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# DENTAL PUMICE AS A SOURCE OF CROSS CONTAMINATION IN LABORATORIES: A MICROBIOLOGICAL STUDY.

Dental Science

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## ABSTRACT

**Aim and objective:** This study aimed to determine the presence and level of microbial contaminants in pumice powder and slurry used in Dental laboratories.

**Materials and Methods:** Ten active private Dental laboratories of Vellore entered in the 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 type of Microorganisms present.

**Results:** Results of this study showed, 100% contamination rate for pumice powders and 100% for pumice slurries.
Frequencies of Gram positive and Gram-negative Bacteria isolated from the powders were 60% and 39% respectively.
Frequencies of Gram positive and Gram-negative Bacteria isolated from the slurry were 64% and 34% respectively.
This study showed that polishing pumice in the form of powder or slurry were contaminated with different oral and non oral Bacteria and also Fungi.

**Conclusion:** 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.

**Clinical Significance:** The risk of cross-infection in Dental clinics and laboratories using contaminated pumice in polishing process of Dental prostheses may result in a non-safe environment for Research and Medical care.

## KEYWORDS:

Bacteria; Contamination; Dental laboratory; Pumice.

## INTRODUCTION:

The spread of infection through aerosol had long been considered. “Aerosol” and “Splatter are two different terms. Aerosols are defined as particles less than 50 micrometers in diameter. Splatter is defined as airborne particles larger than 50 µm in diameter[1].

Aerosols produced within sources of contamination. Aerosols produced within polishing procedure of prostheses using contaminated pumice also can cause eye infectious, conjunctivitis and other diseases to Technicians and Dentist.

Health professionals, especially in Dentistry, are involved at high risk of Microbial cross contamination. If preventing and controlling measures are not mentioned, transmission of diseases during treatment between patients and Dentists, Auxiliary personnel and Dental laboratory Technicians can occur [2].

There are many studies that had reported the risk of cross-contamination in Dental clinics as well as transmission of Microorganisms in Prosthetic laboratories [3].

Dental patients and Dental health care personals (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 (Rossand 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).[4]

In Dental laboratories, pumice is used for polishing of prostheses. The pumice as the last step of prosthesis finishing has been reported 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 remains 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 are 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 risk of Pneumonia (Williams et al., 1985; Vojdani and Zibaei, 2006). [4]

Dentures received from implant and immediate dentures of people, who have fresh ulcers, are very dangerous. Prostheses, which are contaminated with gram negative bacillus and Enterobacter, may cause cross-contamination and especially Oropharyngeal and Pneumonia infections [5].

Since some prostheses used for polishing in Dental laboratories are contacted with patient's mouth, saliva and possibly blood. It is necessary to improve the awareness of the Dental laboratory technicians and Dentists to microbial cross contamination in order to protect them from possible routes of transmission which were frequently ignored in the past [6].

The aim of this research was to determine the bacterial and fungal contamination present in pumice powder and slurry used in Dental laboratories and to evaluate the role of pumice in the cross contamination of Dental laboratories.

## MATERIALS AND METHODS:

### Survey area

The study area were Dental laboratories of Vellore; a district of Tamilnadu state of India included both rural and urban areas.

### Sample collection

This study was conducted during June 2016 to July 2016 in ten randomly selected Dental laboratories of Vellore.

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), sterile spatula and a10-ml syringe from ten Dental laboratories.

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. The samples of pumice powder and pumice slurry (after shaking the slurry) were transferred to the container by a sterile syringe and sealed. The samples were immediately transported to the

Microbiology laboratory for isolation and identification of likelihood Bacterial and Fungal Microorganisms.

**Microbial Processing:**

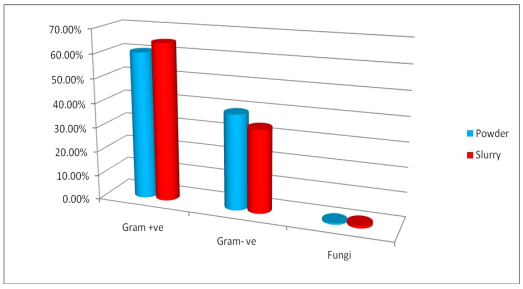
In the laboratory 1 Gram of pumice was aseptically weighed and a suspension in 9 ml sterile normal saline was prepared in a small test tube. The tubes were mixed well for 30 sec. Following that 1 ml of the suspension was cultured on Blood agar (-HIMEDIA Laboratories Pvt. Ltd, India.) with 5% defibrinated sheep blood cell for all bacteria and standard microbiology methods (biochemical tests) for identification of Gram positive Bacteria, on McConkey agar (SISCO research Laboratories Pvt. Ltd, India) for isolation of Gram negative Bacteria, on Manitol salt agar (HIMEDIA Laboratories Pvt. Ltd, India) for isolation of Staphylococcus aureus and on Sabouroud dextrose agar (HIMEDIA, Laboratories Pvt. Ltd, India) for detection of fungi. 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[13] (2006).

