Microbiological contamination of composite resins used in a Dental school clinic

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ABSTRACT

The aim of this study was to verify the microbiological contamination in composite resins used at one Dental school clinic. This is an experimental/laboratory study, in which samples of 10 tubes of composite resin were collected, one of which was the negative control. Portions of composite resin inside the tubes were collected and dipped in test tubes containing nutrient broth for subsequent seeding on to plates and staining for the characterization of bacterial and fungal colonies. All samples revealed contamination, including the tube containing culture medium used as control for handling the experiment. These contaminations may be related to failures in biosafety measures employed in the Dental school clinic and to the transport and storage methods for the tubes of composite resin. Therefore, it is necessary to raise awareness among students and teachers to adopt specific biosafety measures for the handling of composite resins.

Descriptors: Containment of Biohazards. Composite Resins. Dental Clinics. Biological Contamination.

1 INTRODUCTION

The risk of transmitting infectious diseases is inherent to the practice of dentistry[1]. By the end of 2019, the possibility of becoming infected by the viruses Hepatitis B and C, and Acquired Immune Deficiency (HIV) was determining biosafety strategies in dental clinics and offices[2]. On March 11, 2020, the World Health

Organization declared a pandemic due to COVID-19, caused by the new Coronavirus, designated as SARS-CoV-2[3]. The understanding of the evolution of the disease showed Dentistry to be the profession with the highest risk of contagion. On March 15, 2020, the New York Times published the article "The workers who face the greatest coronavirus risk", in which it explains that dental surgeons are the workers most exposed to the risk of infection by SARS-CoV-2, much more so than professionals in the areas of Nursing and Medicine[4]. This new work landscape has led teams to refine their routines and take significant steps towards countering this disease. Various recommendations have been published for dental surgeons and dentistry students, such as greater adherence to the use of personal protective equipment, avoiding or minimizing procedures that could produce drops or airborne particles, such as the use of low- or high-volume saliva ejectors to reduce the production of droplets or aerosols[5]. The consolidated scientific knowledge indicates that the transmission of the virus mainly occurs through the airways, but that it can also survive for a period of time on the hands, objects or surfaces exposed to infected saliva[6].

Preventing cross-infection in dental offices has always been a major challenge for dental surgeons, researchers and microbiologists. Microorganisms often succeed in circumventing the safety measures adopted, putting at risk professionals and patients alike. On the other hand, the lack of care exercised by some professionals, in terms of biosafety, contributes to the intensification of the cycle of cross-infection in the dental environment[6].

Some consumables, specifically resin-based composites, encounter obstacles when it comes to biosafety as they cannot be physically or chemically sterilized or disinfected[7]. The risk of contamination exists from both the restorative materials and the outer surface of the tubes, as all the parts of objects handled before, during and after treatment may be sources of infection. The presence of microorganisms inside the tubes and on the outer packaging, added to the incorrect handling of the material, may constitute a route to cross-infection in dental offices[8].

Given the above, checking for the presence of microorganisms on the surface of composite resins used in dental school clinics is important for proposing potential protective measures to prevent cross-infection and improve safety for all those involved in dental care. Therefore, the aim of the present study is to check for the contamination of composite resins used in a dental school clinic.

2 MATERIALS AND METHODS

Ten tubes of composite resin, all from the same manufacturer, were used in the study, of which one, still sealed in its original wrapping, was used as a negative control and nine were used during treatment, selected at random. All the tubes were collected using sterile gloves and tweezers and were transported in closed, sterile packaging to the Microorganism Biology and Microbiology laboratory.

The samples, isolated from the outside environment in sterile packaging, were transported to the laminar flow and inspected. The caps were removed from the tubes of resin, one by one, and with the aid of sterile scalpels small quantities of resin were collected from the bottom of the inside of the tube. These samples were then dipped in test tubes containing a

nutrient broth culture medium which allows for ample growth of bacteria and fungi. They were incubated at 37°C in a chamber with an orbital shaking (Shaker KS4000i IKA©, Staufen, Baden-Württemberg, Germany), at 180 rpm.

In all, 11 test tubes were used, one of which contained the culture medium used as the quality control for the procedure in order to discard potential false-positive results relating to bad handling of the material or contamination of the cabin that was used. To this end, the tube was opened inside the same laminar flow where the samples were handled and submitted to the same uncapping/capping process. Of the remaining 10 tubes, one contained material originating from the still unused tube of resin and nine were used to seed the material derived from the resins in use in the dental clinic.

