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Full length research paper

Microbiological study pumice used in dental laboratories

Farzaneh Firoozeh[1], Mohammad Zibaei[2]*, Abolfazl Zendedel[3], Hushang Rashidipour[4], Aziz Kamran[5]

[1]Department of Microbiology and Immunology, School of Medicine, Kashan University of Medical Sciences, Kashan, I.R. Iran
[2]Department of Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Iran
[3]Department of Internal Medicine, School of Medicine, Lorestan University of Medical Sciences, Iran
[4]Department of Endodontics, Dental School, Lorestan University of Medical Sciences, Iran
[5]Department of Public Health, Faculty of Health Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

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Dental appliances 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 used dental laboratory pumice powder and pumice slurry in order to evaluate necessary disinfection control procedure in the dental settings. Twenty four active dental laboratories of Khorram Abad entered 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 of the microorganisms. Both the 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 cloaceae* (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 in the predispose people such as technicians, dentists and patients.

Keywords: Contamination; Dental laboratory; Pumice

INTRODUCTION

Cross contamination is a serious problem in dentistry and may occur among dental staff and patients (Kugel et al., 2000). Dental patients and dental personnel (dentists, dental laboratory technicians and dental assistants) can be exposed to pathogenic microorganisms such as hepatitis B virus (HBV), hepatits C virus (HCV), HIV, *pseudomonas*, *Acinetobacter*, *Diphteroids*, *Lactobacilli*, *Staphylococci*, *Streptococci*, *Mycobacterium* and other microorganisms that colonize in the oral cavity and respiratory tract. These organisms can be transmitted to dental settings through direct or indirect contact (Ross and Clarke, 1981; Connor, 1991; Jamani et al., 1995; Al-Kheraif and Mobarak, 2008).

The risk of transmission of microorganisms in dental laboratories has been reported worldwide (Nutt et al., 1999; Parisi and Glick, 2003). In dental laboratories, pumice are used for polishing of prostheses. The pumice as the last step of prosthesis finishing has been reported

*Corresponding Author: E-mail: zibaeim@sums.ac.ir
Tel: + 98- 661- 6200133

as the greatest source of contamination and also a transmission potential source for infection (Powel et al., 1990; King and Matis, 1991; Vojdani and Zibaei, 2006). During polishing prosthesis contaminated aerosol particles spread and remain in the air for a long time and cause high risks for dental staff and patients. Aspiration and inhalation of these aerosols for elderly immunocompromised patients, patients with endocarditis and respiratory disease is really hazardous (Witt and Hart, 1990; Chris et al., 1998). The prosthesis contaminated by potentially pathogenic microorganisms such as Gram negative bacilli can cause serious diseases if it penetrates the oropharyngeal area and increases pneumonia (Williams et al., 1985; Vojdani and Zibaei, 2006). 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 the cross contamination of dental laboratories.

Materials and Methods

Survey area

Khorram Abad, the capital of Lorestan province is located in the southwestern of 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 6,233 km^2. The study site (48° 21', 30° 43') is the largest city in Lorestan province.

Sample collection

This study was conducted between June and September 2012 in Twenty-four dental laboratories of Khorram Abad. Samples collected randomly was placed in sterile containers and immediately transferred to Microbiology Laboratory for isolation of microorganisms.

Preparation, cultivation and identification

In the laboratory 1 Gram 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 well for 30 sec. Following that 1 mL of the suspension was cultured on Blood agar (ATD, ANTEC Co., UK) with 5% defibrinated sheep blood cell for all bacteria and standard microbiology methods (biochemical tests) for identification of Gram positive bacteria, on McConkey agar (MERCK, Darmstadt, Germany) for isolation of Gram negative bacteria, on Manitol salt agar (MERCK, Darmstadt, Germany) for isolation of *Staphylococcus aureus* and on Sabouroud dextrose agar (HIMEDIA, Laboratories Pvt. Ltd, India) for detection of fungi and . The cultured plates were incubated 24-48 hours 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 as described by Vojdani and Zibaei (2006). In addition, coagulase, catalase, sugar fermentation test, KOH and Hemolysis test were carried out according to the method described by Baron and Finegold (1990) and Washigton et al. (2006). Controls were used to assure the sterility and reliability of the techniques.