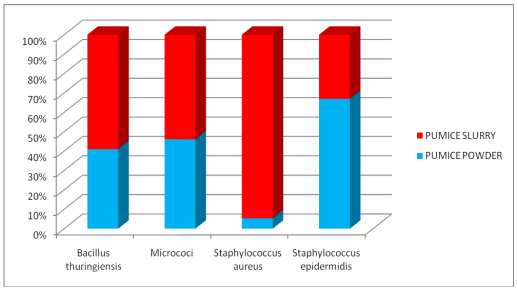
One milliliter of the prepared solution was added to the blood Agar-Agar (-HIMEDIA Laboratories Pvt. Ltd, India.), 45˚C, and mixed with gentle rotation placed at 37˚C for 24 hours, then counted based on colony forming units (CFU).In order to identify the bacteria causing contamination, the samples were cultured in the plates which contained agar, Trypticase Soy Agar and Eosin Methylene Blue (EMB) Agar for 24 hours at 37˚C. Then, the colonies were inoculated again and identified by the differential media. For initial identification of the bacteria, the lawns 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 were 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 and the descriptive results were presented in Table 1 and Graph 1,2,3,4.

**TABLE 1. INDICATES THE ISOLATED MICROORGANISM.**

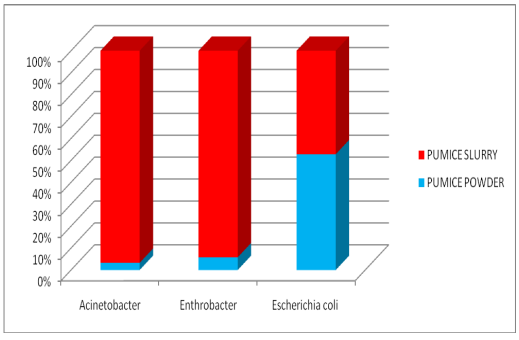
| Mean CFU isolated/gram in | | | | |
|---|---|---|---|---|
| **ISOLATED ORGANISM** | **PUMICE POWDER** | **PERCE NTAGE** | **PUMICE SLURRY** | **PERCE NTAGE** |
| **Bacteria +ve** | | | | |
| | **60.4%** | | **64.7%** | |
| *Bacillus licheniformis* | **0** | **0 %** | **$2.2\times10^4$** | **1.03 %** |
| *Bacillus mycoidus* | **0** | **0 %** | **$2\times10^3$** | **0.09%** |
| *Bacillus subtilis* | **$1.2\times10^3$** | **0.19%** | **$6\times10^4$** | **2.81%** |
| *Bacillus thuringiensis* | **$2\times10^3$** | **0.32%** | **$1\times10^4$** | **0.46%** |
| *Corynebacterium* | **0** | **0%** | **$1\times10^3$** | **0.046 %** |
| *Diphtheroids* | **0** | **0%** | **$4\times10^5$** | **18.73 %** |
| *Micrococi* | **$1\times10^4$** | **1.60%** | **$4\times10^4$** | **1.87%** |
| *Staphylococcus aureus* | **$1\times10^4$** | **1.60%** | **$6\times10^5$** | **28.10 %** |
| *Staphylococcus epidermidis* | **$6\times10^5$** | **94.90 %** | **$10\times10^5$** | **46.83 %** |
| **Bacteria –ve** | | | | |
| | **39%** | | **34%** | |
| *Acinetobacter* | **$1\times10^3$** | **0.24 %** | **$8\times10^5$** | **7.11%** |
| *Enthrobacter* | **$1\times10^3$** | **0.24%** | **$0.44\times10^5$** | **3.91%** |
| *Escherichia coli* | **$4\times10^5$** | **99.50 %** | **$10\times10^5$** | **88.96%** |
| **Fungi** | | | | |
| | **0.61%** | | **1.1%** | |
| *Aspergillus* | **$1.2\times10^3$** | **19.04 %** | **$2\times10^3$** | **5.44%** |
| *Candida albicans* | **0** | **0 %** | **$1.7\times10^3$** | **4.63 %** |
| *Cladosporium* | **$1.1\times10^3$** | **17.46 %** | **$3\times10^4$** | **81.74 %** |
| *Penicillium* | **$3\times10^3$** | **47.61 %** | **$1.5\times10^3$** | **4.08 %** |
| *Rhizopus* | **$1\times10^3$** | **15.87 %** | **$1.5\times10^3$** | **4.08 %** |