After 24 hours, the 11 test tubes were inspected to see if there was any turbidity in the culture medium and an inoculant was then collected using 100µl of total volume, using micropipettes equipped with new, autoclaved tips, seeded in BHI (Brain Heart Infusion) Agar with the aid of a duly sterilized Drigalski spatula, in order to carry out the dispersion of the colonies across the whole plate. The seeding was repeated three times to observe the reproducibility of the test. After 24 hours, the plates were macroscopically analyzed in order to characterize the colonies and were then sent for Gram staining to observe the bacteria, or for lactophenol cotton blue staining, for the observation of possible fungi.

The colonies were viewed under a microscope using Gram methodology and the surface composition was classified as either positive or negative. In addition, the colony arrangement and morphology were observed. For this purpose, isolated colony smears were fabricated: the glass slides were quickly passed over the flame of a Bunsen burner and then, with the aid of a bacteriological loop (platinum loop), a drop of distilled water was placed on the slide while the sample to be stained was collected with the aid of a needle-shaped, platinum loop. Subsequently, the sample was homogenized in the drop of distilled water previously placed on the slide, and was left to dry. To ensure the smear was properly mounted, the slide was quickly passed through the flame.

Once the smear was fabricated, all the material placed on the slide was doused with a crystal violet solution for one minute and quickly rinsed using distilled water. The slide was then covered with Lugol's iodine, again for one minute, and was rinsed once more. Then slide was tilted and acetone alcohol or absolute alcohol was dropped on to it for 15 seconds. Once again, the slide was rinsed and covered with Gram basic fuchsine for 30 minutes. A final rinse in water was carried out and the slides were viewed under an optical microscope using a lens with 100x magnification, with the aid of immersion oil.

3 RESULTS

Except for the tube containing the culture medium used as the control in the handling experiment, the samples of resin in all the tubes showed signs of contamination.

The sample obtained from the (new, unopened) tube used as the negative control, contained a large colony, irregularly-shaped and apparently filamentous or cottony with a dry-looking, white and opaque, elevated protuberance; characteristics indicative of a fungus (figure 1a). Six tubes presented with slightly elevated,

circular-shaped grouped colonies, with edges that were smooth, opaque, off-white in color and viscous (figures 1b and 1c), all of the same type and indicative of bacteria. Both types of colony, as described above, were found in the other three tubes (figure 1d).

Slides were fabricated using the growths on BHI agar with cotton blue staining for those cultures indicative of the presence of fungus and Gram staining for those indicative of bacteria. The colony displayed in figure 1a possessed an ostensibly yeastlike, microscopic structure (figure 2).

Gram staining revealed the presence of Gram-negative bacteria in the shape of bacilli, both in isolation and in long chains on all the plates where there was bacterial growth, as well as isolated cocci (figure 3).

