10.5005/jp-journals-10051-0148

ORIGINAL ARTICLE

Analysis of Bacterial Colonization due to Salivary Contamination on Gypsum Casts and Effect of Various Means of Disinfection of Dental Casts on Bacterial Colonization: An *in vitro* Study

[1]Sweta Pisulkar, [2]Trupti Dahane, [3]Ashok J Pakhan, [4]Surekha R Godbole, [5]Rajiv Bhola

ABSTRACT

Statement of problem: Prosthodontics patients, dentist, and staff are at a high risk for cross-contamination and disease transmission from each other. Addressing the above problem, two identifiable concerns are: (1) How the dentist and his staff can be protected from disease acquisition and disease transmission to patients and (2) steps taken to help to minimize cross-contamination with prosthetic instrumentation. The recovery of microorganisms from the dental casts may be a medium of cross-contamination between patients and dental personnel.

Aim: To determine whether saliva contamination contributes to bacterial growth on dental cast over a period of time and whether cleaning or disinfecting can minimize contamination and to evaluate the effectiveness of various chemicals disinfectants.

Materials and methods: Five type III gypsum casts were contaminated with saliva. Blood agar plates were inoculated and incubated at 37°C for 72 hours. Standardized dental stone cylinders were contaminated with 25 µL of saliva and treated by rinsing in tap water, soaking in 2% glutaraldehyde, 0.525% sodium hypochlorite, 0.5% phenol, or as controls with and without saliva contamination. The treated dental stone cylinders were placed in individual test tubes containing 2.5 mL of sterile phosphate-buffered solution and a final dilution of 10^{-4} was achieved. Colony-forming units (CFU) were counted after 24 hours.

Results: Rinsing the dental cast with tap water can diminish bacterial growth, but it cannot be considered as a reliable method of disinfection of the gypsum cast, as it may also sometimes even lead to further contamination. Immersion of the gypsum cast in 2% glutaraldehyde for 5 minutes completely eliminates bacterial colonization in almost all the instances.

Conclusion: Bacterial contamination of dental casts can occur, and requires an effective method of disinfecting.

[1,2]Associate Professor, [3]Professor and Dean, [4]Professor and Head, [5]Assistant Professor

[1-5]Department of Prosthodontics, Crown & Bridge, Sharad Pawar Dental College & Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe), Wardha Maharashtra, India

Corresponding Author: Sweta Pisulkar, Associate Professor Department of Prosthodontics, Crown & Bridge, Sharad Pawar Dental College & Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe), Wardha Maharashtra, India, Phone: +919881215746, e-mail: drsweta15@gmail.com

Keywords: Bacterial colonization, Chemical disinfection, Gypsum cast, Salivary contamination.

How to cite this article: Pisulkar S, Dahane T, Pakhan AJ, Godbole SR, Bhola R. Analysis of Bacterial Colonization due to Salivary Contamination on Gypsum Casts and Effect of Various Means of Disinfection of Dental Casts on Bacterial Colonization: An *in vitro* Study. Int J Oral Care Res 2018;6(1):47-53.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

An increased concern and attention to infection control in dental practice has resulted from increased awareness of the importance of infectious diseases and recognition of the potential for transmission of numerous infectious microorganisms during dental procedure.

Recent dental literature identifies the potential hazards of dentists transmitting or acquiring infectious diseases during the delivery of dental care. Cross-contamination is a problem confronting all members of the dental profession in varying degrees.[1] acquired immunodeficiency syndrome and hepatitis B are serious diseases due to their poor prognosis, especially hepatitis B, as it is transmissible through saliva.

In dental practice, materials, impressions, casts, and intraoral prosthesis should be cleaned and disinfected before being handled, adjusted, or sent to a dental laboratory according to the American Dental Association (ADA) council on scientific affairs ADA council. Among all these, dental cast is relayed numerous times between dental office and the dental lab.

Contamination of the cast can occur multiple times during each appointment, during fabrication of the prosthesis. To improve the basis of risk assessment and find a suitable strategy for reducing cross-contamination risk, epidemiological studies of the presence and persistence of microbial contamination of dental impressions and gypsum casts are needed.[2]

From professional, medical, and legal points of view, it is essential to develop an effective means of disinfecting dental casts. The disinfection of impressions is an

important issue in clinical practice before they are sent to the dental laboratory.[3] On dental impressions and gypsum casts, the persistent presence of opportunistic pathogens was analyzed. This investigation showed that patient-derived dental impressions and gypsum casts are contaminated with numerous microbes, which are known pathogens responsible for nosocomial and/or life-threatening infection in the immunocompromised host including *Candida*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*.[4]

Through constant exposure to debris, plaque, and saliva, which harbor pathogenic organisms that adhere to dental prosthesis, the dental personnel have an increased chance of infection. An effort to prevent these cross-contamination should be made to reduce the exposure of dental personal and the patient to microbial health hazards. One of the methods used to prevent cross-contamination from the prosthesis is by chemical disinfectants.

