

# Evaluation of bacterial contamination on irreversible hydrocolloid impressions before and after disinfection

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• **Conflicts of interest:** none declared.

## ABSTRACT

**Objective:** to evaluate microbial contamination and characterization of irreversible hydrocolloid impressions, as well as the antimicrobial efficacy of 1% sodium hypochlorite and 2% chlorhexidine solutions in decontamination for 5 and 10 minutes. **Material and Methods:** we made upper arch impressions from 50 dentistry students of the *Escola Superior São Francisco de Assis*, the sample from each impression was collected with a sterile cotton swab and inoculated in BHI broth, being transferred to solid blood agar for evaluation of the contamination. Subsequently, the impressions were washed or disinfected with 1% hypochlorite or 2% chlorhexidine, a new collection was made to evaluate the degree of decontamination by spraying. **Results:** data regarding the number of CFU before and after the decontamination procedures were tabulated, we compared the results using the Wilcoxon signed rank test. Thus, we verified that the microbial load was not reduced after washing the impression with sterile water. In contrast, decontamination using both chemical agents proved to be effective, for 5 and 10 minutes. Moreover, from the characterization of the microorganism test, we noticed that most bacteria are common within the oral microbiota. However, we also found *Staphylococcus aureus* in 5 impressions (8.33%), a pathogenic bacterium that is not common in the oral microbiota. **Conclusion:** decontamination procedures are effective to remove microorganisms present in impressions.

**Keywords:** Disinfectants; Dental Impression Materials; Disinfection.

## Introduction

There is a considerable risk of transmission of infectious diseases within dental clinics, every day the dentist is exposed to a variety of microorganisms present in the oral cavity, which are often pathogenic.<sup>1</sup> The transmission of microorganisms between patients, professionals, auxiliaries and prosthesis laboratories is called cross-contamination.<sup>2</sup>

Several microorganisms may be transmitted by cross-contamination, many of them pathogenic to humans, such as: *Staphylococcus aureus*, *Streptococcus spp.*, *Candida albicans* and even Enterobacteria. Viral particles such as the Human Immunodeficiency Virus (HIV) and Hepatitis B and C viruses may also be transmitted.<sup>3</sup> Many of these microorganisms can survive in different areas of the office through spills and aerosols, even with little moisture.<sup>2,4</sup>

Raising awareness about the risks of cross-contamination during appointments is important in all dental practice activities. However, some studies point to the lack of care from some dental surgeons regarding biosafety and the disinfection/sterilization procedures, especially when performing non-invasive procedures, such as in impressions.<sup>5-7</sup>

Dental impressions consist of registering the anatomy of the mouth to be evaluated. During this procedure there is direct contact of the material with saliva, bacterial plaque and sometimes blood of the patient. The most used imprint material is alginate (irreversible hydrocolloid). When used for an imprint procedure, it collects many microorganisms since it is a hydrophilic material. For this reason, it becomes a means of contamination in dental offices and laboratories.<sup>8</sup>

Given this context, this study examined bacterial con-

tamination and the characterization of microorganisms existing in irreversible hydrocolloid impressions before and after disinfection through collection with sterile swabs. In addition, we evaluated the effectiveness of different disinfectant agents on the decontamination of these impressions.

## Material and Methods

The project was sent to and approved by the Research Ethics Committee no. 47149015.0.0000.5070, CEP/Hospital Meridional S/A (Cariacica, ES, Brazil). The volunteers who participated in the study had to sign an Informed Consent Form.

This is a microbiological laboratory study, of descriptive and comparative nature, the objective was to evaluate the contamination of irreversible hydrocolloid impressions before and after a disinfection procedure. For such, we made impressions of the upper arch of 50 volunteer students enrolled in the undergraduate course of Dentistry of the *Escola Superior São Francisco de Assis* (ESFA) in Santa Teresa, Espírito Santo, Brazil.

## Impression Procedure

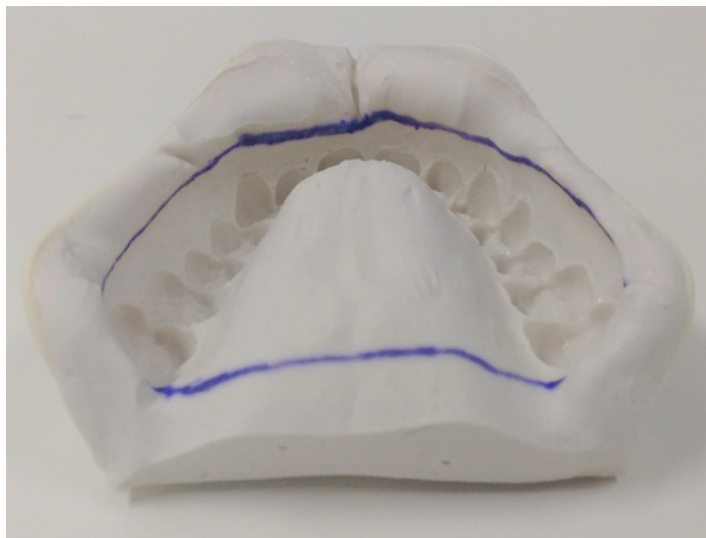
We performed the impression procedure on 50 students of the undergraduate course of Dentistry of the ESFA. The imprints were made in the upper arch of the participants using irreversible hydrocolloid (sodium alginate), which contained chlorhexidine in its composition, AVAGEL (Dentistry, Milford, DE, USA). The impression material was handled according to the instructions of the manufacturer and inserted into a plastic tray (Maquira, Maringá, PR, Brazil). The trays were previously sterilized in an autoclave accord-

ing to the time recommended by the manufacturer.

