Original Article
Journal of Pierre Fauchard Academy (India Section), *Vol 37(1-2), DOI: 10.18311/jpfa/2023/32268, January-June 2023 p. 17-25*
ISSN (Print) : 0970-2199
ISSN (Online) : 2405-772X

Fomite Contamination of Alginate Impressions and the Role of Herbal and Chemical Disinfectants

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Abstract

Introduction: Contamination of alginate impressions is reported and various methods to disinfect them have been studied. However, contamination of the impressions from the rubber bowl is not known. The transfer of microorganisms to the oral cavity by the alginate mixed in an unsterilized rubber bowl is a possibility. The objective of this study is to find out the fomite contamination of alginate impressions from rubber bowls and evaluate the role of chemical and herbal disinfectants against them. **Methodology**: Thirty volunteers were included in the study and they were grouped into six groups (one control and five study groups). Three chemical disinfectants and two herbal disinfectants were used to disinfect the rubber bowl before impressions. The presence of pathogens and the number of colony-forming units were evaluated. **Results:** Harmful pathogens like Staphylococcus species, *E. coli*, Pseudomonas species, Bacillus species, and Klebsiella species have been detected under a light microscope on the surface of the rubber bowl. There was a significant decrease in the number of colony-forming units on alginate impressions after the rubber bowl was disinfected. Herbal disinfectants showed a profound decrease in the transmission of organisms compared to chemical disinfectants. **Conclusion:** The presence of fomite contamination in the routine impression has been confirmed in the study. All the disinfectants showed a significant reduction in colony-forming units when the rubber bowl was disinfected. Both the plant extracts showed an effective decrease in Colony-Forming Units (CFU) per ml and were as effective in eliminating pathogens as the chemical disinfectants. Hence, plant extracts of Neem and Babool can be a viable alternative to chemical disinfectants on rubber bowls.

Keywords: Cross-Contamination, Fomite, Herbal Disinfection, Impressions, Rubber Bowl Disinfection
(**Article chronicle: Received:** 19.04.2023; **Revised:** 31.05.2023; **Accepted:** 08.07.2023)

1. Introduction

The dental clinic environment possesses great potential for the transmission of pathogenic organisms from staff to patient and vice versa through improperly sterilized instruments and contaminated body fluids. Many studies clearly indicate that the impression surfaces when taken out of the mouth are polluted with bacteria[1]. In prosthodontics, impressions, casts, impression trays, record bases, occlusal rims, articulators, and dental prostheses contain pathogenic microorganisms and can cause infection to the dental personnel and the patient. Tap water rinsing of dental impressions has reduced the microbial count to 48.5% and still acted as a potent cross-contaminant[1].

Runnels[2] stated that blood taken from dentists shows an average of 12.9-24 % blood markers, which is a shred of evidence that those being tested had sufficient exposure to Hepatitis B Virus to cause a manifestation of the disease through the production of antibodies or antigens. Prosthodontists are more prone to tuberculosis since the organism is transmitted via sputum. Opportunistic

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pathogens like *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* rates of 55.6, 25.9 and 5.6 %, respectively were isolated from the surface of impressions[3].

The American Dental Association recommended Sodium Hypochlorite, Iodophors, synthetic phenols and aldehydes such as Formaldehyde and Glutaraldehyde as disinfectants for alginate impressions[3]. Though disinfection of impressions, casts, and alginate can prevent cross-contamination there lies a possibility of fomite contamination from the rubber bowl and the introduction of opportunistic as well as pathogenic organisms into the oral cavity via the alginate impressions.

A fomite is any inanimate object, that when contaminated with or exposed to infectious agents, such as pathogenic bacteria, viruses, or fungi, can transfer disease to a new host[4]. In the hospital fomites include patient care items and environmental surfaces. Such fomites in the dental office and operatory settings may serve as reservoirs for a variety of bacteria including multi-drug resistant organisms, and pathogens such as Methicillin-Resistant *Staphylococcus Aureus* (MRSA), vancomycin-resistant *Enterococci* and *Clostridium difficle*. When these surfaces are touched, they can get transmitted to other surfaces or the patient, they act as fomites, resulting in cross-infection[5]. A rubber bowl used for mixing alginate can get contaminated from the other surfaces as it is used indispensably several times while making alginate impressions and thus act like a fomite.