Hence, potential contaminated dental casts need to be routinely disinfected by an easy-to-use, inexpensive, and nondamaging method. Thus, the purpose of this study was to determine whether saliva contamination contributes to bacterial growth on the surface of the dental cast over a time period and whether cleaning or disinfecting the casts can minimize the results of contamination from saliva, and to evaluate the effectiveness of various chemicals disinfectants used for disinfection.

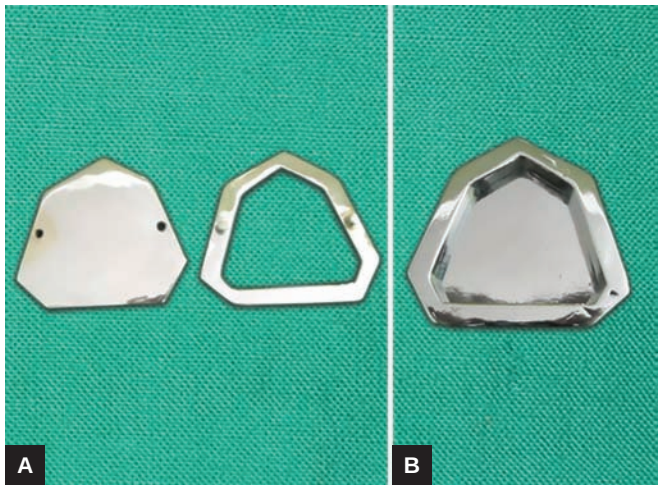
MATERIALS AND METHODS

To evaluate the persistent and time-dependent growth in the amount of bacterial colonization due to salivary contamination and effects of various methods of disinfection on the bacterial colonization, the methodology was divided into five phases as below: ·

- Phase I: Fabrication of custom-made devices

1. Fabrication of two-piece metal base former (Fig. 1)

A dental stone block of 20 mm thickness having seven sides and flat upper and lower surfaces was prepared.



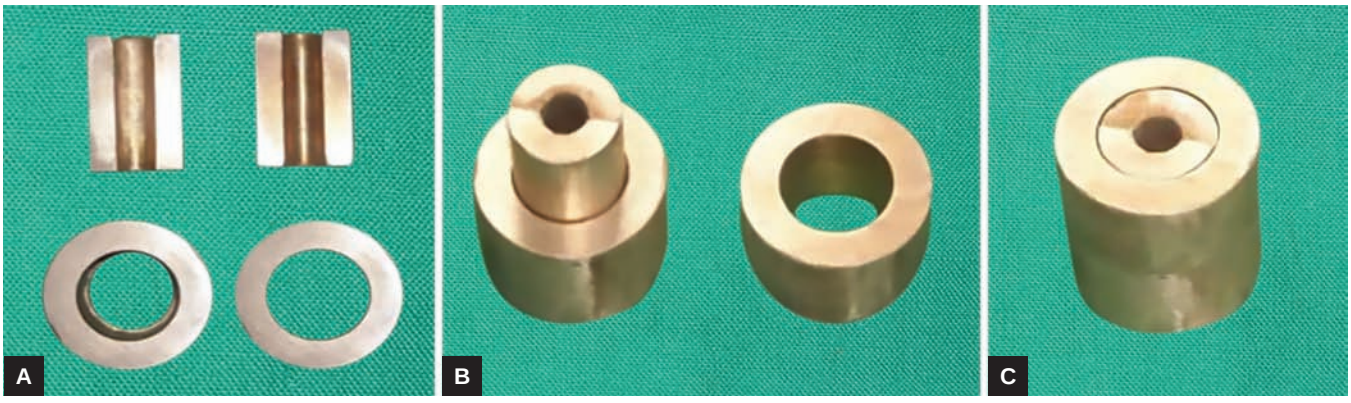
Figs 1A and B: Two-piece metal base former

This block represented a dental stone cast used for the study. This block was casted to fabricate a two-piece aluminum mold (part a: i.e., base and part b: i.e., counterpart) for the ease of the retrieval of the samples. These two parts precisely fitted into each other to form a single-piece mold. With the help of this standardized metal base former, five identical dental blocks were prepared which represented the type III gypsum casts.