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 were collected from dental laboratories in Khorram Abad are reported in Figures 1 and 2. The results indicated that the most rate belonged to *Bacillus cereus* (18.7%) and lowest one was *Enterobacter cloaceae* (4.3%). *Candida albicans* (36.7%) was the highest rate of isolated fungi and *Penicillium spp*. (9.8%) was the lowest respectively (Figure 3).

Discussion

Pumice used for polishing of prosthesis as the last step, could be a potential source of contamination of dental staff (Miller and Micik, 1978; Chris et al., 1998; Agostinho et al., 2004). Dentures of patients who are diseased or immunocompromised have been reported to have higher levels of contamination (Henderson et al., 1987), since most denture users are elderly people, so the risk for infection in them is high. Many of bacterial species isolated from pumices in this study were the same ones reported by Verran et al. (1997). However, they also isolated bacteria such as Micrococcus.

The results of present study revealed a strong oral and non oral contaminating source in polishing pumices. Also most of fungi and bacteria isolated in our study were not pathogenic in healthy people, but some of them like *S. aureus* and *Streptococcus viridans* could be harmful for immunoicompromised and elderly patients as well as healthy people. *S. viridans* is considered as the oral cavity normal flora. The principal significance of this bacterium relates to subacute endocarditis. Because *Streptococci* are present in the throat and around the teeth, even minor traumas may result in their entry into the blood stream and initiate subacute endocarditis especially in patients predisposed. *Viridans streptococci* cause 30-40% of cases of subacute bacterial

Table 1. The microorganisms isolated from dental laboratories

No. Lab	Microorganisms													
	E.co	S.ae	S.vi	B.ce	P.ae	Dipht	E.co	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 spp.; C.al: Candida albicans; Other: other yeasts; Fusa: Fusarium spp.; Aspe: Aspergillus spp. and Peni: Penicillium spp.

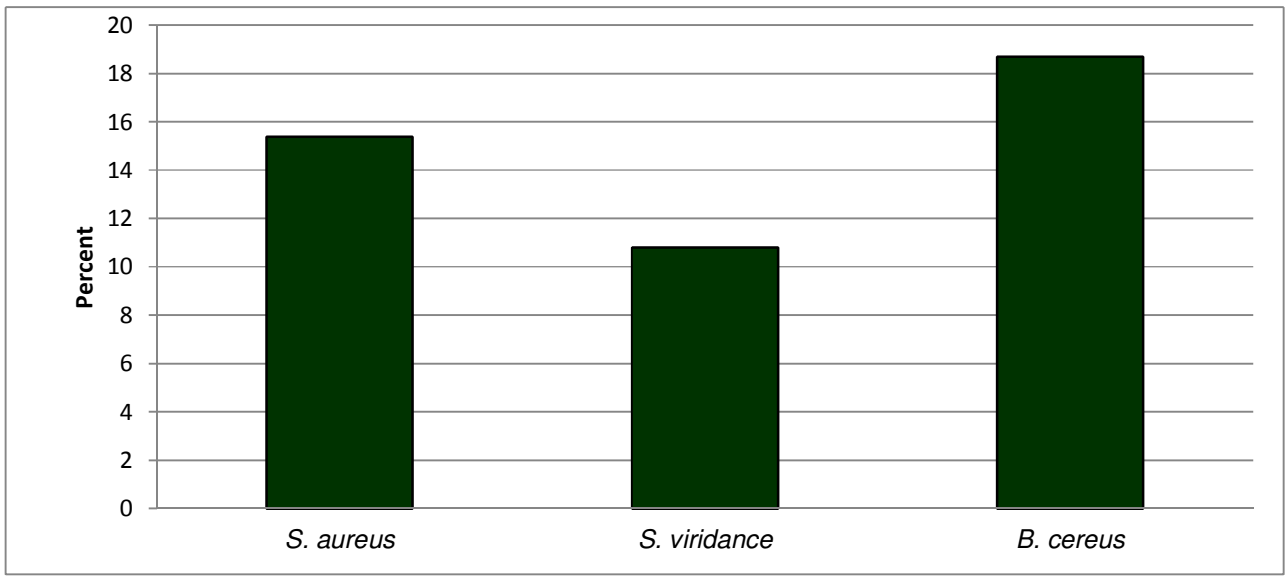

Figure 1. Frequency of Gram Positive bacteria isolated from pumice samples of dental laboratories.

