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# **Evaluation of Presence and Levels of Contamination in Pumice Powder and Slurry Used in Clinical Dental Laboratories**

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**Abstract:** In polishing process of dental prostheses using contaminated Pumice may resulted a harmful cycle of cross-contamination involving dentists, laboratory technicians, patients and auxiliary personnel. The aim of this study was to determine the presence and level of microbial contaminants in pumice powder and pumice slurry used in dental laboratories of Yazd, Iran. Ten samples of pumice powder and slurry were randomly collected from 10 randomly selected dental laboratories of Yazd, cultured on selective fungal and bacterial media in order to quantitatively analyze the total colony-forming units (CFU) and also determine the genus or species of the agents in the samples. Fungi were recovered both from the pumice powder (mean = 1050 cfu gG<sup>1</sup>) and pumice slurry (mean = 6350 CFU gG<sup>1</sup>) and a wide range of bacteria was present in the pumice powder (mean = 450 cfu gG<sup>1</sup>) and pumice slurry (mean = 18700 CFU gG<sup>1</sup>). Statistical paired samples t-test showed a statistically significant difference between bacterial contamination of pumice slurry and pumice powder (p = 0.015), but the difference in the fungal contamination was not significant (p = 0.315). Pumice, especially in the form of slurry, which is used for polishing of dental prostheses is a potential source of bacterial and fungal contamination in dental laboratories and therefore improved techniques are required for disinfecting and controlling possible microbial infections.

Key words: Cross-contamination % dental laboratory % pumice % bacteria % fungi

# INTRODUCTION

Health professionals, especially in dentistry, are involved at high risk of microbial cross contamination. If preventing and controlling measures are not mentioned, the transmission of diseases during treatment between patients and dentists, auxiliary personnel and dental laboratory technicians can be occurred. There are many studies that reported the risk of cross-contamination in dental clinics as well as transmission of microorganisms in prosthetic laboratories [1-2]. Pumice especially pumice slurry is one of the contamination sources of oral and non-oral microbial agents in dental laboratories [3].

In clinics, more than 60% of the prostheses received from laboratories are contaminated with pathogenic microorganisms, such as *Acetinobacter*, *streptococci*, *lactobacilli*, *diphtheroids*, which originated from the oral cavity of other patients [2]. There are reported 10<sup>9</sup> colony forming units of microbial contamination in each gram of pumice slurry after 3 days using for polishing. *Staphylococci*, *Candida* sp. and other yeast were the most commonly isolated agents from pumice slurry [3].

In dental laboratories, lathes and pumice, which widely being used for polishing procedures and finishing of prostheses have been known as the most important sources of contamination [4]. Aerosols produced within polishing procedure of prostheses using contaminated pumice also can cause eye infectious and conjunctivitis of technicians and dentist. Dentures received from implant and immediate denture of peoples, who have fresh ulcers, are very dangerous. Prostheses, which contaminated with gram negative *bacillus* and *Enterobacter*, may cause cross-contamination and especially Oropharyngial and pneumonia infections. Aspiration and inhalation of aerosols contaminated with these bacteria is very dangerous for elderly, hospitalized and immunosupressed patients [5].

Since some prostheses used for polishing in dental laboratories were contacted with patient's mouth, saliva and possibly blood, it is necessary to improve the

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awareness of the dental laboratory technicians and dentists to microbial cross contamination in order to protect them from possible routes of transmission frequently ignored in the past [6]. Little attention has been focused to infection control policy in dental laboratories usually as a result of lack of appropriate training, lack of relevant research, more controlled researches are necessary to determine the potentially dangerous techniques and for assessment of risk factors [6, 7].

The general purpose of the present study was to determine the bacterial and fungal contaminations of pumice powder and pumice slurry using in Yazd dental laboratories to show the role of pumice for cross contamination in dental laboratories.

## MATERIALS AND METHODS

**Sample collection:** Ten pumice powders and ten pumice slurry samples were collected from 10 randomly selected dental laboratories in Yazd, using sterile spatula and sterile containers, ransferred to microbiology laboratory for isolation and identification of likelihood bacterial and fungal microorganisms.

