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# Microbial contamination of a University dental clinic in Brazil

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## Abstract

Pathogens of the oral cavity of a patient can be transferred to the dental office surfaces by direct contact, aerosol instruments and blood or saliva. The objective of this study was to investigate the microbiological contamination presents in the stands, chairs and spittoons in the University Nilton Lins dental clinics, in Manaus, Amazonas. Samples were collected with sterile swabs and seeded in different microbiological culture media for the isolation of microorganisms collected from each room. Then, assays were carried out for identification of strains isolated from each environment, such as: Gram stain, DNA purification, Amplification of 16s rRNA genes and sequencing. All these experiments were performed in the LBS / ILMD / FIOCRUZ. It was found 40 CFU / mL in the stands, 43 on the chairs and 47 in the spittoons and it was also possible to identify microorganisms like *Klebsiella pneumoniae*, *Shigella sonnei* and *Staphylococcus aureus*. The greatest number of CFUs was found in Clinic 3 and it was observed that the spittoon was the dental surface with the highest number of CFUs. Some of the bacterial species isolated are opportunists, suggesting that more severe biosecurity measures must be taken in order to prevent cross-infection.

**Keywords:** students, dental; biosecurity; exposure to biological agents.

## Introduction

Oral microbiota are a potentially significant source of contamination and cross-infection in the dental clinic[1]. The oral cavity is a natural habitat for a large number of microorganisms which, during dental practice, can be transferred to the equipment and instruments used in routine clinical activities of the dentist, posing a risk of cross contamination and infection and may even cause systemic infections[2].

Dental health care students and professionals are at a risk of diseases including HBV, HCV, herpes simplex virus type 1, HIV, influenza, rubella, and besides that, fixed dental units, water lines and handpieces can become vehicles of cross-infection in dental offices[3-5].

The main bacteria that can cause infection hazards linked to dental practice are *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Legionella pneumophila* and *Pseudomonas aeruginosa*[6,7]. This

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aspect is critical in the dental field, where there is constant daily exposure to a wide variety of microorganisms of the oral microflora of patients[8]. Even in non-invasive procedures[4], such as X-rays, potentially pathogenic fungal species are found in X-ray apparatus, which can serve as reservoirs or fungal vectors representing a risk of acquisition of cross-infection for the patient, as well as for the dental team[5].

During a dental appointment, there is dispersion of splashes and aerosols containing pathogenic microorganisms that can be transmitted through saliva, blood and oral secretions onto countertops and materials, furniture and the dental unit itself[6]. Aerosols can be a source of infection for dentists and an indirect cause of occupational hazards at work[7].

To avoid infection in the oral cavity, cleaning methods or disinfection and sterilization of the dental material to be used in the patient are recommended[8]. The professional must follow standard procedures such as risk evaluation and patient protection, personal protection, sterilization and chemical disinfection, sterilization of equipment, appropriate waste disposal, among others[9].

The aim of this study was to investigate the microbiological contamination presents in the stands, chairs and spittoons in the University Nilton Lins dental clinics, in Manaus, Amazonas, through the use of PCR 16s rRNA.

## Materials and methods

### Study Model

Samples (a total of 9 chairs, 9 benches and 9 spitters) were collected in dental clinics of the University Nilton Lins and the experiments were performed in the Biodiversity Laboratory of the Health Institute Leonidas and Maria Deane of the Oswaldo Cruz Foundation (LBS / ILMD / FIOCRUZ).

### Study Population

Three chairs, 3 benches and 3 spitters were collected from each of the three dental clinics.

Inclusion criteria: chairs, benches and spitters in perfect condition, used daily by the students.

Exclusion criterion: chairs with some defect, making it impossible for students to use it.

### Sampling

The study was conducted in dental clinics of the Nilton Lins University, where samples were collected in triplicate from the stands, chairs and spittoons with sterile swabs. The sample collection was carried out in different areas in order to cover the entire clinic. Prior to this, the surfaces were disinfected with 70% alcohol. All handling was performed using sterile gloves.

### Bacteriological Assays

Samples were immediately inoculated in assay tubes containing 3 mL of brain heart infusion (BHI - Difco) and incubated at 37ºC for 24 hours.

After incubation (24 hours / 37˚C), the tubes that showed turbidity of the culture medium had the material placed in Petri dishes containing MacConkey agar, PIA agar, BHI agar, Mueller-Hinton agar, Sabouraud agar culture media. The plates were incubated (24 hours / 37˚C)[8].

Plates were then analyzed by counting the number of grown colonies, their size, hemolysis and staining. Following this, Gram staining of each selected strain was performed.

