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Microbial Contamination of Pumice Powder and Slurry in Dental Laboratories of Hamadan

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Background: Using contaminated pumice in polishing process of dental prostheses may result in cross-contamination of dentists, laboratory technicians, and patients.
Objectives: This study aimed to determine the presence and level of microbial contaminants in pumice powder and slurry used in dental laboratories of Hamadan city.
Materials and Methods: Forty specimens, including 20 pumice powders and 20 pumice slurries were collected from 10 randomly selected dental laboratories and inoculated onto selective and non-selective media in order to count the total colony-forming units (CFU). Isolated fungi and bacteria were identified using Gram-stain and deferential diagnostic tests.
Results: Results of this study showed 85% contamination rate for pumice powders and 100% for pumice slurries. Frequencies of Gram-positive and Gram-negative bacteria isolated from the powders were 68% and 32% respectively. For the slurries the frequencies were 61% Gram-positive and 39% Gram-negative. Organisms detected in pumice powders composed of *Staphylococcus epidermidis*, *E. coli*, *Acinetobacter*, *B. cereus*, *Enterobacter*, *Candida*, and diphtheroids. Organisms detected in pumice slurries included *Staphylococcus epidermidis*, *E. coli*, *Citrobacter*, *S. aureus*, *Enterobacter*, *B. cereus*, *B. proteus*, *Candida*, and diphtheroids.
Conclusions: According to this study, pumice powder and slurry used in dental laboratories of Hamadan are contaminated. Therefore, the Laboratory staff should be aware of the hazards posed by the presence of pathogens in dental laboratories.

Keywords: Laboratories, Dental; Pumice; Equipment Contamination; Cross Infection; Dentures; Hygiene; Disinfection; Dentistry

1. Background

Dentistry personnel are exposed to a wide range of pathogenic microorganisms in the blood and saliva of the patients. Because of the spread of diseases such as tuberculosis, hepatitis and AIDS, the disinfection and sterilization procedures are more important in dentistry. Today the ethical and legal issues in infection control require more attention than before to prevent the contamination (1). In spite of some studies carried on the isolation of bacteria from impressions and dentures, there are few studies about isolation of bacteria from the appliances used in prosthetic treatment (e.g. occlusal rims and try-in dentures). And as these appliances are returned to the dental laboratories, they become a source of cross-contamination (2).

In other studies, the risk of cross-contamination transmission from laboratory contaminants to the clinical dentistry was mentioned (3, 4). The most important contamination agents in dental laboratories are pumice and lathe used to polish the prostheses (5). The pumice, and especially the slurry pumice, is one of the most important sources of oral bacteria and the other bacteria in the dental laboratories (2). Aerosols resulted from polishing procedures may cause eye infection in technicians. Aspiration and inhalation of these aerosols are very hazardous for the old, hospitalized and the patients with immunosuppression (6). The immediate denture and implants are the most contaminated cases. Some prostheses polished in dental laboratories may be exposed to the oral mucosa, saliva and blood of the patients, so technicians and dentists should be aware of cross-contamination in order to control the possible contaminating routes (7).

All the personnel in dentistry should have information to prevent and control the infection as well as the ability to perform the preventive measures. The studies show that the level of awareness, belief and performance of dentists in infection control are more than before, but it is not sufficient yet, so training courses are necessary to inform about preventive measures, and to control the infection. These courses should be repeated in order to offer new techniques (8).

2. Objectives

This study aimed to investigate the extent of bacterial contamination of the pumice in dental laboratories in Hamadan.

3. Materials and Methods

This cross-sectional descriptive study was performed in the dental laboratories in Hamadan in winter 2012.

3.1. Sample Collection

After offering the consent form and getting permission from the chief of laboratories, the samples were collected in a sterile condition using sterile latex surgical gloves, removable containers (sterilized by irradiation) and a 50-mL syringe from 10 randomly selected dental laboratories in Hamadan. At first, the piston was separated from the syringe, and it was filled with pumice powder, then the piston was put back on the syringe. A total of 4 samples, 2 pumice powders and 2 pumice slurries, were collected from each laboratory. Following transferring some pumice powder and pumice slurry (after shaking the slurry) to the container by a sterile syringe and sealing the container, the samples were immediately transported to the microbiology laboratory of the health center in Hamadan in a cold box.

3.2. Microbial Processing

Forty-five milliliter of ringer's solution in a 100-mL sampling container was sterilized in autoclave at 121˚C for 15 minutes, then 5 g pumice powder was added to reach a 1:10 dilution. In a similar condition for pumice slurry, 5 mL of ringer's solution was added to 45 mL ringer's solution to reach a 1:10 dilution. One milliliter of the prepared solution was added to the blood agar, 45˚C, and mixed with gentle rotation placed at 37˚C for 24 hours, then counted based on colony forming units (CFU).