Culture of samples: A suspension of 1 gram each pumice sample in sterile normal saline was prepared in a sterile Petri dish. 10 µL of the suspension solution was then cultured onto Sarbouraud agar (For isolation of fungi) (Oxoid, UK), Blood agar (For isolation of gram positive bacteria) (Merk, Germany), Egg-yolk agar (For isolation of bacillus's) (Oxoid, UK), Manitol salt agar (For isolation of Staphylococcus's) (Liofilchem, Italy) and EMB as well as Mackonkey agar (For isolation of gram negative and Enthrobacteriacea) (Oxoid, UK) plates. The inoculated plates were incubated 48 h at 37°C, the isolated bacterial and fungal colonies were then counted based on colony forming units in each gram of Pumice powder and Pumice slurry. These bacterial and fungal colonies were also identified using macroscopic, microscopic and microbiological analysis to genus and species level.

### RESULTS

**Pumice powder contamination:** Aspergillus, penicillium, cladosporium and Rhizopus were the prevalent fungi isolated from most of pumice powder samples on Sabouraud dextrose agar (mean = 1050 cfu gG<sup>1</sup>). Bacillus subtilis, Bacillus thuringiensis and Staphilococcus epidermisus were the isolated bacterial (mean = 450 cfu gG<sup>1</sup>) in pumice powder samples (Table 1).

|                            | Mean CFU <sup>1</sup> isolated/gram in |                     |
|----------------------------|----------------------------------------|---------------------|
| Isolated organism          | Pumice powder                          | Pumice slurry       |
| Fungi                      |                                        |                     |
| C. albicans                | 0                                      | 1.7×10 <sup>3</sup> |
| Non-albicans Candida       | 0                                      | $1.4 \times 10^2$   |
| Aspergillus                | $1.2 \times 10^{3}$                    | 2×103               |
| Penicillium                | 3×10 <sup>3</sup>                      | 1.5×103             |
| Cladosporium               | 1.1×10 <sup>3</sup>                    | 3×10 <sup>4</sup>   |
| Rhizopus                   | 1×10 <sup>3</sup>                      | 1.5×103             |
| Bacteria                   |                                        |                     |
| Bacillus subtilis          | 1.2×103                                | 6×10 <sup>4</sup>   |
| Bacillus thuringiensis     | 2×10 <sup>3</sup>                      | $1 \times 10^{4}$   |
| B. licheniformis           | 0                                      | $2.2 \times 10^{4}$ |
| B. mycoidus                | 0                                      | 2×103               |
| Staphilococcus epidermidis | 1.3×10 <sup>3</sup>                    | 3×10 <sup>4</sup>   |
| E. coli                    | 0                                      | $1 \times 10^{4}$   |
| Enthrobacter               | 0                                      | 2×103               |
| Aerobacter                 | 0                                      | $1 \times 10^{4}$   |
| Micrococi                  | 0                                      | $4 \times 10^{4}$   |
| Corynebacterium            | 0                                      | 1×103               |

Table 1: The microbial contamination agents isolated from pumice samples

<sup>1</sup>Colony forming unit per each gram

**Pumice slurry contamination:** *C. albicans*, non-*albicans Candida* species, *Aspergillus*, *Penicillium*, *Cladosporium* and *Rhizopus* were the most isolated fungi from pumice slurry (mean = 6350 cfu gG<sup>1</sup>). In pumice slurry *E. coli*, *Enthrobacter*, *Aerobacter*, *Micrococi*, *Staphylococcus epidermis*, *Corynebacterium*, *Bacillus thurinyiensis*, *Bacillus subtilis and B. licheniformis* were also isolated on EYA and Blood agar (Table 1).

Contamination of pumice slurry was much higher than pumice powder (mean = 18700 cfu gG<sup>1</sup>), especially with oral opportunistic yeast, *Candida albicans*. There was seen a statistically significant differences between the complete CFU of microbial contamination in pumice slurry and pumice powders (p = 0.05).

