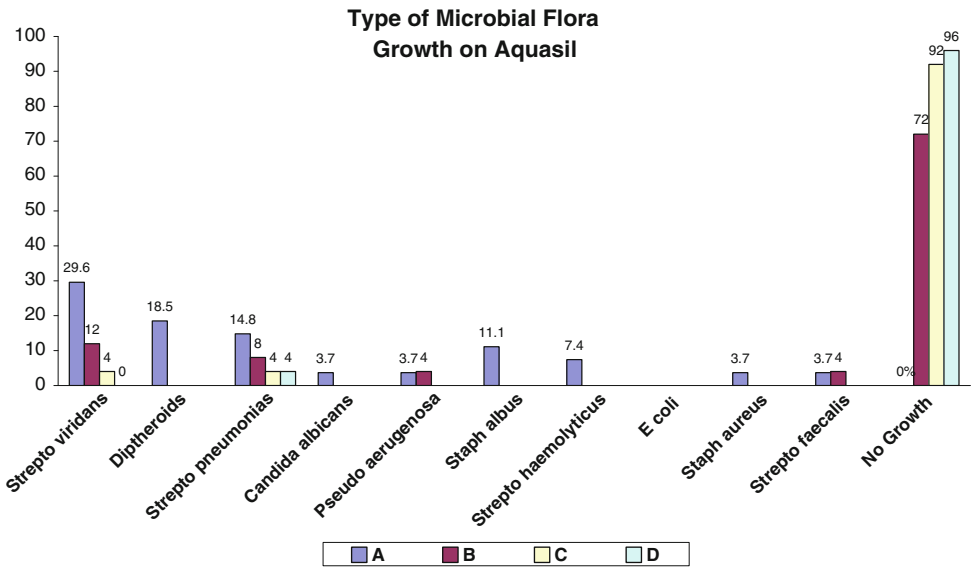

Fig. 6 Comparison of microbial flora with different disinfectants on Aquasil Addition silicone sample

Minagi et al. [9] also concluded that hypochlorite at low concentration could function as an anti adhesion for Candida species but did not affect their pathogenic characteristics. 5.25% NaOCl was found to be sufficient to control the virulent effect of Candida species.

A number of methods for disinfection have been investigated and recommended including antimicrobial immersion system and/or spraying and then sealing in a bag or spraying with a Hygojet system [10]. It has been suggested that immersion system is better than spraying as the latter leads to the pooling effect and its effect is localized. Irreversible hydrocolloids tend to imbibe saliva and blood so immersion system is preferable as it assures coverage of all the surfaces of the impression. Disinfection in the ultraviolet chamber [6] has also been closely studied but a comparative evaluation with other systems was not made.

Effectiveness of UV rays as a method of disinfection depends on a number of factors. Among these were time, intensity, humidity and direct access of UV bulb rays to the microorganisms. Five minute exposure of UV light causes the formation of thymine centaminary photo products in the DNA of the cells. This causes the cells to die. UV radiation though is a powerful bactericidal agent but the shadowing effect in certain areas allows for the survival of unexposed micro-organisms. Residual blood and organic material on dental prostheses, as well as dust particles present in the environment have been found to reduce the already poor penetrability of UV radiation.

Immersion system is considered to be time consuming by some dentists while ultraviolet chamber which is considered a cleaner and much easier method of disinfection. The American Dental Council on Dental Materials in 1996 [4]

Table 1 Total no. of samples in which microbial growth seen in Algin gum hydrocolloid impression sample

Type of microorganisms	A		B		C		D	
	No.	%	No.	%	No.	%	No.	%
Strepto viridans	15	25.5	1	4	2	8		
Diptheroids	13	22.1			1	4	1	
Strepto pneumoniae	9	15.3	2	8				4
Candida albicans	7	11.9	3	12	1	4		
Pseudo aerugenosa	4	6.8						
Staph albus	2	3.4	1	4				
Strepto haemolyticus	2	3.4						
E. coli	0	0						
Staph aureus	1	1.7						
Strepto faecalis	5	8.3						
No growth	0		18	72	21	84	24	96

A Control group, *B* Glutaraldehyde, *C* Sodium Hypochlorite, *D* Ultraviolet chamber

There was presence of more than one microbial growth in each impression

Table 2 Total no. of samples in which microbial growth seen in Vignette alginate impression sample

