Microbial contamination of pumice used in dental laboratories

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Abstract

Dental appliances as well as sending and receiving prosthesis from laboratories are potential sources of cross-contamination for technicians, dentists, patients and can transmit different infectious agents as well. This study was conducted to determine the types of the microorganisms in pumice powder and pumice slurry used in dental laboratory in order to evaluate necessary disinfection control procedure in the dental settings. Twenty-four active dental laboratories of Khorram Abad participated in our study. Samples were randomly collected from prosthesis polishing containers in sterilized condition and were immediately sent to microbiology laboratory. Specimens were cultured on selective bacterial and fungal media in order to determine the microorganisms. Both oral and non-oral bacteria were recovered from pumice samples as follows: *Staphylococcus aureus* (15.4%), *Streptococcus viridance* (10.8%), *Bacillus cereus* (18.7%), *Pseudomonas aeruginosa* (12.8%), *Diphtheriods* (7.3%), *Enterobacter cloace* (4.3%), *Escherichia coli* (13.1%), *Klebsiella pneumonia* (5.4%), and *Acinetobacter* spp. (12.2%). The isolated fungi included *Candida albicans* (36.7%), other yeasts (17.3%), *Fusarium* spp. (13.8%), *Aspergilus* spp. (22.4%) and *Penicillium* spp. (9.8%). This study showed that polishing pumices in the form of powder or slurry were contaminated with different oral and non-oral bacteria and also fungi. Therefore, the chance of cross-contamination still severely exists, and measures should be conducted to prevent the contamination of predisposed people such as technicians, dentists and patients.

Introduction

Cross-contamination is a serious problem in dentistry and may occur among dental staff and patients.[1] Dental patients and dental personnel (dentists, dental laboratory technicians and assistants) can be exposed to a wide variety of pathogenic microorganisms in the blood and saliva, such as hepatitis B virus (HBV), hepatits C virus (HCV), HIV, *pseudomonas*, *Acinetobacter*, *Diphteroids*, *Lactobacilli*, *Staphylococci*, *Streptococci*, *Mycobacterium* and other microorganisms that colonize the oral cavity and respiratory tract. These organisms can be transmitted to dental settings through direct or indirect contact.[2,3]

Most recent literature has focused on cross-contamination of dental prostheses in the dental laboratory.[4,5] In dental laboratories, pumice is used in prostheses polishing. The pumice – as the last step of prosthesis finishing – has been reported to be the greatest source of contamination and also a transmission potential source for infection.[6,7] During prosthesis polishing, contaminated aerosol particles spread and remain in the air for a long time causing high risks for both dental staff and patients. Aspiration and inhalation of these aerosols for elderly immunocompromised patients, patients with endocarditis and respiratory disease is really hazardous.[8,9]

The bacteria, such as *Acinetobacter, Pseudomonas* and *Moraxella*, which are not part of normal oral flora, can cause serious diseases if passed to patients whose dentures are polished with contaminated material and to the technician by exposure to contaminated aerosol.[10]

The prosthesis contaminated by potentially pathogenic microorganisms such as Gram negative bacilli can cause serious diseases when it penetrates the oropharyngeal area and increases pneumonia incidence.[7] Despite rigorous need for sterilization and disinfection of dental instruments, prosthetic appliances do not receive adequate infection control.[11] The sterilization has to be performed with suitable validated procedures so that the success of these procedures can be monitored and safety and health of patients, users, and other persons guaranteed.[12] An earlier research from Shiraz area (Iran) reported the microorganisms isolated from pumice in dental laboratories.[7] The aim of this research was to determine the bacterial and fungal contaminations present in pumice powder and slurry used in Khorram Abad dental laboratories to evaluate the role of pumice in cross-contamination of dental laboratories.

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Materials and Methods

Survey area

Khorram Abad, the capital of Lorestan province is located in the south-west Iran, bordering with the provinces of Markazi, Hamedan, Kermanshah, Khuzestan, Ilam, and Isfahan. The estimated population of Khorram Abad is 540,000. The district covers an area of approximately 6233 km^2. The study site (48°21'S, 30°43'W) is the largest city in Lorestan province.

Sample collection

This study was conducted between June and September 2012 in twenty-four dental laboratories in Khorram Abad. Samples randomly collected were placed in sterile containers and immediately transferred to the microbiology laboratory for isolation of microorganisms.

