

Frequency of Bacteria and Fungi Isolated from Pumice in Dental Laboratories

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Abstract

Background: This study was conducted to determine the species of oral and non-oral microorganisms in dental laboratory pumice in order to undertake the necessary disinfection control protocols.

Methods: Fourteen active and well-known dental laboratories of Tehran entered our study. Samples of pumice were collected from polishing containers in a completely sterilized and controlled condition. They were immediately sent to Microbiology laboratory and were cultured in specific media in order to identify the aerobic and anaerobic bacteria and also genera of fungi.

Results: Microorganisms recovered from pumice were shown in frequency order as follows: *Acinetobacter lowffi*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Diphtheroids*, *Serratia mercerscens*, *Enterobacter aerogenes*, *Morganella morgani*, *Providencia rettgeri*, *Staphylococcus albidus* and *Streptococcus Sanguis*. Therefore both oral and non-oral bacteria were visible. The isolated fungi were *Aspergillus niger*, *fusarium sp.*, *Aspergillus flavous*, *Cephalosporium sp.* and *penicillium sp.*

Conclusion: This study showed that polishing pumice was contaminated by both oral and non-oral bacteria and fungi. Therefore, the chance of cross-contamination still severely exists, and measures should be conducted to prevent the contamination of technicians, dentists and patients.

Keywords: Dental laboratories, Pumice, Infection control

Introduction

Cross contamination is a severe problem that involves health professionals, especially in dentistry. The transmission of diseases during treatment between patients and dentists, auxiliary personnel and dental laboratory technicians can occur if preventive measures are not undertaken. The risk of cross contamination in dental clinics as well as transmission of microorganisms in prosthetic laboratories has been reported in various studies (1, 2). Although control of infection is important in

all steps of appliance and denture construction, but are of special importance in two stages of impression and prosthesis disinfection. The last step of preparing and finishing of prosthesis or an appliance is polishing, which is usually done by brushes, wheels and pumice. Therefore, pumice as the last step of prosthesis finishing, could be a potential source of cross-contamination among technicians, dentists, patients and also a transmission source for different oral and non-oral infections (3-5).

Pumice microorganisms might spread all around the laboratory as aerosols and splatter during polishing processes and caused

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many risks for those working in that environment (6). The risk of contaminated dentures and appliances for patients seeking for implants or immediate dentures and having new and open wounds is of great importance. Contaminated restorations are dangerous for patients with underlying diseases. For examples, such prostheses in patients with problems such as endocarditis are hazardous (5-7). Those prostheses contaminated by microorganisms such as gram-negative Bacilli or *Entrobacters* could penetrate the infection into oropharyngeal area and increase the risk of pneumonia (3). This may be an especially serious factor in elderly patients, among those who were admitted in hospitals, in patients with immune deficiency problems, in subjects with respiratory diseases and also in dental technicians (7, 8). It was shown that non-oral bacteria were able to remain in pumice slurries for months (9).

Acinetobacter moraxella, *Alcalgines*, *Pseudomonas* SP., *Bacillus* were detected from laboratory pumice (3).

Despite rigorous need for sterilization and disinfection of dental instruments, prosthetic appliances do not receive adequate infection control. Sixty one percent of the dental technicians reported not to use disinfectant in the pumice, and 93% did not disinfect the polishing instruments (10).

The aim of this study was to evaluate bacterial and fungal contaminations of pumice used in the dental laboratories to help the prevention of cross-contamination if the pumice slurries were severely infected.

Materials and Methods

During 2005, used dental laboratory pumice was collected from fourteen well known laboratories in Tehran, capital of Iran. The amount of time the pumice had been in use was undetermined, since none of the personnel in the laboratories could recall with any accuracy the time of its last change. In each laboratory, pumice samples, (several grams)

were taken from ten randomly selected areas of each pumice pan. The samples were placed into sterile containers and immediately transferred to the Microbiology Laboratory for examination. In the laboratory, one gram of pumice aseptically weighted, placed in 9 ml of sterile saline in a small test tube and mixed on a vortex mixer for 30 sec. The tubes placed in a tube rack and left undisturbed for 30 min to allow settling of pumice.