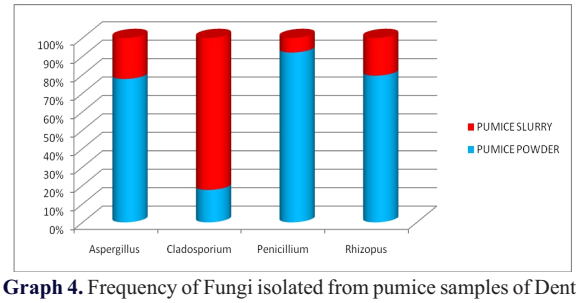

**Graph 1.** Frequency of Gram Positive and negative bacteria isolated from pumice samples of Dental laboratories.




**Graph 2.** Frequency of Gram Positive bacteria isolated from pumice samples of Dental laboratories.




**Graph 3.** Frequency of Gram negative bacteria isolated from pumice samples of Dental laboratories.




**Graph 4.** Frequency of Fungi isolated from pumice samples of Dental laboratories.

**RESULTS:**

All the Ten Dental laboratories were contaminated for Microorganisms. These microbial contaminations were detected in the pumice powder and pumice slurry in Dental laboratories.

Gram-positive bacteria, including Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium, Micrococi, Bacillus sp, Diphtheroids.

Gram-negative bacteria, including Acinetobacter, Enterobacter, Escherichia coli.

Fungi including Aspergillus, Candida albicans, Cladosporium,, Penicillium. Rhizopus,

100 percent of pumice powder samples and pumice slurry were contaminated.

The highest and lowest colony-forming units (CFU) of gram positive in the pumice powder were Staphylococcus epidermidis 95 % (6 × 105) and Bacillus subtilis 0.2%(1.2×103) respectively. Table 1.

The highest rate of isolated colony-forming units (CFU) of gram negative in the pumice powder were 99.50 %( 4 × 105) Escherichia coli and lowest one was 0.24 %(1 × 103) Acinetobacter and Enterobacter respectively. Table 1.

The highest rate of isolated colony-forming units (CFU) of fungi in the pumice powder were 47.61 %( 3×103) Penicillium and lowest one was 16 % (1×103) Rhizopus.

The highest colony-forming units (CFU) of gram positive in the pumice slurry were Staphylococcus epidermidis 47 % (10 × 105) and lowest one was 0.046 % (1×103) Corynebacterium.

The highest rate of isolated colony-forming units (CFU) of gram negative in the pumice slurry were 89 % ( 10 × 105) Escherichia coli and lowest one was 4 % ( 0.44 × 105) Enterobacter.

The highest rate of isolated colony-forming units (CFU) of fungi in the pumice slurry were 81.74 % (3×103) Cladosporium and lowest one was 4.08 % (1.5×103) Penicillium and Rhizopus respectively.

**DISCUSSION:**

When Dental 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.

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.

The hazard of infection transmission via pumice powder and slurry, their Bacterial and Fungal contamination was investigated in Dental laboratories in Vellore, and the following Bacteria were isolated: Gram-positive Bacteria, including Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium, Micrococi, Bacillus sp, Diphtheroids.

Gram-negative bacteria, including Acinetobacter, Enterobacter, Escherichia coli.

In the present study it was found that pumice powder and pumice slurry were equal with regard to CFU, but contamination was 64.7% in pumice slurry and 60.4% in pumice powder.

Jafari et al., [7] 2006 had reported that bacterial contamination was higher in pumice slurry compared to pumice powder, thus in both studies pumice slurry showed relatively more contamination than pumice powder.

Agostinhoet et al., [8] 2004 had reported that Pumice used for polishing of prosthesis as the last step, could be a potential source of contamination to the Dental staff.

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 the present study were non pathogenic in healthy people, but some of them like S.aureus and Streptococcus viridians could be harmful for immunocompromised and elderly patients as well as healthy people.

S. viridansis 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.

Washington et al., [9] 2006 had reported that Viridans streptococci cause 30-40% of cases of subacute bacterial endocarditis In a microbiologic study by Williams et al., [14] 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 Katberg et al., [10] 1974 had reported E. coli and hemolytic and non-hemolytic Staphylococcus, alpha and beta Enterobacter in the pumice samples.

In the other study, Witt et al., [11], 1990 had isolated non-oral bacteria such as E. coli, Bacillus and Staphylococcus epidermidis from pumice samples.