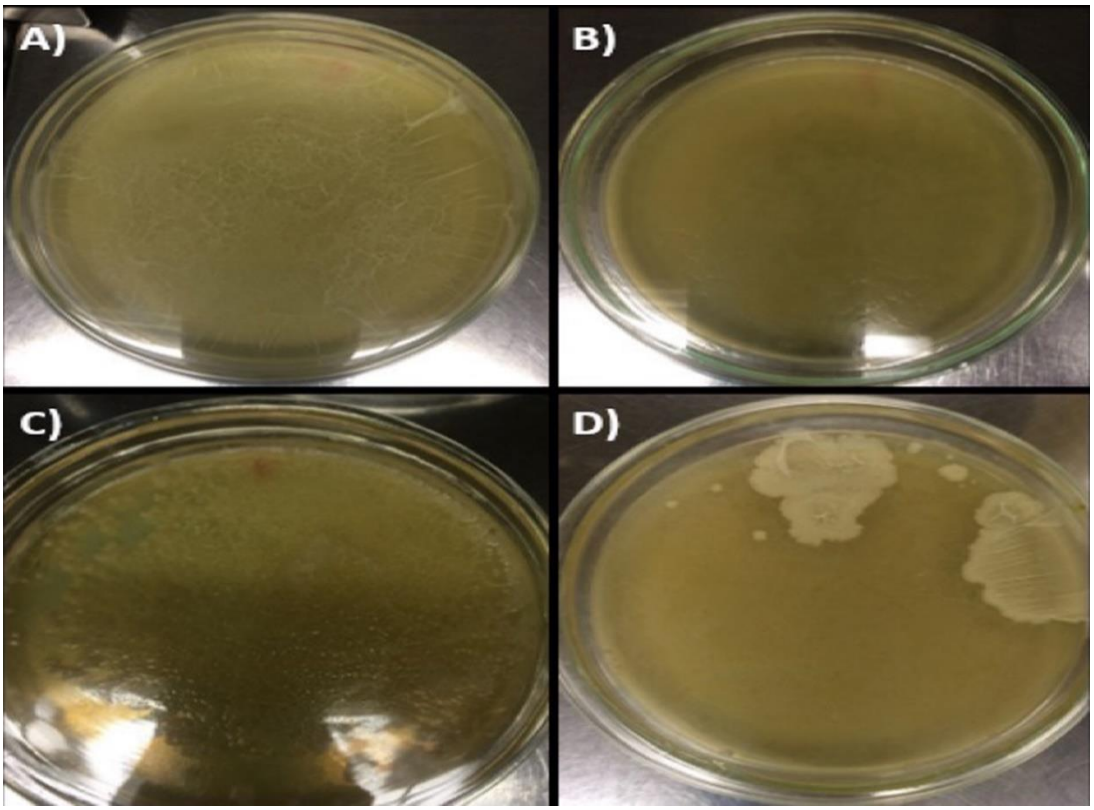

Figure 1. Morphological appearance of the colonies found on the plates containing BHI culture medium. Characteristics indicative of a fungus (a), bacteria (b and c), and both fungus and bacteria (d).

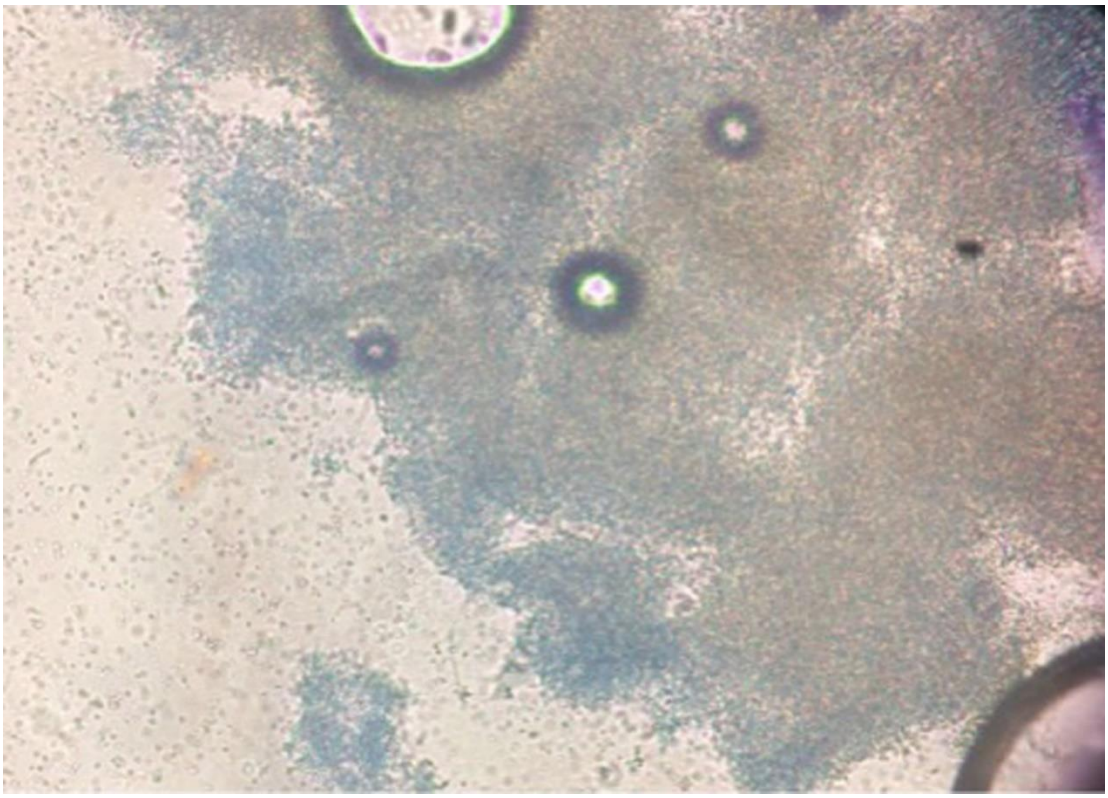

Figure 2. Slide fabricated using cotton blue stain (lactophenol) demonstrating the possible presence of yeast. Magnification of 400x.