In order to identify the bacteria causing contamination, the samples were cultured in the plates which contained gar, trypticase soy agar and eosin methylene blue (EMB) agar 24 hours at 37˚C. Then, the colonies were inoculated again and identified by the differential media. For initial identification of the bacteria, the lams were prepared using the cultured colonies on the blood agar and EMB media then the bacteria morphology, Gram-negative and Gram-positive, was determined by Gram staining. There are three types of hemolysis on the blood agar: 1-β-hemolysis (complete), 2-α-hemolysis (incomplete) and 3-γ-hemolysis, since different bacteria have different types of hemolysis, this classification could be used to identify them. The colonies were studied in EMB by staining, and the final identification was performed using biochemical tests based on the interaction. The colonies were identified using differential media such as Simmons citrate, urea broth, SIM, VP, MR, indole, ONPG, lysine, and OF. The CFU was counted by eye. The diagnostic tests were Gram staining, catalase test, mannitol test, salt agar test and TSI. The data was analyzed using SPSS software version 13, and the descriptive results are presented in Table 1 and Figure 1.

4. Results

These microbial contaminations were detected in the pumice powder and pumice slurry in dental laboratories: Gram-positive bacteria, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacter*, diphtheroids, and *Bacillus*; and Gram-negative bacteria, including *Acinetobacter*, *E. coli*, *Citrobacter*, *Proteus*, and *Enterobacter*. Also *Candida* was found (Table 1). Eighty-five percent of pumice powder samples and 100% of pumice solution were contaminated. The identified bacteria in pumice powder were as follows: 70.5% *S. epidermidis*, 23.6% *E. coli*, 11.8% *Acinetobacter* and *B. cereus*, *Enterobacter*rand diphtheroids each 5.9% (Figure 1). The detected bacteria in pumice powder were 68% Gram-positive and 32% Gram-negative.

The identified bacteria in pumice slurry were as follows: 45% *S. epidermidis*, 35% diphtheroids, 30% *E. coli*, 20% *Citrobacter*, 15% *Staphylococcusaureus*, 10% *Enterobacter*, 5% *Citrobacter,* and *Proteus*. Sixty-one percent of the bacteria found in pumice slurry was Gram-positive and 39% Gram-negative. The highest and lowest colony-forming units (CFU) in the pumice powder were *Acinetobacter* (4.5×10^5) and *Enterobacter* (1×10^3), and in the pumice slurry were *B. cereus* (9×10^5) and *Enterobacter* (0.54×10^5) (Table 1).

5. Discussion

Regarding the hazard of infection transmission via pumice powder and slurry, their bacterial and fungal contamination was investigated in dental laboratories in Hamadan, and the following bacteria were isolated:

S. epidermidis, *S. aureus*, *Enterobacter*, *Bacillus*, *Acinetobacter*, *E. coli*, *Citrobacter*, *Proteus, Enterobacter,* and diphtheroids.

The non-oral bacteria were as follows: *Acinetobacter*, *Bacillus cereos*, *Citrobacter*, *Proteus*, *Staphylococcus* sp, *Enterobacter*, *E .coli* and the oral bacteria were *Enterobacter* and diphtheroids.

The isolated bacteria in pumice powder and slurry were oral and non-oral. In a microbiologic study by Williams et al. on pumice, both oral and non-oral bacteria were reported: non-oral bacteria such as *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Moraxella*, *Micrococcus* and *Staphylococcus aureus*, and the oral bacteria like *Enterobacter* and diphtheroids (9). Katberg et al. reported *E. coli* and hemolytic and non-hemolytic *Staphylococcus*, alpha and beta *Enterobacter* in the pumice samples (10). In the other study, Witt isolated non-oral bacteria such as *E. coli*, *Bacillus* and *Staphylococcus epidermidis* from pumice samples (11). In the study of pumice samples by Jafari, non-oral bacteria such as *Bacillus*, *Staphylococcus epidermidis*, *E. coli* and *Enterobacter*, as well as oral bacteria were reported (6).