Osama Al Jebrah[6] conducted a study to determine the effect of four disinfectants on three commercially used impression materials, alginate, polyether, and polyvinyl siloxane with the results stating that the disinfectants were able to eliminate microorganisms carried by the impressions. For those undisinfected specimens, the results showed that untreated alginate impressions appear to carry more microorganisms (P<.05) than the other two rubber impression materials used in the study. Saso Ivanovski[7] evaluated the effectiveness of disinfecting solutions incorporated into dental stone casts. Irreversible hydrocolloid impressions were contaminated individually with *Escherichia coli*, *Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa, Klebsiella*, Pneumonia, *Actinobacteria calcoaceticus, Bacillus subtilis, Mycobacterium phlei and Candida albicans*. Four readily available disinfecting solutions (glutaraldehyde, povidone-iodine, chlorhexidine, and sodium hypochlorite) were added to the die stone mix used to pour up the impressions. The set cast surfaces were swabbed at 1h and 24h, the samples plated on agar and incubated at 37 °C for 24h and 3d for *M. phlei*. Subsequently, colony forming units were counted. The results showed that only glutaraldehyde and povidone-iodine killed all contaminating microorganisms within 1h, while the 15 dilution of sodium hypochlorite solution was equally effective after 24h. Two percent glutaraldehyde was the most effective disinfectant with the least adverse effects on the physical properties of the set cast.

Till date several studies have been carried out on disinfection of impressions. However, no study on the contamination of alginate impression through the rubber bowl is found in the literature. The need for disinfection of the rubber bowl is emphasised in the study. The purpose of this study is to find out the presence of fomite contamination from rubber bowls to alginate impressions and to evaluate the antibacterial efficacy of chemical and herbal disinfectants on it. Hence, the null hypothesis is that there is no contamination of the alginate impression surface from the rubber bowl and the disinfectants are not effective against the organisms.

2. Material and Methods

Thirty healthy undergraduate students from both genders of age 19 to 20 years were included in the study. The subjects were all students and are exposed to the clinical environment. All the subjects were explained about the study protocol and signed a consent form. The thirty subjects were divided into six Groups (A-F) with five subjects in each group (Table 1). It is an analytical experimental study.

2.1 Preparation of Plant Extracts

Six to eight kilos of leaves of *Acaia nilotica* (babool) and *Azadirachta indica* (neem) were collected from the forest area around Theni district, Tamil Nadu. The leaves were air-dried at room temperature for 37 °C for 2 weeks and ground individually to form a uniform powder of 40 mesh size. 25 g of air-dried *A. nilotica* leaves were packed separately into a Soxhlet apparatus and were extracted with 100 ml of Ethanol (EA). The organic extracts were dried over a vacuum desiccator and the solvent was removed in a vacuum (40-60 °C) in a Soxhlet extractor (Figure 1) for 18-20 hours. The solution was then evaporated to dryness. The *Acacia* extract and

Table 1. Group categorization according to disinfection technique

Group	Disinfectants Used	Method of Disinfection	Time
A	(Control)	None	
B	Glutaraldehyde 2%	Immersion	15 mins
C	Sodium Hypochlorite 0.5%	Immersion	15 mins
D	Hydrogen Peroxide 3%	Immersion	15 mins
E	Leaf Extract of *Acacia nilotica*	Wiping	15 mins
F	Leaf Extract of *Azadirachta indica* (Neem)	Wiping	15 mins

neem extract (Figure 2) were concentrated with 25 gm of powder in 100 ml of ethanol and stored in separate plastic containers at room temperature.