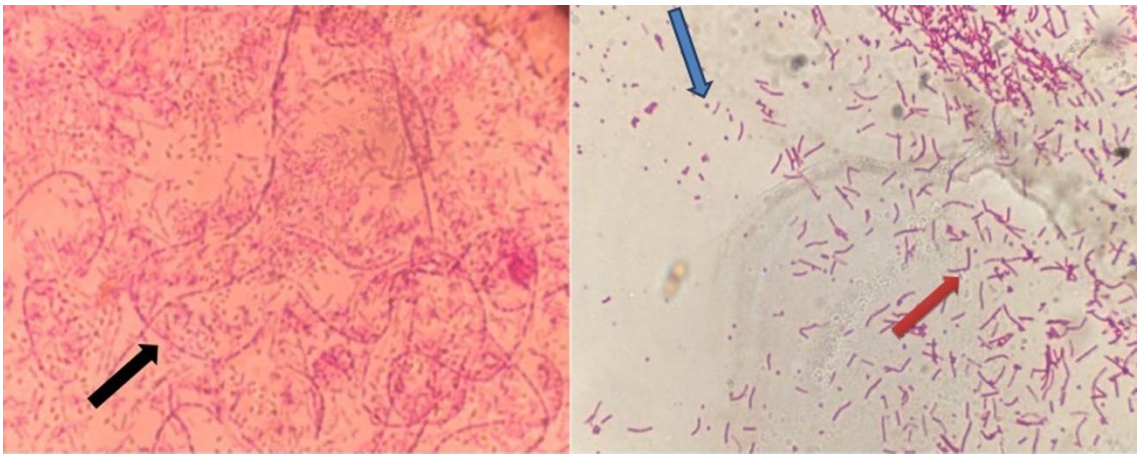

Figure 3. Gram stain showing Gram-negative bacteria. The black arrow shows the bacilli in long chains, the blue arrow shows the isolated cocci and the red arrow shows the presence of the isolated bacilli.

4 DISCUSSION

The present study showed a presence of microorganisms in all the composite resin samples. Colonies were also found in the sample used as the negative control, suggesting possible contamination, which was not expected as its packaging was suitably sealed. However, for a more emphatic affirmation of the contamination found on the sealed tube, a larger sample would be required as well as an inspection of the resin storage location and the manufacturing batch, as the whole process of distribution and storage of this material is liable to contamination. This outcome may be explained by the storage conditions of these composite resins. According to the manufacturer, this material must be stored in a location with a temperature between 2ºC and 27ºC, away from bright light and other materials that may contain eugenol. However, these procedures were not observed in the dental school clinic where the tubes were collected.

In an experiment conducted in the graduation clinics at the Dental Faculty of the Federal University of Juiz de Fora (FO/UFJF), involving a sample of 50 tubes of composite resin, (46 in the test group and 4 used as a negative control), in which bacterial growth was observed via the turbidity method. It was found that there was no turbidity in the tubes used as a negative control, while in the test group only two tubes presented with turbidity. However, these contamination data were not statistically significant. According to the results obtained, it is believed that the biosafety techniques employed in the FO/UFJF graduation clinics were effective with regard to the contents of the tubes of composite resin[9].

It is believed that there is a standard for the control of infection in teaching institutions, but the materials for dental use are handled by a variety of students within a short period of time, so precautions concerning preventive measures must be intensified in order to prevent contamination and cross-infection. No studies were found concerning the presence of SARS-CoV-2 on the surface of materials for dental use, however, it is recommended that restoration techniques should be performed in absolute isolation, that all dental instruments be sterilized, that personalized equipment be used, with special attention being paid to the use of protective goggles and N95/PFF type masks, with disinfection and protection of the surface of collectable materials as well as the disinfection of insertion spatulas for every increment performed, with the aim of protecting against infectious diseases[6,10].