## DISCUSSION

Dental laboratory technicians are particularly exposed to oral and non-oral microbial crosscontamination from dentures, pumice powder and particularly pumice slurry, which are used for polishing of dental prostheses [8].

The results of Pumice powder and slurry culture conducted in present study revealed massive bacterial and fungal contamination particularly in pumice slurry. There wasn't seen any pathogenic fungi and bacteria in pumice and usually the saprophyte and opportunistic fungi and bacteria were isolated that could be harmful for immunocompromised and elderly debilitating people. *C. albicans* is an oral opportunistic fungus [9] that isolated from pumice slurry showed cross contamination between patients' denture and pumice. *Bacillus licheniformis, E. coli* and *Staphylococcus epidermisus* were isolated in present study the same as Witt and Hart study however they also reported the isolation of pathogenic bacteria such as *Pseudomonas, B. cereus, Streptococcus viridans, Staphylococcus areus* and *Nisseria* species that weren't seen in current study [10].

The process of polishing using Pumice and high-speed lathes can transmit bacteria to the laboratory technician, dental clinicians and patient. Polishing lathes and brushes are considered to be a source of contamination in prostheses laboratories. It is necessary to perform the effective infection control measures to reduce the cross contamination of oral and non-oral microorganisms in polishing procedure of dentures. This research determined the colony forming units (CFU) of microorganisms in pumice powder and slurry, which used for polishing of prostheses in Yazd dental laboratories.

There is possibility of transferring microorganisms from patient prostheses to sterile prostheses in most prosthesis laboratories where, pumice and polishing cones are not changed or disinfected regularly between procedures on different prostheses [1-10]. In a study, Kahn *et al.* [11] reported a mean transfer of  $5.0 \times 10^5$  CFU mlG<sup>1</sup> bacteria of patients' dentures to sterile dentures, particularly the pathogenic microorganisms such as Staphylococcus aureus, Escherichia coli, Candida albicans and "-hemolytic streptococci. Since the denture users are usually elderly people with lowered resistance to immunological infections: crosscontamination caused increasing risk for development of infections in them. However in current study mostly the non-oral opportunistic and saprophyte bacteria and fungi (except C. albicans) were isolated, these bacteria and fungi could be dangerous for immunocompromised and elderly people.

There is possibility of microorganisms transmission to the dental professional laboratory by aerosol contamination produced during the polishing process with contaminated Pumice. Oral microorganisms such as *Streptococcus mutants* and non-oral potentially pathogenic microorganisms such as yeast and Gramnegative bacteria were found in aerosol and Splatter, which can cause eye and respiratory infections [12]. The *Enterobacter* isolated from cultivated pumice slurry is one of the acquired pneumonia agents in debilitated, Immunosuppressed, alcoholism and drug users [13, 14]. There are reports for isolating of *Acinetobacter*, *Micrococcus*, *Pseudomonas*, *Moraxella* and *Alcaligenes* from pumice in commercial laboratories, which can cause serious infections if transferred to patients, whose dentures were polished, as well as the technician by exposure to contaminated aerosol [5, 15], whereas mostly the non-oral bacterial of soil, water and air flora were isolated in this study.

In spite of the fact that the elimination of all contamination sources is not possible in the dental laboratories, dental laboratory personnel should have awareness to the microbial contamination of pumice. It is necessary to have a series of prevention and controlling strategies to decrease these levels of contamination. Disinfection of dentures before sending them to the laboratory and also before returning to the dental clinic [16-17] using sterile pumice and brushes or the association of disinfectants with pumice for polishing, using barriers during polishing are important alternatives to significantly reduce cross-contamination in the dental laboratory [18].

## CONCLUSIONS

In conclusion, pumice especially pumice slurry are massively contaminated with microorganisms and can serve as the primary source of the microbial cross infection cycle in dental laboratories. Disinfection of dentures b efore polishing, using disposable gloves, associating of disinfectants with pumice, disinfection of polishing cones and prevention of aerosols production can control cross contamination in dental laboratories.

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