In this study, the required concentration employed for disinfection with *Azadirachta indica* extract is 2500 µl in 100 ml of saline and for *Acacia nilotica* is 250 mg in 1 ml saline. So, the extracts are diluted to the required concentration and then were used for disinfection. The neem extracts were prepared based on the study done by Maragathavalli and the extracts of *Acacia nilotica* were done based on the study by Kalaivani mentioned in this study.

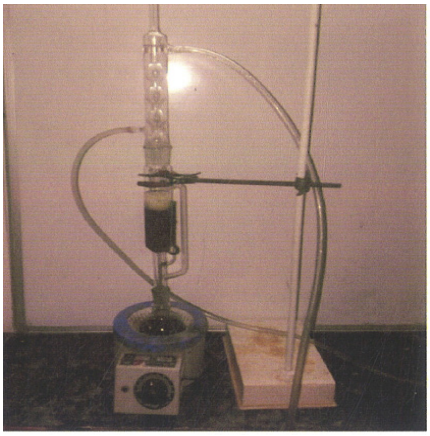


Figure 1. Soxhelt extractor.

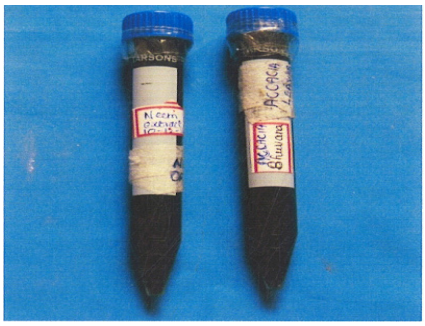


Figure 2. Acacia and azardicata extract.

2.2 Sample Collection

From each subject, four samples were collected. 1) before disinfecting the rubber bowl, 2) after disinfecting the rubber bowl, 3) the sample is collected from the alginate surface before impression making, 4) the sample is collected on the surface of the alginate after the impression is made. So, for each group, 20 samples were collected and in total 120 samples were collected in the study.

Sample 1

The sample was collected from the inner walls of the rubber bowl before disinfecting it. Two wet sterile swabs were used and each was rolled on each half of the inner wall of the rubber bowl. One swab was directly inoculated into the blood agar plate and the other was directly inoculated into 1.5 ml of brain heart infusion broth present in a sterile test tube. The contents were thoroughly mixed in the broth. The swab was removed after thoroughly mixing and squeezing on the inner wall of the test tube to liberate the contents.

Sample 2

The sample was collected from the inner walls of the rubber bowl after disinfection. The rubber bowl is treated with disinfectants. Glutaraldehyde 2% for Group B, Sodium Hypochlorite 0.5% for Group C, Hydrogen Peroxide 3% for Group D, the leaf extract of *Acacia nilotica* (2.5 mg/ml) for Group E, Leaf extract of *Azadirachta indica* (2500 µg/ml) for Group F for the mentioned contact time (15 mins). After the contact time is over, the disinfectant was discarded and a pair of the swab is rolled in each side of the rubber bowl.

Sample 3

The sample was collected from the alginate surface before impression making. Before each impression, the alginate powder used for the study was sterilized in a hot air oven

at 160 ˚C for 1hr. 18 gm of alginate powder is mixed with 40 ml of sterile water for each impression in the disinfected rubber bowl. The mix was then loaded on the sterile impression tray. A pair of swabs was rolled over the mixed alginate surface, one swab on the right half of the alginate surface and the other swab on the left half of the alginate surface.

Sample 4

The samples were collected over the set alginate impression surface once after the impression is made. The impressions were made in the subject's oral cavity, and once the alginate is set, the impression is removed from the oral cavity and was washed in running water to eliminate debris. A pair of swabs were used to roll over each half of the impression surface. One of the two swabs collected from each sample is directly inoculated in the blood culture plate and incubated at 37 ˚C overnight in aerobic conditions. After 24 hours, the plates were verified for the presence of the pathogen. If the pathogen was detected, then it is subjected to gram staining and confirmed with an array of biochemical tests. The swabs were transported to the microbiological lab with a buffered glycerol saline transport medium.