Table 1. Bacterial Contamination of Pumice in Dental Laboratories in Hamadan

Kind of Pumice	Contamination Type	Number of Contaminated Samples	Amount of Contamination (Number of Colonies)			
			Max	Min	SD	Mean
Pumice Powder						
	S. epidermidis	12	10×10^5	10	3.5×10^5	2.67×10^5
	E. coli	4	$10^5 \times 6$	10	$10^5 \times 2.9$	1.75×10^5
	Acinetobacter	2	$10^5 \times 8$	$10^5 \times 1$	$10^5 \times 4.9$	$10^5 \times 4.5$
	Enterobacter	1	-	-	-	$10^5 \times 10$
	Enterobacter	1	-	-	-	$10^5 \times 4$
	Diphtheroids	1	-	-	-	$10^5 \times 2$
	Enterobacter	1	-	-	-	$10^3 \times 1$
	Total	22	$10^5 \times 10$	10	$10^5 \times 3.5$	$10^5 \times 2.9$
	Candida	1	-	-	-	$10^5 \times 8$
Pumice Slurry						
	S. epidermidis	9	$10^5 \times 10$	$10^5 \times 5$	$10^5 \times 3.4$	$10^5 \times 2.7$
	E.coli	6	$10^5 \times 10$	$10^3 \times 9$	$10^5 \times 4.2$	$10^5 \times 5.13$
	Enterobacter	1	-	-	-	$10^5 \times 9$
	Diphtheroids	7	$10^5 \times 4$	$10^3 \times 1$	$10^5 \times 1.54$	$10^5 \times 0.9$
	Enterobacter	2	$10^5 \times 1$	$10^3 \times 8$	0.65×10^5	$10^5 \times 0.54$
	Proteus	1	-	-	-	$10^5 \times 1$
	S.aureus	3	$10^5 \times 6$	$10^3 \times 8$	$10^5 \times 3.25$	$10^5 \times 2.26$
	Citrobacter	4	$10^5 \times 10$	$10^3 \times 5$	$10^5 \times 3.86$	$10^5 \times 3.75$
	Total	33	$10^5 \times 10$	$10^5 \times 5$	$10^5 \times 3.4$	$10^5 \times 2.86$
	Candida	1	-	-	-	$10^5 \times 10$

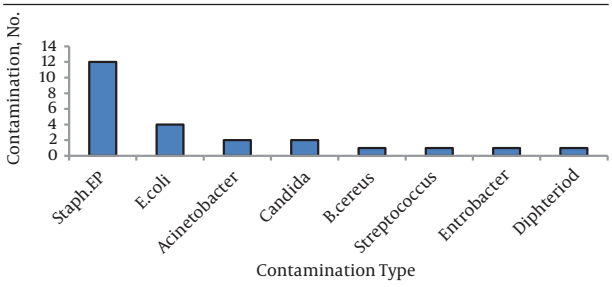

Figure 1. Frequency Distribution of Fungi and Bacteria in the Contaminated Pumice Powder in Dental Laboratories in Hamadan

Oral organisms need temperature and nutrition similar to the oral condition, so the number of oral organisms in pumice samples got declined, and the number of non-oral microorganisms increases in time. Kahn indicated that in clean pumice containing a few non-oral bacteria, after polishing denture and culturing the pumice samples the detected oral bacteria were dominant (12). There are four sources, which lead to various contaminations in the pumice slurry: 1- patient's denture; 2- technician's hands, nose, and mouth; 3- aerosols suspended in the environment; 4- water.

Twenty-five percent of people have *Acinetobacter* on their skins, so the skin of laboratory personnel could be a source of *Acinetobacter* contamination. *Staphylococcus aureus* may be spread by air or dust. Diphtheroids could be transferred to the saliva via technician's hands or contaminated denture. *Enterobacter* may be transferred by the skin or saliva. Gram-negative bacteria could be transferred to the pumice via denture of hospitalized patients also water may be a source of *Bacillus* (9). The Gram-positive bacteria, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacter*, diphtheroids and *Bacillus*, the Gram-negative bacteria, *Acinetobacter*, *E. coli*, *Citrobacter*, *Proteus* and *Enterobacter* showed similar distribution in pumice powder and pumice slurry. However, in Williams's study, the aerobic Gram-negative bacteria were dominant in pumice samples of laboratories (13). The contamination rate of all samples was 92.5% (37 of 40 samples). In all 40 samples, only 3 samples contained microbial contamination with 85% in pumice powder and 100% in pumice slurry that was similar to Jafari's results (6).

In the current study, *Staphylococcus epidermidis* showed the highest frequency in both pumice powders (70.5%) and pumice slurries (45%), and the other bacteria had lower frequency. The fungal contamination, *Candida*, was found in 2 pumice powders samples and 1 pumice slurry sample. In Williams's study, the most frequent fungi were *Aspergillus* and *Fusarium* and *Cephalosporium*, *Penicillium* and *A. flavus* were lower (14). The common con-

taminants were *Candida* and the other yeasts in pumice slurry in Verran's study (15). The most common fungi reported by Jafari were *Aspergillus*, *Rhizopus*and *Penicilliu-m*in pumice powder and *Candida albicans* and the other *candida*, *Aspergillus*, *Penicillium*, *Cladosporium* and *Rhizopus*in pumice slurry (6).We found that pumice powder and pumice slurry were equal with regard to CFU, but contamination was 100% in pumice slurry and 85% in pumice powder. Jafari et al. reported that bacterial contamination was higher in pumice slurry compared to pumice powder (6), thus in both studies pumice slurry showed relatively more contamination than pumice powder.