2. Fabrication of brass cylindrical die (Fig. 2)

Brass metal master die of 24 mm length and 6 mm (Fig. 2C) diameter was fabricated. To ensure ease of the retrieval of the models from the brass die, it was fabricated in four parts (a, b, c, and d) with a precise fit of each part with one another (Fig. 2C). Parts a and b were split from the center in the vertical direction (Fig. 2A) for easy separation after the dental stone has set to retrieve the samples without any damage. In order to hold part a and part b with absolute precision, they were confined using brass rings; the other parts, i.e., part c and part d (Fig. 2B), split from each other in a horizontal direction. This die was used to prepare 120 cylindrical samples that were used for testing the efficacy of the various methods of disinfection.

- Phase II: Collection of saliva (Fig. 3)



Figs 2A to C: Brass cylindrical die

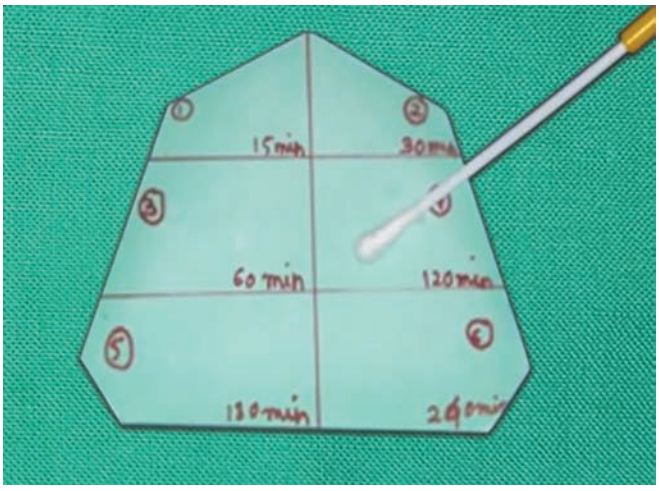


Fig. 3: Gypsum model representing cast to evaluate time-dependent increase in bacterial colonization

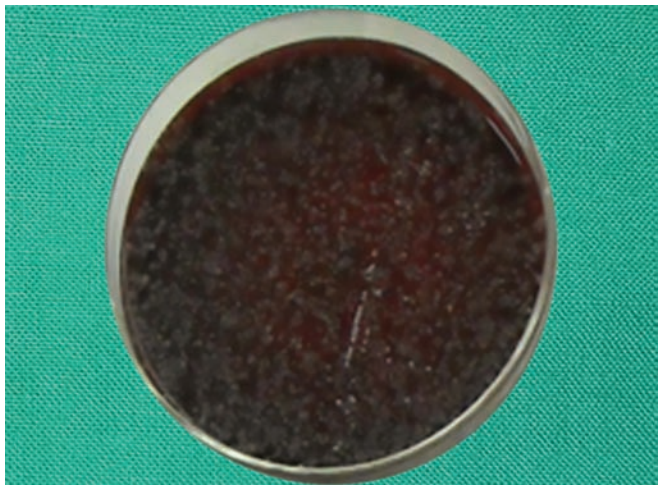


Fig. 4: Time-dependent increase of CFU after 24 hours of salivary contamination of the gypsum model

A volume of 5 mL of unstimulated saliva of a completely edentulous patient who reported to the Department of Prosthodontics was collected in a sterile disposable saliva collector. In order to maintain the standardization, all the samples were dipped in the saliva collected from this same patient. The patient was ruled out for any medical history and was ensured that he was completely alright without any infection or medical problem. The saliva collected by the above method was used for treatment of all samples.

- Phase III: Evaluation of time-dependent increase in bacterial colonization

To ensure complete sterilization and to rule out any environmental contamination before carrying out the study, the metal base former and the brass die were autoclaved and the samples were poured taking into consideration all aseptic measures. Type III dental stone was mixed in a clean rubber bowl and a plaster spatula according to the instructions given by the manufacturer and was poured in the metal base former representing the dental cast. The flat stone casts were allowed to ensure complete setting and retrieved from the model for further testing. Under all aseptic precautions, the entire surface of five flat dental casts were contaminated with the collected saliva with the help of sterile disposable swab to determine if salivary contamination is retained on the surface of dental cast. Each flat stone cast was divided into six sections and each section was numbered from 1 to 6 according to the time duration at which the swab will be taken, such as 15, 30, 60, 120, 180, and 240 minutes respectively (Fig. 3). At a designated time, a different section was swabbed with sterile swab wetted in sterile water at 15, 30, 60, 120, 180, and 240 minutes respectively.