For detection of fungi, one tenth ml of the supernatant fluid was transferred onto plates of yeast extract glucose chloramphenicol agar (YGC Agar). The plates were incubated at 25 °C for a period of two weeks and checked daily. At the end of the 14th day of incubation period, the different types of colonies were subcultured onto fresh YGC agar plates to obtain pure cultures. Pure cultures of the fungi isolates were identified based on their appearance in slide culture. Identification of isolates was confirmed by different mycological references (11).

To recovery of opportunistic and/or pathogenic bacteria, the supernatant was inoculated onto duplicate plates of 5% sheep blood agar (BA), eosin methylene blue (EMB) and manitol salt agar (MSA). Growth on the blood agar plates were used for all bacteria, eosin methylene blue for the recovery of gram negative bacteria and manitol salt agar for detection of *Staphylococci*. All plates were incubated at 37 °C for 48 to 72 h. Morphologically, different colony types were described and subcultured for isolation and purification. The purified colonies were subjected to gram staining and characterized using biochemical tests. Tests included coagulase, catalase, fermentation of manitol salt agar, KOH, hemolysis produced on blood agar and other biochemical tests (12).

Results

The rates of isolated bacterial colony types recovered from dental laboratory pumice collected from fourteen dental laboratories

are indicated in Table 1. Based on this data the most and the least rate belonged to *Acinetobacter lowffi* (19.1%) and *Streptococcus sanguis* (4.3%), respectively. Non-oral bacteria (87.72%) and oral bacteria (12.28%) were the highest and lowest ones, respectively. Isolated oral bacteria were *Diphtheroids* and *Streptococcus sanguis* and other were non-oral ones.

Fungi recovered from used dental pumice samples are shown in Table 2. *Aspergillus niger* (40.8%) encompassed the high rate of isolated fungi.

Table 1: Frequency of isolated bacteria from dental laboratory pumice, Tehran, 2005

Isolated bacteria	n	%
<i>Acinetobacter lowffi</i>	18	19.1
<i>Acillus cereus</i>	15	16.0
<i>Stephylococcus aureus</i>	13	13.8
<i>Pseudomonas aeruginosa</i>	10	10.6
<i>Diphteroid</i>	7	7.5
<i>Serratia marcescens</i>	5	5.3
<i>Entrobacter aerogines</i>	6	6.4
<i>Morganella morganii</i>	5	5.3
<i>Providencia rettgeri</i>	5	5.3
<i>Staphylococcus albidus</i>	6	6.4
<i>Streptococcus sanguis</i>	4	4.3
Total	94	100

Table 2: Frequency of isolated fungi from pumice in dental laboratories, Tehran, 2005

Isolated fungi	n	%
<i>A.niger</i>	40	40.8
<i>Fusarium</i>	22	22.4
<i>Cephalosporium</i>	18	18.4
<i>A.flovous</i>	9	9.2
<i>Penicillium</i>	9	9.2
Total	98	100

Discussion

The results of this study showed that polishing pumice was contaminated by some types of oral and non-oral microorganisms; there-

fore cross-contamination possibility should not be ignored. Most of non-oral bacterial species recovered from dentures by Agostinho et al. were similar to our study (5). So it is important to know that dentures could severely be contaminated after polishing by pumice and pilli wheels (8).

In a study α -hemolytic *Streptococcus*, β -hemolytic *Streptococcus*, *Klebsiella oxytoca*, *Pseudomonas* sp. were recovered from pumice, showing that non-oral types were able to remain in pumice solution for months (9). In another study, special bacteria were located on the polishing wheels. These bacteria were isolated from the nasal cavity and the mouth of patients in waiting room (6).

Public health importance of *Pseudomonas aeruginosa* and *Acinetobacter lowffi* previously has been shown (12-14). It seems that metal particles, such as amalgam or chrome cobalt and other metal particles used in prostheses may make individuals susceptible to *Acinetobacter* infections (15). Although bacteria particularly gram negative bacilli are dangerous for elderly, hospitalized patients with chronic diseases or AIDS and persons with respiratory disorders, but it must be noted that these infections are not only limited to such individuals. For examples, the incidence of acquired pneumonia caused by *Acinetobacter* has been increased in recent years (16,17). The increasing trend of community-acquired *Acinetobacter* diseases alone is sufficient cause, to take steps to reduce the level of bacterial contamination in pumice.