The incubated nutrient broth mixture is diluted in the following manner: From the samples, serial dilution was made using saline. From each dilution, 100 µl of the sample was transferred to the surface of sterile dry Blood Agar plates. The samples were spread using L- Rod and plates were allowed to dry and incubated at 37 ˚C for overnight. Following incubation, the plates were screened for CFU/ml. From each test tube, 100 µl sample was taken and inoculated using an L loop in the blood agar plate separately and incubated at 37 ˚C for 24 hours.

Figure 3 explains the methodology of the study. The colonies were identified, and the readings were done in the next 24 hours (Figure 4).

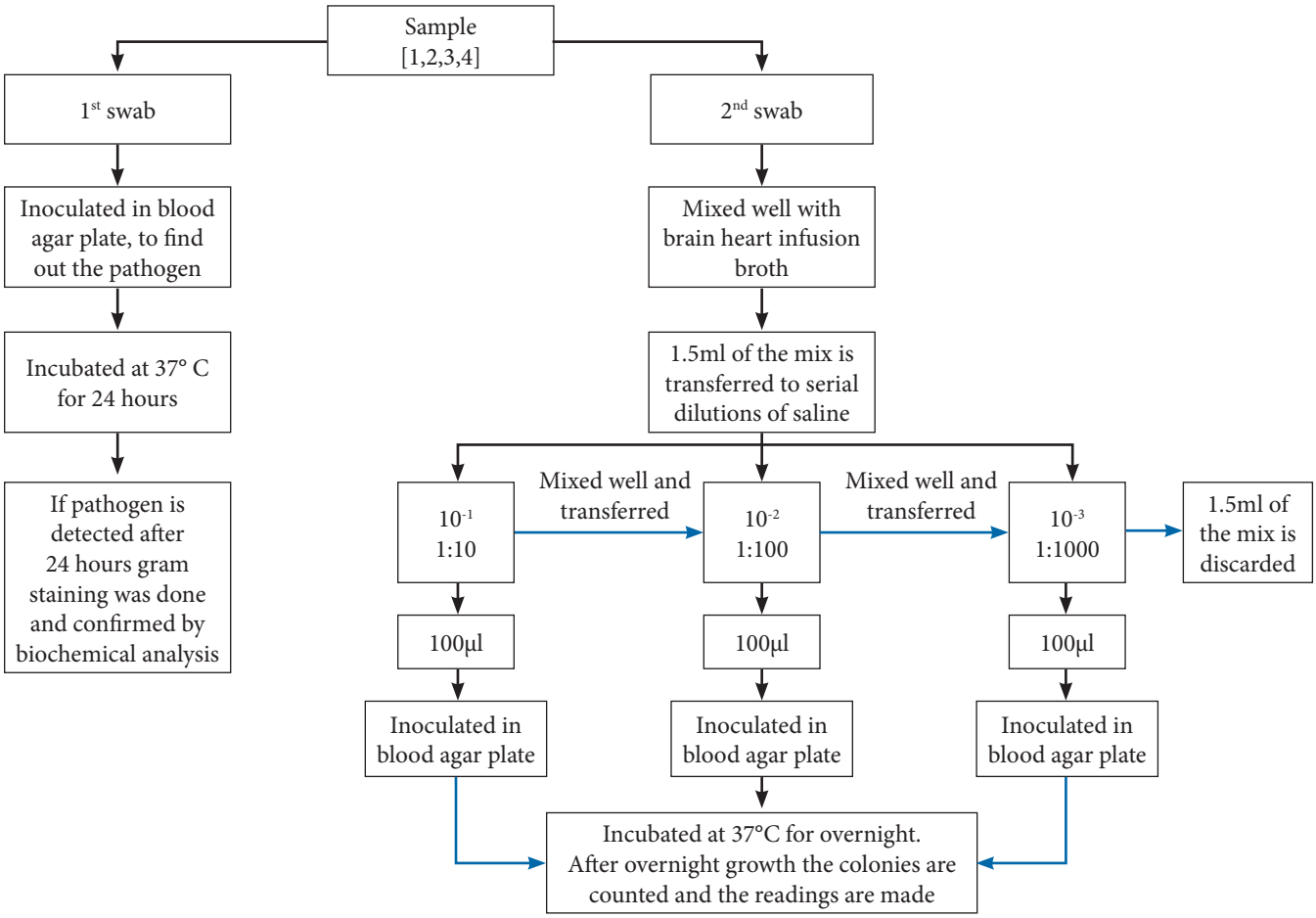


Figure 3. Explanation of the microbiological procedure.