### DNA Purification

Each isolated strain was grown in Luria Bertani liquid (24 hours / 37˚C) with stirring. The culture was transferred to microtubes and centrifuged (16000 g / 10 min / 4˚C). The supernatant was discarded and the pellet resuspended in 300 uL (microliter) of buffer and homogenized by vortexing. After adding 30 uL of lysozyme the samples were incubated (30 min / room temperature). They were then added to samples of 50 uL of triton X-100 10% 20 uL of 3 M NaCl. Following this they were heated in a dry bath (5 min / 60˚C) and 2 uL RNA were added, and then incubated in an oven (15 min / 37˚C). 25 uL of 10% SDS were added to the samples and homogenized by vortexing, adding 3 uL of proteinase and then incubated (15 min / 37˚C). Thereafter, 500 uL of phenol was added and the mixture was stirred manually for 5 minutes. The sample was centrifuged (16000 g / 10 min) at room temperature. The supernatant was removed, recovering the aqueous phase and replacing the microtube by adding 400 uL of chloroform, followed by manual shaking for 5 minutes. Once again the samples were centrifuged (16000 g / 10 min) at room temperature and the supernatant was discarded. Then the microtube was replaced and 25 uL of 3M NaCl was added. Finally, there was the careful addition of 1 mL of 100% ethanol at 20˚C. The samples were centrifuged (16000 g / 10 min / 4˚C), resuspended in 70% ethanol and homogenized by vortexing, centrifuging again under the same conditions, discarding the supernatant carefully. The DNA microtube was dried under flow and resuspended in 200uL of Buffer (10mM Tris-HCl, pH 7.5 + 1 mM EDTA), and maintained at -20˚C. The extraction protocol was performed as described in literature, however with modifications[10,11].

### Amplification of 16s rRNA genes and sequencing

The reaction was performed under the following conditions: 40 ng of DNA, 10X0.25 mM buffer of dNTPs, 2.5 mM of MgCl, 5 mM of each primer, 2.5 U Taq polymerase and sterile deionised water to a total volume of 25 uL. The primers used were: 530F and 1492R of the 16s rRNA gene. The Polymerase Chain Reaction (PCR) program used in a thermocycler (Eppendorf) comprised the following steps: initial denaturation (5 min / 95˚C); followed by a 45 times cycle with a denaturing step (15 sec / 95˚C), annealing of primers (20 sec / 65˚C) and an extension step of the tapes (2 min / 72˚C); in addition to the final extension (2 min / 72˚C). It was used agarose gel electrophoresis to visualize the PCR products and observe the effects the different annealing temperatures had on the PCR reaction[12].

The samples were then sequenced by the Fiocruz / ILMD platform, using the automatic sequencer ABI PRISM 3100 Genetic Analyzer ™ (Applied Biosystems) (Figure 1).

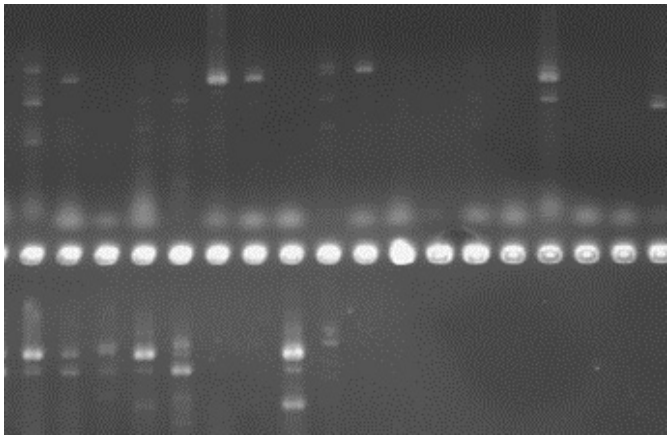

**Fig. 1 -** Electrophoretic profile of genes in multiplex PCR found in the samples in 1.5% agarose gel.

## Results

The sequences obtained were processed for removal of poor quality sequences using the "Phred / Phrap" program. Each sequence was compared to sequences deposited in the "Genebank" of National Center for Biotechnology Information and with sequences deposited in the Ribosomal Database Project.

A total of 27 samples distributed in 3 bench samples, 3 chair samples and 3 spittoon samples for each clinic were analyzed. Bacteriological analysis of samples taken showed that 27 (100%) samples had microbial growth.

The results obtained in this study showed variations in total CFU / mL between clinics and dental units (Figures 2 and 3).

Figure 2 shows the total number of CFUs found in each university dental clinic. Thus, the greatest number of CFUs was found in Clinic 3, indicating that the decontamination process was less efficient in relation to the others.