Preparation, cultivation and identification

Initially, 1 g of pumice was aseptically weighed and a suspension in 9 mL sterile normal saline was prepared in a small test tube.

the tubes were mixed for 30 sec. afterwards, 1 ml of the suspension was cultured on blood agar [automatic tank dewater (ATD); Antec International Ltd., Sudbury, UK] with 5% defibrinated sheep blood cell for isolation of all bacteria, on McConkey agar (MERCK KGaA, Darmstadt, Germany) for isolation of Gram negative bacteria, on manitol salt agar (MERCK KGaA) for isolation of *Staphylococcus aureus,* and on Sabouroud dextrose agar (HiMedia Laboratories Ltd., Mumbai, India) for detection of fungi. The cultured plates were incubated 24-48 h at 37°C for bacterial isolation and at 25°C for 2 weeks for fungi. The plates were checked daily for detection of microorganisms. Morphologically different bacterial and fungal colonies were subcultured, and isolated colonies were identified to genus and species levels using microscopic and macroscopic characters.[7] In addition, coagulase, catalase, sugar fermentation test, KOH and hemolysis test were carried out according to the standard methods.[13] To assure the sterility and reliability of the techniques, 24 non-treated pumices (with denture) for each dental laboratory were considered as the control group.

Results

Of the 72 samples collected from 24 dental laboratories, 16 (66.7%) dental laboratories were contaminated for microorganisms (Table 1). The isolated microorganisms from cultures of pumice samples collected from dental laboratories in Khorram Abad are reported in Figures 1 (Gram positive bacteria) and 2 (Gram negative bacteria). The results indicated that the highest rate belonged to *Bacillus cereus* (18.7%) and the lowest one was *Enterobacter cloace* (4.3%). *Candida albicans* (36.7%) was the highest rate of isolated fungi and *Penicillium* spp. (9.8%) was the lowest (Figure 3).

Discussion

Pumice used as the last step in prosthesis polishing could be a potential source of contamination to dental laboratory technicians.[10] It was shown that in patients with immune deficiency problems, dentures have higher levels of contamination,[14,15] since most denture users are elderly people, so the risk of infection is even higher. In this study, we found a great part of bacterial species from pumices. Most interestingly, this is consistent with report by Verran *et al.*,[16] although they also isolated *Micrococcus* from pumice slurry.

Results obtained in the present study revealed a strong oral and non-oral contaminating source in polishing pumices. Most of the fungi and bacteria isolated in our study were not pathogenic in healthy people, but some of them, such as *Staphylococcus aureus* and *Streptococcus viridans,* can be harmful both for the immunoicompromised and elderly patients as well as for healthy people.

Viridans streptococci are part of the oral cavity normal flora. The main significance of these bacteria relates to their ability to cause 30-40% of cases of subacute bacterial endocarditis.[14] Since the organisms are most abundant in the mouth, minor trauma may lead to their entry into the bloodstream and initiate of endocarditis especially in predisposed patients. Witt *et al.*[8] notified a similar situation. They found *Streptococcus viridians* in cultures of pumice from laboratories.

When prosthesis is polished with pumice,

Table 1. Microorganisms isolated from dental laboratories.

No.	Lab.Microorganisms													
	S.vi.	*S.ae*	*B.ce.*	*P.ae*	*Dipht.*	*E.co.*	*E.cl.*	*K.pn.*	*Acin.*	*C.al.*	*Other*	*Fusa.*	*Aspe.*	*Peni.*
1	+	+	-	-	-	-	-	+	-	+	-	-	-	-
2	-	+	-	+	+	-	-	-	+	+	+	-	+	+
3	-	-	-	+	+	-	+	-	+	+	+	-	+	-
4	-	-	+	+	-	-	+	-	+	+	-	+	+	-
5	-	-	+	+	-	-	+	-	+	+	+	+	-	-
6	-	+	-	+	+	+	+	-	+	+	+	+	+	-
7	-	+	-	+	+	+	-	-	+	-	-	+	+	+
8	+	+	-	+	-	-	-	+	+	-	-	+	+	+
9	-	-	+	-	+	+	-	-	-	+	-	-	-	+
10	-	-	+	-	-	-	-	-	-	+	+	-	+	-
11	-	-	+	-	-	+	-	-	-	-	+	-	+	-
12	-	-	+	-	-	+	-	-	-	+	+	-	+	-
13	-	+	-	-	-	-	-	+	-	+	-	-	+	+
14	-	-	-	-	-	-	-	+	-	+	-	-	+	-
15	-	-	-	-	+	+	-	+	-	+	+	+	-	-
16	-	+	-	+	-	+	-	-	-	-	-	+	-	-
17	-	-	-	+	+	+	-	-	-	-	-	+	-	-
18	-	-	-	-	-	+	+	-	-	-	-	+	-	+
19	-	-	-	-	+	+	+	-	-	-	-	-	+	-
20	-	-	+	+	+	-	+	-	-	-	-	-	-	-
21	+	+	-	+	-	-	+	-	-	-	-	-	-	-
22	-	+	-	-	-	-	+	-	+	+	-	-	-	+
23	-	+	-	+	-	-	-	-	+	-	-	-	-	+
24	-	-	-	+	+	-	-	-	+	+	+	-	-	+