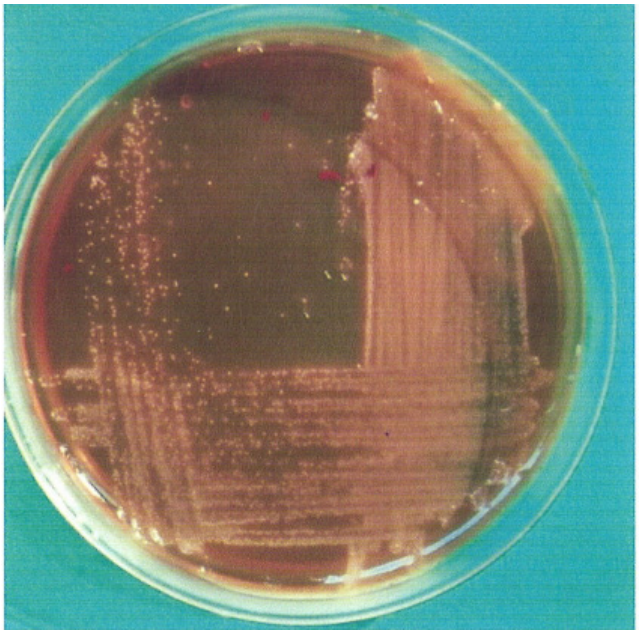


Figure 4. Colonies formed in control group (Group A).

2.3 Microbiological Analysis

The pathogen was identified as per standard microbiological procedures (Mackie and McCartney)[8]. The tests performed to identify the organism include catalase, oxidase, motility, IMViC, Triple sugar iron agar, of test, urease production, amino acid fermentation – LAO, carbohydrate fermentation tests. (Mackie and McCartney).

3. Results

In this study, the number of colonies forming units in each group and the presence of pathogens in each group is found and compared (Table 2).

The average mean value of the microbial colonies in CFU/ml on the surface of the rubber bowl in the control group without disinfection was 2.35E+09 CFU/ml. There was also a significant increase in CFU/ml after mixing the alginate in the rubber bowl, 3.1E+09 CFU/ml, which confirms the contamination of the alginate surface from the rubber bowl (Table 2).

Three chemicals and two herbal disinfectants were used to check their efficacy in reducing the number of colonies. All the disinfectants were effective in reducing the number of colony-forming units on the surface of the rubber bowl. There was a significant decrease in the microbial count after disinfection (Table 2). However, there is a propulsive increase in the microbial count once the impression is exposed to the oral cavity.

The herbal disinfectants were found to be more effective in colony reduction than chemical disinfectants.

Using gram staining techniques, the presence of microorganisms like *Staph aureus*, (Figure 5) *E. coli, Bacillus sp., Pseudomonas sp.,* and *Klebsiella sp.* have been detected under the light microscope on the surface of a rubber bowl (Table 3).

Table 2. Mean value of microbial colonies (CFU/ml) experimental groups

Samples No	N	Group A	Group B	Group C	Group D	Group E	Group F
Sample 1 (S1)	5	2.35×10^9	2.12×10^9	2.32×10^9	2.07×10^9	2.32×10^9	2.35×10^9
Sample 2 (S2)	5	--	1.88×10^3	$2.70x\ 10^3$	1.37×10^3	1.45×10^4	2.03×10^4
Sample 3 (S3)	5	3.1×10^9	1.53×10^4	2.44×10^4	1.24×10^4	1.34×10^4	1.56×10^4
Sample 4 (S4)	5	4.4×10^9	3.05×10^9	2.69×10^9	3.06×10^9	2.03×10^9	2.14×10^9