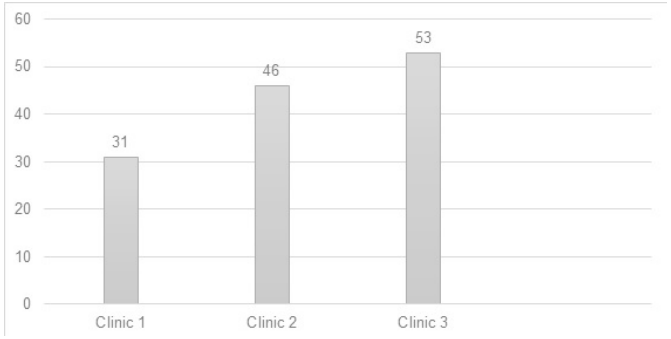


**Fig. 2 -** Correlation CFU / mL between the clinics.

Figure 3 shows the total number of CFUs found in each type of surface of the dental equipment, and it was observed that the spittoon was the dental surface with the highest number of CFUs, due to the surface having direct contact with the secretions produced by the patient.

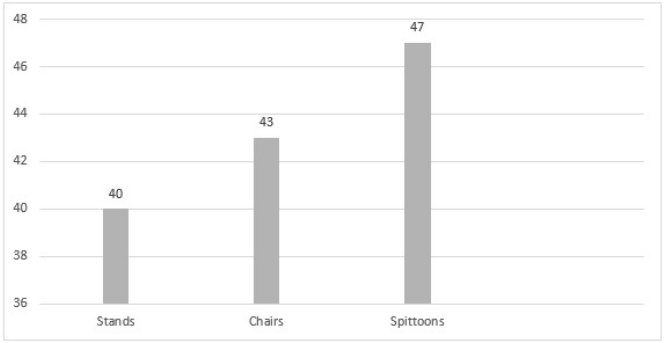


**Fig. 3 -** Correlation CFU / mL between the sites collected.

The microorganisms found on the surfaces of the dental clinics of the university are listed in Table 1 and it was possible to identify 16 bacterial species and 3 genera.

**Table 1 -** Correlation between clinics, places and species / bacterial genus found.

| | | |
|---|---|---|
| Clinic 1 | Chair | Bacillus Subtilis, Exiguobacterium sp |
| | Stand | Rhizobium sp |
| | Spittoon | Bacillus pumilus, Bacillus cereus |
| Clinic 2 | Chair | Klebsiella pneumoniae, |
| | Stand | Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus |
| | Spittoon | Staphylococcus warneri, Enterobacter cancerogenus, |
| Clinic 3 | Chair | Staphylococcus saprophyticus, Bacillus megaterium, Bacillus safensis, Acinetobacter sp |
| | Stand | Shigella sonnei |
| | Spittoon | Staphylococcus pasteuri, Enterobacter amnigenus, Citrobacter freundii, Escherichia coli |

## Discussion

The highest concentration of microorganisms in the dental office is found in the patient's mouth[13]. The hands of dental professionals, once contaminated with saliva, sulcular fluid and / or blood, are the major vehicles for contamination of surfaces[14]. In this context, the microbial contamination of dental clinics, such as Dental Unit Water Systems it was already found P. aeruginosa and Legionella spp. 7* So, the use of barriers, such as cellophane paper or plastics, are recommended and do not interfere in clinical procedures[15].

In multidisciplinary clinics, as seen in dentistry courses, biosecurity measures for infection control play a major role in daily care. Staphylococcus aureus is found in human skin, especially nares and perineum and it has been linked to different types of infection, including pneumonia, meningitis, osteomyelitis, infections of the skin and soft tissue[16].

The Bacillus genus comprises about 50 species of chain forming Gram-positive bacilli which are also capable of forming spores. Bacteria which are usually found in the Bacillus subtilis environment are used as biological indicators for testing the

sterilization effectiveness of autoclaves[17].

Escherichia coli is a gram-negative bacillus mobile belonging to the Enterobacteriaceae family. All Enterobacteriaceae are potentially pathogenic and responsible for various types of infections such as diarrheal diseases, urinary tract infections and sepsis[18].

Acinetobacter spp. are non-fermentative bacteria, and potentially pathogenic environmental contaminants[19].

Shigella sonnei is a bacterium that is an important agent of diarrheal infectious diseases that usually affects children, the elderly and immunocompromised patients. Shigella ssp. has the ability to invade human intestinal mucosa and cause dysentery, spreading efficiently through a low dose of fecal-oral transmission[20].

Klebsiella pneumoniae is a gram-negative bacterium commonly found in the skin and saliva that plays an important role as a resistance mechanism in the general hospital setting and confers resistance to carbapenem antibiotics, and inactivates penicillins, cephalosporins and monobactams[21].

Professionals have to be aware of the inherent risk in their work, and be trained and encouraged to apply biosecurity procedures in order to reduce or eliminate these microorganisms[5].

## Conclusions

Given the results of this study and published reports, it is clear that the contamination of surfaces of dental units exists, and thus it is a matter of great importance that should be discussed among professionals and students of dentistry to search for more effective ways to prevent cross-infection. In order to decrease the risks it is necessary to establish a biosafety protocol in dental clinics which includes the correct preparation procedures of dental units, use of IPE (Individual Protection Equipment) and adequate disinfection of surfaces.

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