Out of gram negative non-spore forming *Bacilli* in pumice, four microorganisms belong to the family of Enterobacteriaceae (12), *S. marcescens* can cause pneumonia, bacteraemia and endocarditis especially in narcotic addicts and hospitalized patients. *P. rettgeri* can cause infections in urinary tract. These two microorganisms are often resistant to antimicrobial therapy (12). *Bacillus cereus* importance in addition to food toxicity was also shown in endocarditis, osteomyelitis,

pneumonia and ocular infection in individuals with immune deficiency (13).

It has been suggested that pneumonia caused by gram-negative bacilli may be initiated as a result of endogenous aspiration of oropharyngeal flora or the inhalation of bacteria-laden aerosols (16, 18).

Staphylococci are the other contaminating factor of pumice. Among them, *Staphylococcus aureus* can cause infection in man more than the other types. This microorganism is also dispersed in air and dust but is usually transmitted by hands and cause wide range of diseases such as various oral and facial lesions (1, 12).

In the present study, non-oral microorganisms were dominant compared to oral ones. It may be due to nutritional and other biological conditions of these microorganisms. Approximately all non-oral microorganisms found in pumice are available in the environment (water, soil and air). They are considered saprophyte and are able to continue their growth under coprotrophic condition. In contrast, a large amount of oral types, such as sensitive *Streptococci* need complicated factors to grow and survive. Although plenty of them may be available on dentures coated with saliva and other body fluids and tissues, they may lose their ability to live quicker than non-oral ones due to lack of nutrient. The other factor that may be effective in reduction of oral bacteria is antagonism or competition between the oral and non-oral bacteria. So, the temperature, limited nutrient and competition are the factors, dominating the non-oral bacteria to oral ones in pumice solution (14, 19).

Aspergillus, *Fusarium*, *Penicillium* and *Cephalosporium* recovered from our samples may be classified as opportunistic pathogens that rarely produce disease in healthy individuals (11). Of fungi recovered in this study, *A. flavus* and *A. niger* are most frequently involved in human infections. The presence of *A. flavus* is important, because some strains

are prolific producers of aflatoxins which are highly toxic and carcinogenic for some animal species (13, 20).

Increased risk of fungal infections particularly by *Aspergillus* sp., has been associated with certain occupations such as farm workers. Small-dose exposure over a long term may lead to complications such as allergic reactions to mycotoxins (11, 20).

Dental technicians spend a considerable amount of time pumicing and polishing dentures and may be exposed to fungal spores contaminating laboratory pumice. Consequently, dental laboratory personnel should be concerned about the risk of fungal infections resulting from contact or traumatic implantation of fungal elements in the eye and through a broken skin. In addition, for the immediate denture and implant patients, the possibility of implanting fungal materials into an open wound is real if the denture or stent is finished with contaminated pumice. Immediate dentures made for debilitated, institutionalized or other patients who may have an increased susceptibility to infection should be processed with special attention given to minimizing the possibility of contamination of the prostheses during processing (19- 22). Regarding the environment and the type of microorganisms transmitted, pumice solutions may be contaminated through four routes such as old dentures, skin, hands, nose and mouth of a technician, aerosols in the air of laboratory and water. Therefore, to control the infections, these four ways should be considered (10, 15).

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. For further protection of the dental laboratory's staffs and to reduce aerosols, an appropriate ventilation system must be provided in the laboratory. All technicians should be vaccinated against hepatitis. Dis-

posable sterilized pumice should be used for denture or prosthesis polishing and the pumice containers must be cleaned after polishing of each denture (8, 19, 22). It was shown that 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 (19, 21).

In conclusion, dental pumice maybe contaminated with microorganisms, so an infection control system seems to be necessary to prevent contamination of dental personnel, dentists and patients.

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