Table 3. Organisms isolated from the samples

Samples		Chemical and Herbal Disinfectants				
	Control Group A	**Glutaraldehyde Group B**	**Sodium Hypochlorite Group C**	**Hydrogen Peroxide Group D**	***Acacia nilotica* Group E**	***Azadiracta indica* Group F**
S1	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*
S2				*E. Coli* *Pseudomonas*	*Pseudomonas*	
S3	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebsiella*	*E. Coli* *Pseudomonas* *Bacillus* *Klebsiella*	*Pseudomonas* *Bacillus*	*E. Coli* *Pseudomonas*	*E. Coli* *Pseudomonas*	
S4	*E. Coli* *Pseudomonas* *Bacillus* *S. aureus* *Klebseilla*	*E. Coli* *Pseudomonas* *Bacillus* *Klebsiella*	*Pseudomonas* *S.aureus*	*E. Coli* *Pseudomonas*	*E. Coli* *Pseudomonas*	

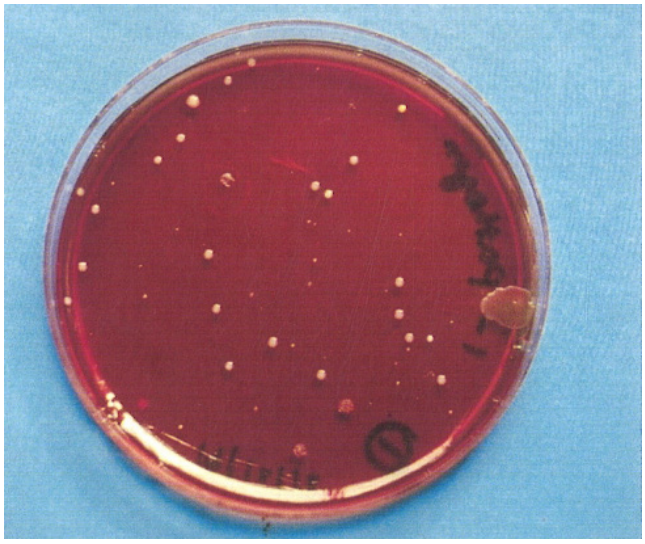


Figure 5. Staphylococcus aureus in blood agar.

4. Discussion

The use of rubber bowl in dental clinics is an absolute necessity. The contamination of the rubber bowl and the risk of infection being carried through an alginate mix to the oral cavity is a possibility. One of the general routes for transmission of microbial agents in dental clinics is indirect transmission of microorganisms from a contaminated intermediate object (fomite). Examples of potential fomites are instruments, impression trays, suction tips, rubber bowl or any inanimate article[4].

The study was conducted to find out the presence of microorganisms on the surface of the rubber bowl and on alginate impressions contaminated from the rubber bowl. This study also compares the effect of various disinfectants against these organisms on the surface of a rubber bowl.

In this study three chemical disinfectants and two herbal disinfectants were used to check their efficiency in reducing the number of colonies. Glutaraldehyde 2%, sodium hypochlorite 0.05%, Hydrogen peroxide 3%, the leaf extract of *Acacia nilotica* (2.5 mg/ml) for Group E, Leaf extract of *Azadirachta indica* (2500 µg/ml) were used as disinfectants. The alginate used in this study was sterilized in a hot air oven at 160 ˚C for 1hr before each impression, this was done to reduce the number of colonies that would be introduced in the oral cavity through this alginate surface.

It was evaluated that the average mean value of the microbial colonies in CFU/ml on the surface of the rubber bowl before disinfection was 2E + 08 CFU/ml. This means that there is a wide range of microbial colonies on the surface of the rubber bowl before it is used for mixing alginate, when not disinfected. The presence of fomite contamination on alginate impression is proven in the study. Hence the first part of the null hypothesis is rejected.