- Phase IV: Inoculation and readings

Immediately after each designated area was be swabbed, a separate blood agar plate was inoculated and immediately incubated at 37°C for 72 hours in an incubator followed by counting of CFU after 24 hours (Fig. 4).

- Phase V: Comparison of efficacy of various methods of disinfection

1. Preparation of cylindrical samples used for disinfection.

Type III dental stone was mixed using a clean rubber bowl and a plaster spatula according to the directions given by the manufacturer and was gradually poured in the brass cylindrical die kept on a clean glass slab in increments with vibration to rule out porosities on the models. The dental stone was allowed to set for 1 hour, and the dental stone cylinders were then retrieved carefully from the die. A total number of 120 dental stone cylinders were poured and used for the study. These dental stone cylinders were preserved at room temperature and humidity for 24 hours in the sterile airtight zip pouches.

2. Grouping of the samples

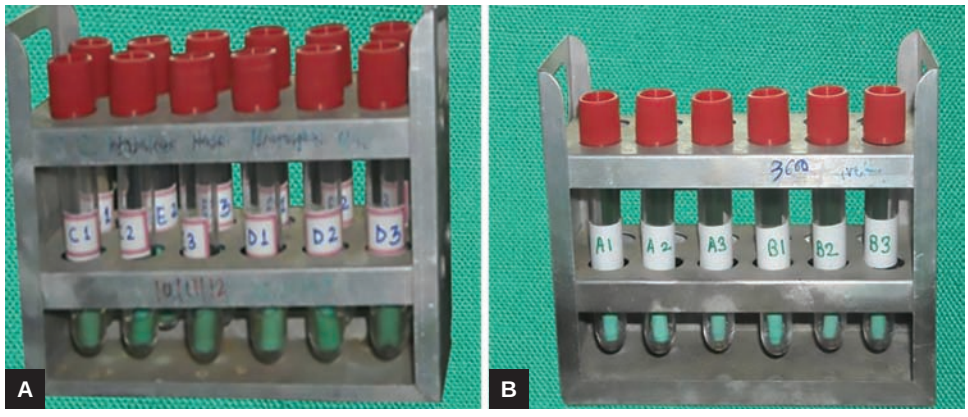
Twenty specimens were randomly picked as the control group, which are without salivary contamination used to rule out the effect of presence of any environmental contaminants and placed in sterile airtight polythene zip pouches and labeled as group I. Remaining 100 specimens used for quantitative bacteriology were immersed for 5 minutes in 25 µL of salvia.