In this study, various concentrations of pathogenic and non-pathogenic organisms were identified in pumice powder and pumice slurry. *Acinetobacter*, *Enterobacter*, and *Staphylococcus aureus* could cause eye infection, and two latters may cause pneumonia. Thus, aerosols containing these microorganisms produced during polishing, could cause ocular and respiratory disease in personnel of dental laboratories. In this regard, using protective glasses and masks as well as air conditioner is necessary in dental laboratories (9). The dentures contaminated during polishing, may transfer organisms to the mouth and pharynx of patients and cause gastrointestinal diseases.

In the present study, *Acinetobacter*, *E. coli*, *Staphylococcus aureus* and *Staphylococcus* were isolated that may cause some gastrointestinal infections. *Enterobacter* which is an opportunistic and pathogenic microorganism was isolated and could cause nosocomial respiratory and urinary infection as well as septicemia. *Proteus* usually causes infections in different parts of body and is resistant to antibiotics so its treatment is more difficult and could cause abscesses in submandibular space and bacterial parotitis. The other bacterial species is *E. coli*that may lead to food poisoning and intestinal problems (16). These bacteria may transfer via denture and cause infections.

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Authors' Contributions

Developing the original idea and the protocol, abstracting and analyzing data, writing the manuscript: Fariborz Vafaee and Masoomeh Khoshhal; and developing the protocol, abstracting data, and preparing the manuscript: Pegah Radan, Farnaz Firouz, Bijan Heidari and Alireza Izadi; data collection: Mehdi Basami.

References

1. Alsaadi A. K. . Bacterial cross-contamination between clinic & dental laboratory during polishing procedure of complete denture. *Marietta Dly J.* 2011;**8**:288–9.
2. Verran J, McCord JF, Maryan C, Taylor RL. Microbiological hazard analysis in dental technology laboratories. *Eur J Prosthodont Restor Dent.* 2004;**12**(3):115–20.
3. Agostinho AM, Miyoshi PR, Gnoatto N, Paranhos Hde F, Figueiredo LC, Salvador SL. Cross-contamination in the dental laboratory through the polishing procedure of complete dentures. *Braz Dent J.* 2004;**15**(2):138–43.
4. Powell GL, Runnells RD, Saxon BA, Whisenant BK. The presence and identification of organisms transmitted to dental laboratories. *J Prosthet Dent.* 1990;**64**(2):235–7.
5. Setz J, Heeg P. Disinfection of pumice. *J Prosthet Dent.* 1996;**76**(4):448–50.
6. Jafari AA, Falah Tafti A, Falahzada H, Yavari MT. Evaluation of presence and levels of contamination in pumice powder and slurry used in clinical dental laboratories. *Middle East J Sci Res.* 2006;**1**(1):50–3.
7. Connor C. Cross-contamination control in prosthodontic practice. *Int J Prosthodont.* 1991;**4**(4):337–44.
8. Infection control recommendations for the dental office and the dental laboratory. ADA Council on Scientific Affairs and ADA Council on Dental Practice. *J Am Dent Assoc.* 1996;**127**(5):672–80.
9. Williams HN, Falkler WA, Jr, Hasler JF. Acinetobacter contamination of laboratory dental pumice. *J Dent Res.* 1983;**62**(10):1073–5.
10. Katberg JW, Jr. Cross-contamination via the prosthodontic laboratory. *J Prosthet Dent.* 1974;**32**(4):412–9.
11. Witt S, Hart P. Cross-infection hazards associated with the use of pumice in dental laboratories. *J Dent.* 1990;**18**(5):281–3.
12. Kahn RC, Lancaster MV, Kate W, WA, Jr. The microbiologic cross-contamination of dental prostheses. *J Prosthet Dent.* 1982;**47**(5):556–9.
13. Williams HN, Falkler WJ, Hasler JF, Libonati JP. The recovery and significance of nonoral opportunistic pathogenic bacteria in dental laboratory pumice. *J Prosthet Dent.* 1985;**54**(5):725–30.
14. Williams HN, Falkler WJ, Smith AG, Hasler JF. The isolation of fungi from laboratory dental pumice. *J Prosthet Dent.* 1986;**56**(6):737–40.
15. Verran J, Kossar S, McCord JF. Microbiological study of selected risk areas in dental technology laboratories. *J Dent.* 1996;**24**(1-2):77–80.
16. Jawetz B, Melnick B, Adelberg M. *Medical Microbiology.*; 2001.