S.vi., Streptococcus viridance; S.ae., Staphylococcus aureus; B.ce., Bacillus cereus; P.ae., Pseudomonas aeruginosa; Dipht., Diphtheriods; E.co., Escherichia coli; E.cl., Enterobacter cloace; K.pn., Klebsiella pneumonia; Acin., Acinetobacter species; *C.al., Candida albicans;* Other, other yeasts; *Fusa., Fusarium* species; *Aspe., Aspergilus* species; *Peni., Penicillium* species.

contaminated aerosol particles of microorganisms such as Gram negative bacteria and fungi, are spread all around the laboratory. This could be a major source for different oral and non-oral infections. Several studies have reported isolation of Gram negative bacteria like *Pseudomonas*, *Moraxella* and *Acinetobacter* from pumice which can be transferred to patients and dental laboratory staff by contaminated aerosols, and cause ocular and respiratory infection especially in persons with chronic respiratory disorders.[16] The entry of Gram negative bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* into the blood of patients can cause a fatal infection especially Gram negative septicemia in debilitated patients.[17] Isolation of Gram negative bacteria in the current study is similar to that obtained by other studies.[7]

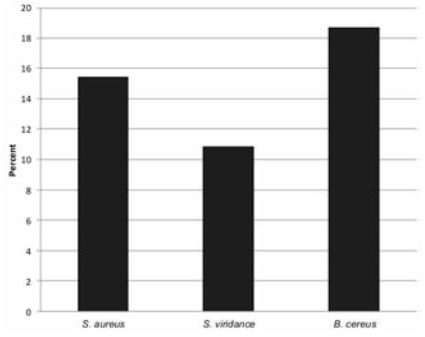

Figure 1. Frequency of Gram positive bacteria isolated from pumice samples of dental laboratories.

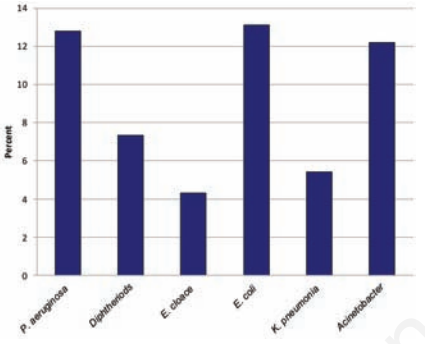

Figure 2. Frequency of Gram negative bacteria isolated from pumice samples of dental laboratories.

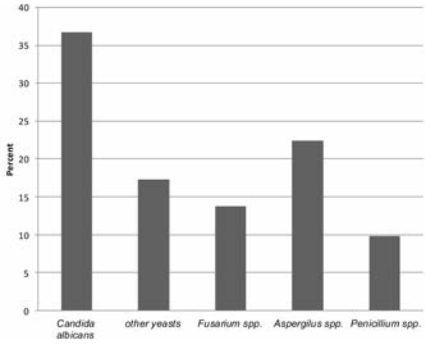

Figure 3. Fungal species isolated from pumice samples.

Fungi recovered from used pumice samples in the current study included *Aspergillus*, *Fusarium*, *Penicillium* and *Candida* that increased risk of fungal infection especially in persons who work for a long period of time in dental laboratories and have been exposed to fungal spores.[18] Some reports support that *Candida albicans* belong to the normal physiology flora of mouth. It is able to grow in pumice and cause infections in humans.[19]

Besides, new studies have been conducted on viral infection transmission especially HBV and HIV in dental laboratories. Occupational infection of the dental laboratory technicians with HBV has been reported. The studies suggest that all healthcare workers working in dental laboratories should be vaccinated against hepatitis B virus.[20]

There are many studies that provide some additional information regarding prosthesis disinfection. A previous study in Brazil showed a transfer of microorganisms from patients prosthesis to sterile prosthesis and in most laboratories pumice was not changed or disinfected between polishing procedures.[10] Jagger *et al.*[21] reported that about 6.1% of dental laboratories used disinfectants in the pumice and 92.9% did not disinfect the polishing instrument. A previous study has proven that pumice slurry freshly made up using disinfectants was reported to be free from most contaminations.[8] Unfortunately, in the present study most of the laboratories did not used a disinfectant while working with pumice, however, it will be good to use such disinfection protocol to minimize the chance of infection among the dental laboratories technicians and patients. It is recommended to disinfect old or used dentures before starting any action. The technician should use sterilized gloves, disinfected protecting glasses, oral masks, brushes and polishing tools to polish prosthesis.

Conclusions

Polishing pumices are potential source of infection in dental laboratories when considering the wide variety of microorganisms in the blood and saliva of patients. Following our study results, low temperature sterilization, such as gas or plasma sterilization, would allow optimal reduction in the number of pathogenic bacteria. The use of sterile pumice or association of disinfectants with pumice for polishing the prosthesis, sterilization of containers after each use with adding of an appropriate disinfectant such as 0.2% chlorohexidine gluconate or 5% hypochlorite sodium to pumice could be effective and daily change of polishing paste is recommended to reduce the hazard of cross-contamination. However, no standard procedures actually exist.

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