3. Disinfection of the samples (Fig. 5)

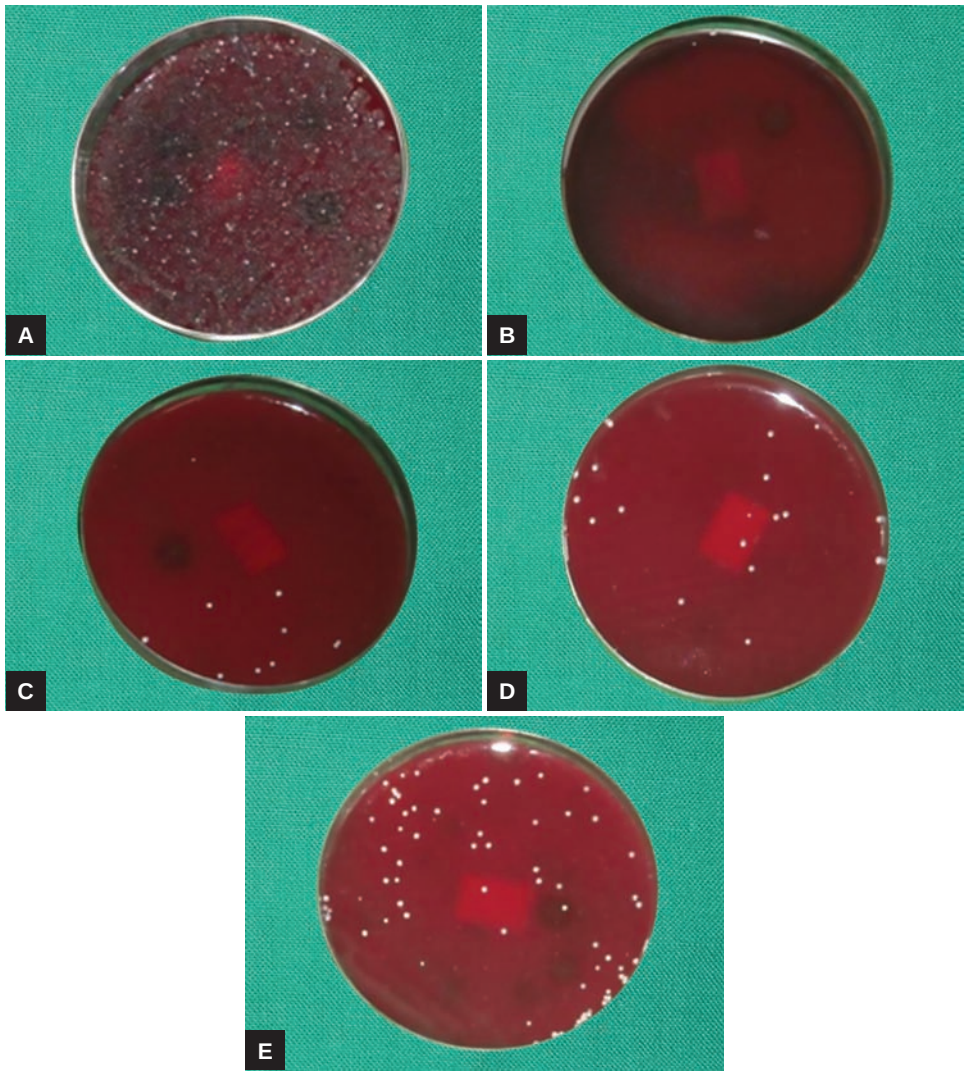
Twenty specimens contaminated with saliva had no further treatment (group II); 20 specimens were immersed in 2% glutaraldehyde for 5 minutes (group III); 20 specimens were immersed in 0.525% sodium hypochlorite for 5 minutes (group IV); 20 specimens were soaked in 0.16% phenol for 5 minutes (group V); and 20 specimens were washed in tap water (group VI).

4. Microbiological Analysis

After this, each of the cylinders was placed in sterile test tubes for 20 seconds containing 2.5 mL of sterile



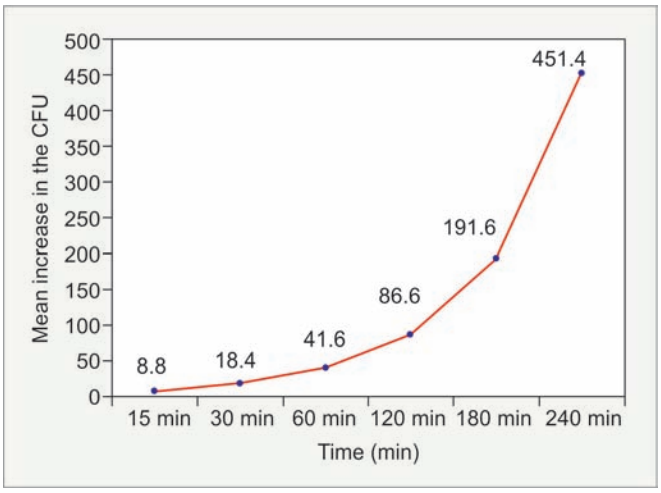
Figs 5A and B: Disinfection of the grouped cylindrical gypsum samples



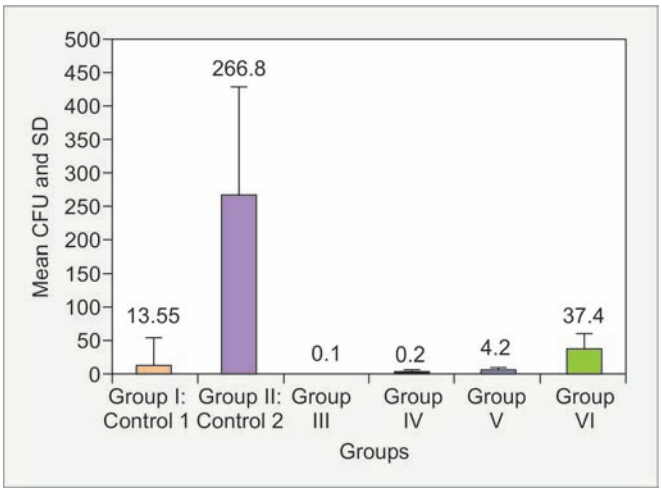
Figs 6A to E: Readings after disinfection of samples using various methods (total number of CFU after 24 hours). (A) CFU for specimens contaminated with saliva had no further treatment (group II). (B) CFU for specimens immersed in 2% glutaraldehyde for 5 minutes (group III). (C) CFU for specimens immersed in 0.525% sodium hypochlorite for 5 minutes (group IV). (D) CFU for specimens soaked in 0.16% phenol for 5 minutes (group V). (E) CFU for 20 specimens were washed in tap water (group VI)