Type of microorganisms	A		B		C		D	
	No.	%	No.	%	No.	%	No.	%
Strepto viridans	15	25.5	1	3.8				
Diptheroids	12	20.4	0					
Strepto pneumoniae	10	17	1	3.8			1	4
Candida albicans	6	10.2	3	11.6	2	8		
Pseudo aerugenosa	5	8.5	2	7.8				
Staph albus	1	1.7	1	3.8				
Strepto haemolyticus	2	3.4						
E. coli	1	1.7						
Staph aureus	0	0						
Strepto faecalis	6	10.2						
No growth	0		16	65.4	23	92	24	96

For explanation see Table 1 footnote

Table 3 Total no. of samples in which microbial growth seen in Ad-sil addition silicone impression sample

Type of microorganisms	A		B		C		D	
	No.	%	No.	%	No.	%	No.	%
Strepto viridans	9	32.1	3	12			1	4
Diptheroids	4	14.4	3	12				
Strepto pneumonias	2	7.1	2	8	1	4		
Candida albicans	5	18						
Pseudo aerugenosa	1	3.6						
Staph albus	3	10.7			1	4	1	4
Strepto haemolyticus	2	7.1						
E. coli		0	1	4				
Staph aureus	1	3.6						
Strepto faecalis	1	3.6						
No growth	0		16	64	23	92	23	92

For explanation see Table 1 footnote

Table 4 Total no. of samples in which microbial growth seen in Aquasil addition silicone impression

Type of microorganisms	A		B		C		D	
	No.	%	No.	%	No.	%	No.	%
Strepto viridans	8	29.6	3	12	1	4		
Diptheroids	5	18.5						
Strepto pneumonias	4	14.8	2	8	1	4	1	4
Candida albicans	1	3.7						
Pseudo aerugenosa	1	3.7	1	4				
Staph albus	3	11.1						
Strepto haemolyticus	2	7.4						
E. coli	1	3.7						
Staph aureus	1	3.7						
Strepto faecalis	1	3.7	1	4				
No growth	0		72		23	92	24	96

For explanation see Table 1 footnote

suggested the use of disinfectants like glutaraldehyde and sodium hypochlorite for disinfection. The commonly used disinfectants used in this study were Korsolex (containing 2% of alkaline glutaraldehyde) and sodium hypochlorite (5.25%). Korsolex was diluted to 1:19 as per the manufacturer's instructions. Sodium hypochlorite solution was diluted 1:10. This resulted in 5000 ppm of available chlorine in the prepared solution. ADA recommended 10 min immersion in sodium hypochlorite with available chlorine of 5000 ppm. Manufacturers' claim a period of 10 min immersion for 99.8% tuberocidal activity.

In this study, overall it was noted that the concentration of the micro organism was almost two folds in the alginate control group as compared to the addition silicone group. This could be explained due to the hydrophilic nature of the alginates. Not much difference was found in the persistence of the microflora on the impression surface of both the Indian and International brands of the impression materials studied.

The results of this are in agreement with Look et al. [11]; Powell et al. [12] who revealed that the microbial load was two fold in alginate impressions as compared to elastomeric impressions. Study by Samaranayake et al. [13] revealed that organisms transferred in alginate impressions are almost three to five times the number of organisms that were transmitted in case of elastomeric impressions under the same conditions.

Table 5 Statistical analysis of the comparison of Microbial flora in different groups

Type of micro organisms	χ^2	df	*p*-Value			
			A	B	C	D
Strepto viridans	6.865	3	0.07	$p > 0.05$	$p > 0.05$	$p > 0.05$
Diptheroids	11.586	3	0.009	$p > 0.05$	$p > 0.05$	$p > 0.05$
Strepto pneumonia	9.547	3	0.02	$p > 0.05$	$p > 0.05$	$p > 0.05$
Kleb pneumonias	6.51	3	0.08	$p > 0.05$	$p > 0.05$	$p > 0.05$
Candida albicans	9.64	3	0.022	$p > 0.05$	$p > 0.05$	$p > 0.05$
Pseudomonas aerugenosa	5.2	3	0.15	$p > 0.05$	$p > 0.05$	$p > 0.05$
Staph albus	1.34	3	0.71	$p > 0.05$	$p > 0.05$	$p > 0.05$
Strepto haemolyticus	0	3	1	$p > 0.05$	$p > 0.05$	$p > 0.05$
E. coli	2.046	3	0.56	$p > 0.05$	$p > 0.05$	$p > 0.05$
Staph aureus	2.04	3	0.56	$p > 0.05$	$p > 0.05$	$p > 0.05$
Strepto faecalis	2.04	3	0.56	$p > 0.05$	$p > 0.05$	$p > 0.05$
No growth						