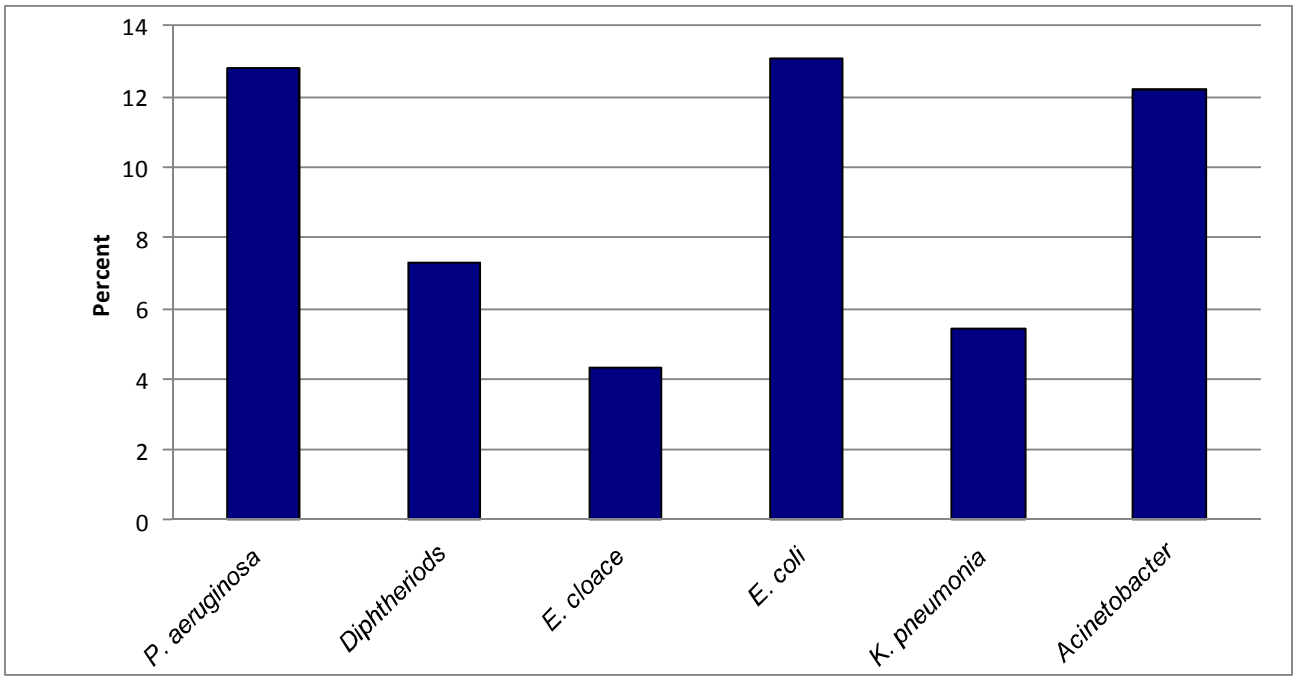

Figure 2. Frequency of Gram negative bacteria isolated from pumice samples of dental laboratories.