phosphate-buffered saline. The suspensions were then agitated. To facilitate CFU, the final volume of 250 µL with 10^{-4} dilution was obtained. To determine the number of bacteria in each dilution, 100 µL from the total solution of 250 µL was transferred to the blood agar plates to obtain a lawn of growth on the surface of the plate.

5. Readings (Fig. 6)



Graph 1: Comparison of time-dependent increase in the amount of bacterial colonization due to salivary contamination on the gypsum cast



Graph 2: Comparison of efficacy of various disinfectants on the bacterial colonization

Table 1: Comparative evaluation of the efficacy of various methods of disinfection on the bacterial colonization

	Source of variation	*Sum of squares*	*Df*	*Mean square*	*f-value*	*p-value*
Group I: Control 1 and Groups III, IV, V, VI	Between groups	18895.04	4	4723.76	10.92	0.000 S, p<0.05
	Within groups	41059.95	95	432.21		
	Total	59954.99	99			
Group II: Control 2 and Groups III, IV, V, VI	Between groups	1065931.84	4	266482.96	50.44	0.000 S, p<0.05
	Within groups	501812.20	95	5282.23		
	Total	1567744.04	99			

S: Significant

The total number of CFU per plate would be equal to CFU × 2.5 × 10^{-4}. The blood agar plates were incubated at 37°C for 24 hours followed by counting of CFU after 24 hours (Figs 6A to E). The data were analyzed using Students paired "t" test, Dunnett "d" test, and the Duncan's range test for specific comparisons.

RESULTS

The results of the first part of the study revealed that the area of the dental cast that was swabbed at 240 minutes after contamination had as much growth as the area of the dental cast swabbed at 15 minutes after contamination (Graph 1).

These results indicated that the contamination of dental casts does not decrease; in fact, the colonization increases even if the casts are allowed to sit for 4 hours before handling. The results of the second portion of this study (Graph 2) was that, all dental stone cylinders contaminated with saliva and not treated had a large number of CFUs covering the blood agar plate. The specimens that were contaminated and rinsed only with water showed a range of 4 to 498 colonies at 10^{-4} dilution.

Most of the blood agar plates that were cultured from the dental stone cylinders and treated by 0.16% phenol for 5 minutes showed a range of 1 to 9 CFUs at the 10^{-4} dilution, followed by treatment with 0.525% sodium hypochlorite for 5 minutes with a range of 0 to 7 CFUs at 10^{-4} dilution.

All the dental stone cylinders that were soaked for 5 minutes in 2% glutaraldehyde yielded no growth. The controls that were not contaminated with saliva had minimal growth on few agar plates. The results of the analysis of variance indicated that there was a significant difference between saliva-contaminated dental stone cylinders cleaned with 2% glutaraldehyde, 0.525% sodium hypochlorite, and 0.16% phenol as opposed to rinsing with water or doing nothing to the contaminated dental stone cylinders (Table 1).

DISCUSSION

Increased awareness of the importance of infectious diseases and recognition of the potential for transmission of numerous infectious microorganisms during dental procedures have led to an increased concern and attention toward infection control in dental practice. Prosthodontics is one field of dentistry where prevention of cross-contamination seems to be an insurmountable problem.

Since infectious disease may be transmitted by blood and saliva, dental persons are exposed directly or indirectly to a wide variety of microorganisms. The mouth is a permanent source of microorganisms that could be

transferred and cause cross-infections. Media coverage of exposure incidents is becoming more intense. The lifetime cost of effective infection control is far less than one malpractice settlement.