In spite of the fact that this study noted the presence of microorganisms inside the tubes of composite resin, earlier studies discovered a high rate of contamination on the outside of the tubes in the test group. Thus, when there is no external disinfection, the restoration material becomes susceptible to contamination, representing a risk to the health of patients and professionals alike[11, 12].

Although the aim of this study was not to evaluate the specific microbiota found in each sample, a variety of forms (cocci, bacilli, isolated, grouped, and yeasts) and stains (Gram-negative and cotton blue) were observed. In a study conducted by Almeida et al. (2010)[7] it was possible to find microorganisms in samples of composite resins, for example coagulase-negative *Staphylococcus* (bacteria), *Bacillus sp* (bacteria) and *Aspergillus* (fungus). These microorganisms may be found in skin microbiota, the environment and in some

mucosa, and can very often cause infection, depending on the microbial load.

In the dental school clinic where the sample was collected, it is necessary to adopt measures to control cross-infection through this restorative material. The use of disinfectant substances on the outer part of the tubes of composite resins, such as 70% alcohol, is effective against Gram-positive and Gram-negative bacteria, some viruses and fungi, however, it is not able to eradicate the spores[13]. 70% alcohol gel was compared with chlorhexidine. The former was found to be more effective against *Staphylococcus aureus*, equally effective against *Escherichia coli* and *Streptococcus mutans* and less effective against *Candida albicans* and *Enterococcus faecalis*[14].

It has been proved that the use of barriers, such as Polyvinyl Chloride (PVC), increases the efficiency of infection control, as it acts as a physical barrier and has to be replaced after each treatment so that there is no direct contact between the operator and the packaging[15].

The difficulty in controlling the contamination of common-use materials is enormous inside a dental school clinic as a large number of people are involved in the medical care process: teachers, students, employees and patients. The important thing is to make all those involved aware about the need to follow instructions in order to avoid a break in asepsis and consequently prevent cross contamination[11].

The presence of the microorganisms found in this study may be explained by the number of people involved in the dental school clinic (patients and students). According to the evidence found, a biosafety method is suggested, with the use of composite resin, using a cocoon or a Dappen dish, in which increments are separated prior to the procedure. This proposal is costly for the daily practice of a dental school clinic[12], moreover the characteristics of the material may be altered due to exposure to light and other environmental influences, such as oxygen[16], however its use should not be discarded.

Thus, this and other measures such as the disinfection of the tubes of resin and protection with paper film at each use, should become a universal measure, as the materials are handled by various students in a short period of time.

5 CONCLUSION

The presence of microorganisms was found in all the collected samples, including the negative control. This contamination may be related to defects in biosafety procedures employed in the dental school clinic, as well as the process of storing the sealed, unused product. Therefore, there is a need to raise awareness in both students and teachers of the adoption of specific biosafety measures for the use of composite resins, as well as measures that will control the proper storage of this material.

RESUMO

Contaminação microbiológica de resinas compostas utilizadas em uma clínica-escola de Odontologia

O objetivo desse estudo foi verificar a contaminação microbiológica de resinas compostas utilizadas em uma clínica-escola de Odontologia. Trata-se de uma pesquisa experimental/laboratorial, na qual foram coletadas amostras de 10 bisnagas de resina composta, sendo uma delas o controle negativo. Porções de resina composta contidas no interior das bisnagas foram coletadas e mergulhadas em tubos de ensaio contendo caldo nutriente e posterior semeadura em

placas e coloração para caracterizar as colônias e observar bactérias e fungos. Todas as amostras apresentaram contaminação, inclusive o tubo contendo meio de cultura utilizado como controle de manuseio do experimento. Essas contaminações podem ter relação com as falhas dos meios de biossegurança empregados na clínica-escola e com os métodos de transporte e armazenamento das bisnagas de resina composta. Portanto, existe a necessidade de conscientização dos estudantes e docentes para a adoção de medidas de biossegurança específicas para o manuseio das resinas compostas.

Descritores: Contenção de Riscos Biológicos. Resinas Compostas. Clínicas Odontológicas. Contaminação Biológica.

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