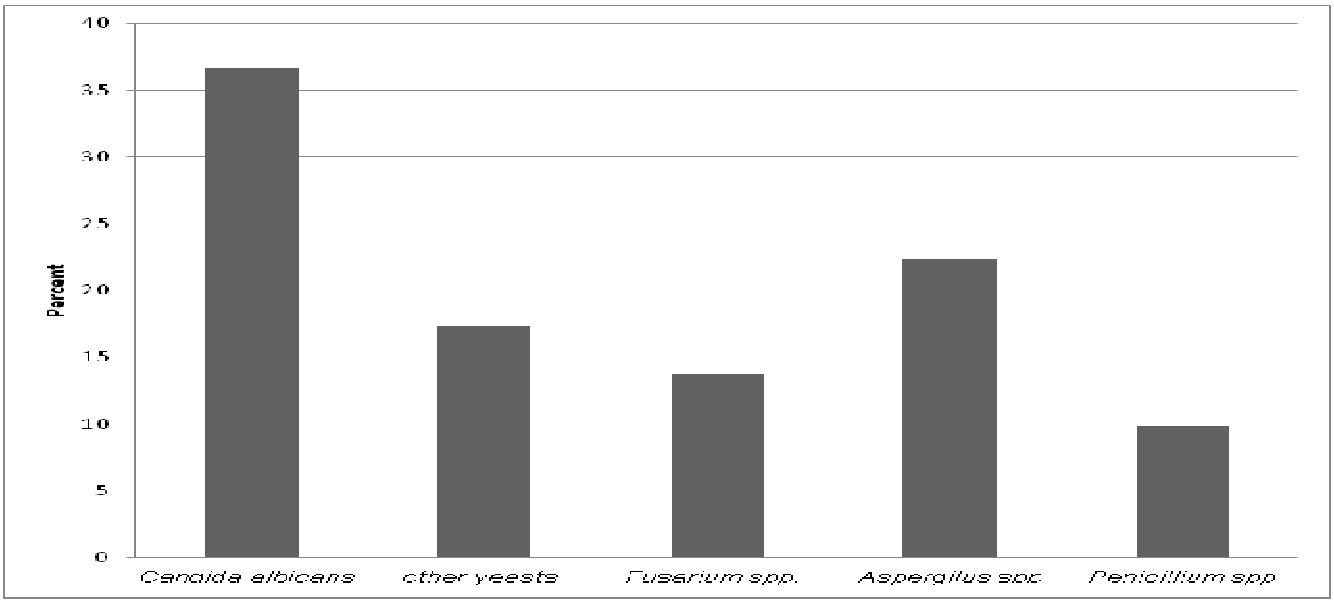

Figure 3. Fungal species isolated from pumice samples.

endocarditis (Washigton et al. 2006). *S. viridans* as one of contaminating bacteria recovered in this investigation is reported by Witt and Hart, (1990), too.

When prosthesis is polished with pumice, contaminated aerosol particles of microorganisms such as Gram negative bacteria and fungi spread all around the laboratory that 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 (Williams et al., 1983; Katberg, 1974). The entry of Gram negative bacteria such as *E. coli*, *Enterobacter* and *Klebsiella* into the blood of patients can cause a fatal infection especially Gram negative septicemia in debilitated patients (Schuoter, 2002). Isolation of Gram negative bacteria in the current study is similar to that obtained by other studies (Vojdani and Zibaei, 2006).

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 (Williams et al. 1986). *Candida albicans* as oral normal flora are isolated from pumice and have shown cross contamination between patients, dentures, and pumice in other surveys (de Resende et al., 2006). New studies have been conducted on viral infection transmission especially HBV and HIV in the dental laboratories. Occupational infection of the dental laboratory technicians with HBV has been reported. The studies suggest that all health care workers who work in dental laboratories should be vaccinated against hepatitis B virus (Al-Dwarai ZN, 2007).Disinfection protocols have been suggested to prevent dental laboratories technicians and patients from infectious diseases (Wakefield, 1980). A previous study in Brazil showed a transfer of microorganisms from patients prosthesis to sterile prosthesis and in the most laboratories pumice was not changed or disinfected between polishing procedures (Agostinho et al., 2004). Jagger et al. (1995) reported that about 6.1% of dental laboratories used disinfectants in the pumice and 92.9% didn't 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 (Witt and Hart, 1990). 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.

In conclusion, polishing pumices is a potential source of infection in dental laboratories such as the wide variety of microorganisms in the blood and saliva of patients. The use of sterile pumice or association of disinfectants with pumice for polishing the prosthesis, sterilization of containers after each use and appropriate ventilation system to reduce infectious aerosols is recommended for prevention of cross contamination.

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