The potential hazards of dentists acquiring or transmitting infectious diseases during delivery of dental care have been identified in recent dental literature.[5-7] The procedure of disinfecting the contaminated dental prosthesis before entering the laboratory is control and Occupational Safety and Health Administration exposure control program.[8]

An investigation on the effect of various disinfectants on tubercle bacilli on various impression materials and the resultant casts[9,10] denoted that bacilli are known to be a contaminant of the local water supply. A principal component of the oral flora is constituted by alpha-streptococci; beta-streptococci may be carried in large numbers in the oropharynx; and gamma-streptococci are minor salivary and oropharyngeal organisms.

Beta-staphylococci are minor oral and oropharyngeal organisms and can be carried in the nasopharynx, and nonhemolytic staphylococci are minor oral and oropharyngeal organisms. Some coliform bacteria exist in trace amounts in the oral cavity, but the bulk of the coliform and nonhemolytic streptococcal contamination probably comes about via the hands of the technicians, the former through poor hygiene and the latter as a normal epidermal inhabitant.[1]

In this study, disinfection of the dental cast made from type III dental stone is considered because it is transferred numerous times between the dental laboratory and the dental office. It has been proved that gypsum casts made from contaminated impressions can be the medium for cross-contamination between patients and dental personnel.

Dental casts can also can be re-infected when the acrylic resin record base is placed intraorally and then replaced on the dental casts and not only cross-contaminated from the contaminated impressions of patients. Contaminated impressions[11] made of dental stone poured against contaminated impressions may be an indirect source of disease transmission for cross-contamination between patients and dental personnel.[12] In this study, the methods of disinfection used are immersion in 2% glutaraldehyde solution, immersion in 0.525% sodium hypochlorite, immersion in 0.16% phenol and rinsing in tap water for a minimum duration of 5 minutes each. Undiluted saliva was used in this study because it is what the acrylic resin bases and dental stone cast would be contaminated with clinically. Saliva was contaminated with specimens for 5 minutes in 2.5 mL solution of phosphate-buffered saline to facilitate counting of CFU, and then dilutions were accomplished to reduce the relative numbers to manageable quantities. To determine the number of bacteria, the total solution was transferred to a blood agar plate. The blood agar plates were incubated at 37° for 24 hours in the incubator. All specimens contaminated with saliva and not treated had a large number of CFU covering the blood agar plate.

A range of colonies grew in the specimens that were contaminated and rinsed with water only, whereas the specimens that were contaminated with saliva and treated with sodium hypochlorite 0.525 and 0.16% phenol had significantly less CFU numbers when compared with that treated with tap water.

The specimens treated with 2% glutaraldehyde had no growth. This pattern of disinfection is observed in this study. The use of water as a disinfectant in this study was justified by the fact that water was readily available at chairside and was easily accessible to all practitioners. The use of tap water alone, however, was not very effective in decreasing the CFU; this was proved from the results obtained in this study. So, the additional step was instituted, i.e., disinfection of the gypsum cast with 2% glutaraldehyde, sodium hypochlorite 0.525%, and 0.16% phenol significantly reduced CFU number in just 5 minutes. The significant p-values obtained in Table 2 prove the fact that there is retention of saliva on the gypsum cast

Table 2: Time-dependent increase in the quantitative bacterial colonization due to salivary contamination on the gypsum cast

	Paired differences							
				95% confidence interval of the difference				
Time interval (min)	*Mean*	*Standard deviation*	*Standard error mean*	*Lower*	*Upper*	*t-value*	*Df*	*p-value*
15–30	–9.60	2.30	1.02	–12.45	–6.74	9.32	4	0.001 S, p<0.05
30–60	–23.20	2.28	1.01	–26.03	–20.36	22.74	4	0.000 S, p<0.05
60–120	–45.00	5.14	2.30	–51.39	–38.60	19.54	4	0.000 S, p<0.05
120–180	–105.00	5.83	2.60	–112.24	–97.75	40.26	4	0.000 S, p<0.05
180–240	–259.80	13.51	6.04	–276.58	–243.01	42.97	4	0.000 S, p<0.05
15–60	–32.80	3.11	1.39	–36.66	–28.93	23.54	4	0.000 S, p<0.05
15–120 min	–77.80	8.10	3.62	–87.86	–67.73	21.46	4	0.000 S, p<0.05
15–180 min	–182.80	3.96	1.77	–187.71	–177.88	103.16	4	0.000 S, p<0.05
15–240	–442.60	17.15	7.67	–463.90	–421.29	57.690	4	0.000 S, p<0.05

S: Significant

Table 3: Comparison of the efficacy of four methods of disinfectants with each other (Duncan's multiple range test)