Microbial analysis revealed the presence of harmful pathogens like *Staphylococcus aureus, E. Coli sp., Pseudomonas sp., Klebsiella sp.,* and bacillus on the surface of the rubber bowl.

In the control group, the number of colony-forming units CFU/ml in the rubber bowl before mixing was 2.35E + 09 CFU/ml and after mixing the alginate it was 3.1E + 09 CFU/ml. After the impressions are made, the CFU/ml on the alginate surface was increased to 4E + 09 CFU/ml. Al Omari[9] stated that alginate carries a significantly higher number of microorganisms. Impressions laden with microorganisms carry these organisms for up to 5 hours. Studies do indicate that alginate impressions produced significantly higher contamination than polyvinyl siloxane and polyether impressions from the same individual ($P < 0.5$). Samaranayake[2], stated that approximately three to four times more organisms are transferred to irreversible hydrocolloids than to elastomers during impression-making. A reason for this may be the irregular topographical features of irreversible hydrocolloids compared with the smooth surfaces of elastomeric impression materials, which are recognized to be superior in the precise reproduction of fine detail.

On exposure to the oral cavity, there was a significant increase in the CFU in both the control and the disinfectant groups. However, the CFU in control (4.4E + 09) is relatively high compared to that of the disinfectant (Table 2). The overall increase in the number of CFU on the impression surface in this study, is explained by Swapan[10]. He stated that adult human saliva is reported to contain approximately 6 billion ($6x10^9$) microorganisms per millilitre. Joana Correia-Sousa[1] stated that after mouth contact, alginate microbial count increased from 1.59 ± 2.79 to $2.68 \times 10^3 \pm 6.19 \times 10^2$ CFU/mm^2.

In this study, the CFU/ml of the experimental groups shows that the chemical and herbal disinfectants were effective in reducing the fomite contamination from the rubber bowl to the impression surface (Table 2). Hence, the second part of the hypothesis is rejected. There is a necessity to routinely disinfect the rubber bowl to reduce the organisms contaminating the alginate surface and subsequently the impression surface.

The use of herbal disinfection has been employed in dentistry. Plants are known to possess various secondary metabolites with profound antimicrobial properties and hence they have been used extensively in traditional medicine for years. Salma Khanam[11], described the disinfectant and antimicrobial properties of several herbs like *Azadiracta indica, Alovera, Euclapyptus robusta, Capsicum annum, Menthopiperertia,* etc. These plants with proven disinfectant activity are referred to as plant antiseptics.

In our study, both the herbal disinfectants *Acacia* and *Azadirachta* showed a steep decrease in CFU compared to the chemical disinfectants, the Acacia showed the highest reduction in CFU compared to other groups (Table 2). The descending order of the disinfectant activity of experimental groups according to the mean value of CFU/ml in this study is *Acacia nilotica, Azadirachta indica*, Sodium Hypochlorite, Glutaraldehyde and Hydrogen peroxide.

The antimicrobial efficacy of aqueous extracts of leaf of *A. indica* against human pathogenic bacteria (*Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis,* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus fumigatus* and *Candida albicans*) was reported by Raja Rathnareddy[12].

Rosina Khan[13] in 2009, found that crude ethanolic extracts of *Acacia nilotica, Cinnamon zeylanicum, Syzygium aromaticum* were effective against multidrug-resistant strains of *E. Coli, Klebsiella Pneumoniae and Candida Albicans.* Kalaivani[14], determined the free radical scavenging, cytotoxic and hemolytic activities of the ethanolic extract of *Acacia nilotica*. Maragathavalli[15], compared gentamycin with ethanolic extract of *Azadirachta indica* and found that the plant extract shows maximum inhibition on *Bacillus pumillus, Pseudomonas aeuroginasa, and Staphylococcus aureus.*

In our study, 2.5 mg/ml of ethanolic extract of *Acacia nilotica* was used to disinfect the rubber bowl. There was a significant decrease in the number of colonies forming units after disinfection (1.4E + 08 CFU/ml) compared to before disinfection (2.32E + 09 CFU/ml). A study conducted by Rwarinda U Angelo[16] revealed that the minimal inhibitory concentration of ethanol extract was low (2.5 mg/ml) as compared to other extracts (10 mg/ml). She concluded that it has an antimicrobial effect on Gram-positive and Gram-negative bacteria as well as antifungal activity in addition to antioxidant activity. Another study evaluated the effectiveness of ethanolic extract of *Acacia nilotica,* Extracts of *A. nilotica* showed the most potent activity against all the microorganisms like *E. faecalis, S. aureus, S. bovis, and S. mutans*[15].