For explanation see Table 1 footnote

Table 6 Comparison of efficacy of glutaraldehyde with control groups

Group A v/s B	χ^2	df	*p*-Value	Significance
Algin gum	25.096	1	<0.001**	HS
Vignette	25.096	1	<0.001**	HS
Ad-sil	23.05	1	<0.001**	HS
Aquasil	23.53	1	<0.001**	HS

** p-value ≤ 0.001

Table 7 Comparison of efficacy of sodium hypochlorite with control group

Group A v/s C	χ^2	*p*-Value	Significance
Algin gum	FE	<0.001**	HS
Vignette	FE	<0.001**	HS
Ad-sil	FE	<0.001**	HS
Aquasil	FE	<0.001**	HS

As the expected cell value was less than 5, Fisher's exact test (FE) was done and the *p*-value calculated

** p-value ≤ 0.001

Table 8 Comparison of efficacy of ultraviolet chamber with control group

Group A v/s D	χ^2	*p*-Value	Significance
Algin gum	FE	<0.001**	HS
Vignette	FE	<0.001**	HS
Ad-sil	FE	<0.001**	HS
Aquasil	FE	<0.001**	HS

** p-value ≤ 0.001

Table 9 Comparison of efficacy of glutaraldehyde with sodium hypochlorite

Group B v/s C	χ^2	DF	*p*-Value	Significance
Algin gum	0.47	1	0.49	NS
Vignette	FE	1	0.13	NS
Ad-sil	4.2	1	0.04	S
Aquasil	FE	1	0.13	NS

Table 10 Comparison of efficacy of glutaraldehyde with ultraviolet chamber group

Group B v/s D	χ^2	*p*-Value	Significance
Algin gum	FE	0.048*	S
Vignette	FE	0.048*	S
Ad-sil	4.2	0.04	S
Aquasil	FE	0.048*	S

* p-value ≤ 0.05

Table 11 Comparison of efficacy of sodium hypochlorite with ultraviolet chamber

Group C v/s D	χ^2	df	*p*-Value	Significance
Algin gum	FE	1	0.348	NS
Vignette	FE	1	1	NS
Ad-sil	FE	1	1	NS
Aquasil	FE	1	1	NS

Storer and McCabe [7] had investigated the effect of 2% glutaraldehyde, sodium hypochlorite (1% available chlorine) and 4% formaldehyde. They concluded that glutaraldehyde was the most suitable form of sterilisation. This was contrary to the findings in this study. The reason could be that amount of available glutaraldehyde for disinfection was much less. The possible explanation for this could be that the material used: Korsolex had formaldehyde and urea plasticizers also present in it. It was containing 7 g of glutaraldehyde which was to be diluted to 1:19 according to the manufacturer's instructions. The material used by the other research workers was pure glutaraldehyde without any other ingredient.

Results by Boylan et al. [5] on disinfection by ultraviolet chamber also showed the same results as this study. They had advocated use of this disinfection method as it reduced surface contamination and did not produce irritating vapours. The drawback of shadowing was countered by use of rotating table. Frequent change of the UV bulb was considered imperative as there is loss of input of rays from the bulb with time. The UV light source in the unit should be surrounded by mirrors to reflect the light from many angles and thus make it more effective [6].

Every member of dental health team has a duty to ensure that all necessary steps are taken to prevent cross infection to both their patients and themselves.

Conclusion

All the three disinfection systems were effective in reducing the microbial load with ultraviolet chamber being the most effective irrespective for the Indian and International brands of impression materials studied. Maximum percentage of micro-organisms like *Streptococcus viridans, Diphtheroids* and *Streptococcus pneumoniae* were found from the tissue surface of impression samples in the control group. Microorganisms found in the descending order of concentration were *Streptococcus viridans, Diphtheroids, Streptococcus pneumoniae* and *Pseudomonas aeruginosa. Candida albicans, Staphylococcus albus, Streptococcus haemolyticus* were also found. *E. coli* was also found but to a lesser extent.

Persistence or carriage of microbial load was twice in alginate impressions as compared to the addition silicone group. This could be explained due to the hydrophilic nature of the alginates. Glutaraldehyde showed reduction of microorganisms but maximum reduction was with sodium hypochlorite and the ultraviolet chamber.

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