Method of disinfection	*n*	*Mean*	*Duncan grouping*
Group III	20	0.10	C
Group IV	20	2.20	D
Group V	20	4.20	E
Group VI	20	37.40	F

surface, leading to bacterial contamination which goes on increasing as the time increases. The comparisons between each disinfectant on each CFU is also depicted in Table 3, which states that immersing the gypsum cast in 2% glutaraldehyde for 5 minutes is the most efficient method of disinfection of the gypsum cast, followed by group IV, i.e., immersing the cast in sodium hypochlorite 0.525% for 5 minutes followed by group V, i.e., immersing the cast in 0.16 % phenol for 5 minutes, and the last is group VI, i.e., rinsing the cast under running tap water. Therefore, the clinical implication from this study is useful in determining the most effective method of disinfection of the type III gypsum cast to prevent cross-contamination due to the bacterial colonization.

CONCLUSION

From this study, based on the findings, the following conclusions were derived:

- It was proved that when the type III gypsum cast was contaminated with saliva, there is persistence of saliva leading to bacterial contamination which increased over a time period and thereby justifying a definite need of disinfection of the gypsum cast in order to prevent cross-contamination.
- To prevent cross-contamination via dental cast in the laboratory, various methods of disinfection can be implemented among which the chemical method of disinfection has amounted for reduction in the bacterial contamination significantly.
- Rinsing the dental cast with the running tap water can diminish the bacterial growth, but it cannot be considered as a dependable method of disinfection of the gypsum cast, as it may sometimes even lead to further contamination through the contaminants in the tap water.
- For the dental casts, various methods of chemical disinfection used for disinfection by immersing it in disinfectants like 2% glutaraldehyde, 0.525% sodium hypochlorite and 0.16% phenol for 5 minutes have proved to produce reduction in the bacterial contamination significantly.
- In this study, among all the methods tested, the immersion in 2% glutaraldehyde for 5 minutes of the gypsum cast have been proved to be the best method, as it completely eliminates the bacterial colonization due to salivary contamination in almost all the instances.

REFERENCES

1. James KW. Cross-contamination via the prosthodontic laboratory. J Prosthet Dent 1974 Oct;32(4):412-419.
2. Infection control recommendations for the dental office and the dental laboratory. J Am Dent Assoc 1996 May;127(5): 672-680.
3. Donald ML, Hariri NM, Duncanson MG Jr, Jacobsen NL, McCallum RE. Quantitative study of bacterial colonization on the dental casts. J Prosthet Dent 1997 Nov;78(5): 518-521.
4. Egusa H, Watamoto T, Abe K, Kobayashi M, Kaneda Y, Ashida S, Matsumoto T, Yatani H. An analysis of the persistent presence of opportunistic pathogens on patient-derived dental impressions and gypsum casts. Int J Prosthodont 2008 Jan-Feb;21(1):62-68.
5. Ralph LL, Stevenson SE. Gypsum casts as a potential source of microbial cross-contamination. J Prosthet Dent 1983 Feb;49(2):210-211.
6. Stern MA, Johnson GH, Toolson LB. An evaluation of dental stones after repeated exposure to spray disinfectants, Part I: Abrasion and compressive strength. J Prosthet Dent 1991 May;65(5):713-718.
7. Samaranayake LP, Hunjan M, Jennings KJ. Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials. J Prosthet Dent 1991 Feb;65(2):244-249.
8. Michael L. Practical Dentun 70:538-540.
9. Ray KC, Fuller ML. Isolation of mycobacterium from dental impression material. J Prosthet Dent 1963 Jan-Feb;13(1): 93-94.
10. Pleasure MA, Duerr EL, Goldman M. Eliminating a health hazard in prosthodontic treatment of patients with pulmonary tuberculosis. J Prosthet Dent 1959 Sept-Oct;9(5):818-824.
11. Beyerle MP, Hensley DM, Bradley DV Jr, Schwartz RS, Hilton TJ. Immersion disinfection of irreversible hydrocolloid impression with sodium hypochlorite. Part 1: Microbiology. Int J Prosthodont 1994 May-Jun;7(3):234-238.
12. Vandewalle KS, Charlton DG, Schwartz RS, Reagan SE, Koeppen RG. Immersion disinfection of irreversible hydrocolloid impressions with sodium hypochlorite. Part II: Effect on gypsum. Int J Prosthodont 1994 Jul-Aug;7(4):315-322.