In our study, 2500 µg/ml of ethanolic extract of *Azadirachta indica* was used as the disinfectant. There was a significant decrease in the number of colony-forming

units after disinfection (2.03E + 08 CFU/ml) compared to before disinfection (2.35E + 09 CFU/ml). A study showed that leaf extract exhibited strong antimicrobial activity against bacteria and fungi at all the concentrations tested (500, 1000 and 2000 µg/ml). The presence of high concentrations of azadirachtins, quercetin, and β-sitosterol in *A. indica* leaves might be responsible for strong antibacterial and antifungal activity compared with bark and seed[17].

In the present study, the pathogens found were *E. coli, Bacillus sp., Staphylococcus aureus, Pseudomonas sp., and Klebsiella* species. The control group had all the above pathogens. Glutaraldehyde, hydrogen peroxide, acacia and *Azadirachta* were effective against *Staphylococcus* spp. Sodium hypochlorite was effective against *E. coli, Bacillus sp., and Klebsiella sp*. Hydrogen peroxide was inactive against *E. coli, and Pseudomonas sp.,* but was effective against *Bacillus sp., Staphylococcus sp., and Klebsiella sp. Acacia nilotica* was active against *Bacillus* and *Klebsiella*. All pathogens were sensitive to *Azadirachta indica.*

A review found that several pathogenic bacteria were present in the complete dentures[18]. The isolation frequencies of the selective bacteria from the highest to the lowest are as follows*: Streptococcus spp., Staphylococcus spp., Enterobacter spp., Klebsiella spp. Pseudomonas spp. and E. coli spp*. These pathogenic bacteria could be an etiological agent for systemic conditions such as endocarditis, respiratory infections as well as gastrointestinal infections[19].

In this study, *Azadirachta* showed effective disinfection of pathogens compared to other disinfectants. This is in accordance with the study done by Abalaka[20] which showed that a minimum inhibitory concentration of 5mg and minimum bactericidal concentration of 50 mg of *Azadirachta indica* will be suitable for the treatment of various infections.

In our study, the Glutaraldehyde and Sodium hypochlorite was effective initially in reducing the number of CFU/ml, but later on, the impression surface reduced the number of CFU/ml in the Acacia group. It could be explained by the ability of chemical disinfectants to be minimized in the presence of extraneous organic matter like alginate that resulted in a comparatively high number of CFU/ml against herbal counterparts. This also adds to the advantageous property of herbal disinfectants that could act better than chemical disinfectants even in the presence of organic matter. Thus, the plant extracts could effectively decrease the number of colony-forming units and eliminate the pathogens. Hence the plant extracts could be considered as a viable alternative to chemical disinfectants.

5. Conclusion

The fomite contamination of alginate impression is proven in this study. The use of chemical and herbal disinfectants has significantly decreased the CFU/ml on the surface of the rubber bowl and alginate impression.

- The descending order of the disinfectants according to the mean value of CFU/ml in this study is *Acacia nilotica (Babool)* 2.5 mg/ml, *Azadirachta indica (Neem)* 2500 µg/ml, Sodium Hypochlorite 0.05%, Glutaraldehyde 2%, and Hydrogen peroxide 3%.
- *Azadirachta nilotica* is effective against all pathogens.
- The plant extract of *Neem and Babool* used as herbal disinfectants are as effective as chemical disinfectants and can be a viable alternative to them.
- Disinfection of rubber bowls can reduce the transfer of organisms to the oral cavity.

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