In the study of pumice samples by Jafari et al.,[7] 2006, non-oral bacteria such as Bacillus, Staphylococcus epidermidis, E. coli and Enterobacter, as well as oral bacteria were reported.

Williams et al., [14] 1986; Katberg et al., [10] 1974 had reported isolation of Gram negative bacteria like Pseudomonas, Moraxella and Acinetobacter from pumice which are transferred to patients and Dental laboratory staff by contaminated aerosols, and cause ocular and respiratory infection especially in persons with chronic respiratory disorders.

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 et al., [12] 2002).Isolation of Gram negative bacteria in the current study is similar to that obtained by other studies (Vojdani M, Zibaei M,13 (2006).

Fungi recovered from used pumice samples in the current study included Aspergillus, Penicillium and Candida that increased risk of fungal infection especially in persons who work for a long period of time in Dental laboratories and had been exposed to fungal spores (Williams et al., [14] 1986). Candida albicans as oral normal flora are isolated from pumice and had shown cross contamination between patients, dentures, and pumice in other surveys (de Resende et al., [15] 2006).

The other bacterial species is E. coli that may lead to food poisoning and intestinal problems. These bacteria may transfer via denture and cause infections.

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 had been reported. The studies suggest that all health care workers who work in Dental laboratories should be vaccinated against Hepatitis virus (Al-Dwarai ZN., [16] 2007).Disinfection protocols had been suggested to prevent Dental laboratories technicians and patients from infectious diseases (Wakefield., [17]1980). A previous study in Brazil showed that a transfer of microorganisms from patient's prosthesis to sterile prosthesis and in the most laboratories pumice was not changed or disinfected between polishing procedures (Agostinhoet et al., [8] 2004).

Jagger et al., [18] 1995 reported that about 6.1% of Dental laboratories used disinfectants in the pumice and 92.9% didn't disinfect the polishing instrument.

Witt and Hart 11, 1990 had proven that pumice slurry freshly made up using disinfectants was reported to be free from most contaminations.
In the present study most of the laboratories did not use 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.

**METHODS OF REDUCING AIRBORNE CONTAMINATION (RECOMMENDATIONS):**

Provision of good ventilation with its diluting effect on the airborne microbial load. Reduction in the emission of bacterial Dental aerosols which can be attained by:

a. Flushing of water from ultrasonic scalar device and turbine hand pieces for 5-10 Minutes in the beginning of the day and for 2 minutes before treatment.

b. Use of Pre-Procedural antiseptic mouth rinse (0.2% Chlorhexdine, Povidone Iodine).

c. Use of aerosol reduction devices (High volume suction apparatus).
d. Purification of airborne microbial pollutants (Disinfection with physical and chemical means)

e. Rubber dam isolation while using turbine hand pieces.

f. Minimizing biofilm formation in Dental unit waterlines

I. Use of sterile water or sterile saline.

II. Drain and flush water for several minutes before beginning clinic each day.

g. Perform periodic chemical treatment as recommended by manufacturers.

h. Other precautions like use of facemask and face shield. Masks should have at least 95% filtration efficiency for particles 3.0-5.0µm in diameter. It should be changed for each patient. Change of mask after

20 minutes in aerosol or 60 minutes in non aerosol environments are recommended.

h. Other precautions for Eye Protection; Protective eye wear or face shield must be worn while treating patients

## SUMMARY AND CONCLUSION:

Cross contamination is prevalent at every procedure involving patients and laboratories. The risk of infection from patients to the professional and vice versa cannot be ruled out. This study was done to estimate contamination in the pumice powder and slurry, which can act as a source of cross infection in the laboratories.

In accordance with international recommendations and in the light of the present study, all appliances including impressions should be thoroughly cleaned of blood, saliva and debris in 1% Hypochlorite solution before delivery to and from the laboratory. The preparation of pumice slurry should be made up with Sodium hypochlorite and changed daily. Brushes for denture polishing should also be treated with sodium hypochlorite. The hydro flask water should be changed and disinfected daily. Laboratories should change to using small size hydro flasks, and the water changed with each use. These recommendations will not only eliminate cross contamination between the Dental clinic and the Dental laboratory or vice versa but will also go some way reducing it and making the environment for the Dental team and patient safer one.

Awareness about the dangers of aerosol and its hazards should be spread, not only to Dental health care workers but also to patients and Dental students. Specific measures should be taken to control and decrease aerosol production. Also, adequate steps should be taken to prevent cross infection between Dental health care workers and patients in the Dental environment.

The results emphasize the importance of strict infection control during Dental polishing procedures.

**ETHICAL APPROVAL**: Not required.

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