The tray containing the irreversible hydrocolloid was placed in the mouth of the student next to the cervical margins to touch the hard tissue and gingiva. After the period of jellification, the tray was removed from the mouth.

### Material Collection and Laboratory Analysis

After the imprinting procedure, we immediately collected the material to be analyzed using a sterile swab in a pre-determined region of the alginate impression, as shown in Figure 1.



**Figure 1.** Region of the impression where we performed the collection

Each swab was then inoculated in Brain Heart Infusion (BHI) broth (OXOID, Pinheiros, São Paulo, Brazil).

We followed the disinfection procedures recommended by the Brazilian Ministry of Health (2000),<sup>9</sup> the impression was washed in sterile water by spraying, each impression was sprayed with the disinfectant solutions of 1% sodium hypochlorite or 2% chlorhexidine. The impressions were stored in plastic bags for 5 or 10 minutes.

After this period, the impression was washed in sterile water again and another collection using a sterile swab was performed. The swabs were inoculated in new BHI broth to continue the microbiological analyses. We also washed the impressions using only sterile water as the control for the

procedure. After the described procedures, we evaluated five groups:

- Group 1: 10 Impressions disinfected with 1% sodium hypochlorite for 10 minutes.
- Group 2: 10 Impressions disinfected with 1% sodium hypochlorite for 5 minutes.
- Group 3: 10 Impressions disinfected with 2% chlorhexidine for 10 minutes.
- Group 4: 10 Impressions disinfected with 2% chlorhexidine for 5 minutes.
- Group 5: 10 Impressions washed only in sterile water for a minute (control group).

Each tube of BHI broth was incubated for 24h at 37°C. After this period, we transferred 100 µL of broth of each sample to a solid blood agar medium and spread by surface scattering technique using a Drigalski spreader. Then, the plates were incubated for 48h at 37°C to promote microbial growth.

After growth on the plates, we manually counted the Colony-forming Units (CFU). We considered as the maximum value the cases in which the count exceeded 300 CFU. Isolated colonies were characterized through Gram stain using optical microscopy increased by 100x, the colonies were identified and classified using Gram stain. For Gram-positive bacteria we used the following tests: Catalase, DNase, hemolytic profile, PYR, bacitracin sensitivity, sulfa-trimethoprim susceptibility and growth on mitis salivarius agar. For Gram-negative bacteria: test series of glucose and oxidase fermentation. Regarding fungal growth, the identification was performed using only morphological analysis using the Gram stain.

We tabulated the data regarding CFU before and after the disinfection procedure. Due to being nominal qualitative data, we performed the comparisons using the Wilcoxon signed rank test.

## Results

We obtained 50 impressions total, they were analyzed for the number of CFU on two different moments: before and after the disinfection procedures. Table 1 shows the median of the results for the count of colonies in the five experimental groups, as well as the comparison, for each group, before and after disinfection.

**Table 1.** CFU count on the impressions before and after the disinfection procedure

Microorganisms	Sterile water 1 min Before washing (n=10)	Sterile water 1 min After washing (n=10)	Chlorhexidine 10 min Before Disinfection (n=10)	Chlorhexidine 5 min Before Disinfection (n=10)	Chlorhexidine 10 and 5 min After Disinfection (n=10)	Hypochlorite 10 min Before Disinfection (n=10)	Hypochlorite 5 min Before Disinfection (n=10)	Hypochlorite 10 and 5 min After Disinfection (n=10)
Enterobacteria	1	1	0	0	0	0	0	0
Coagulase-Negative Staphylococci	5	5	0	3	0	4	3	0
<i>S. aureus</i>	1	1	0	1	0	1	1	0
<i>Streptococcus</i> spp (viridans)	9	8	10	10	0	10	9	0
Yeast	0	0	0	1	0	0	0	0

We can notice that washing with sterile water (positive control) was not effective to reduce the microbial load in irreversible hydrocolloid impressions. On the other hand, all other experimental groups presented satisfactory behavior, regardless of the time of exposure to the disinfectant agent.

Table 2 summarizes the major microorganism groups present in the impressions.

The results of this study showed that after the disinfection procedure, the impressions were free of contamination. Therefore, the disinfectant agents used – 1% sodium hypochlorite and 2% chlorhexidine – were effective in the decontamination of the impressions through the spraying technique. Esteves *et al.*,<sup>11</sup> evaluated the direct antimicrobial action of these agents against *S. mutans* and *S. aureus*

**Table 2.** Characterization of contaminant microorganisms on the alginate impressions before and after the disinfection procedure

Microorganisms	Sterile water 1 min Before washing (n=10)	Sterile water 1 min After washing (n=10)	Chlorhexidine 10 min Before Disinfection (n=10)	Chlorhexidine 5 min Before Disinfection (n=10)	Chlorhexidine 10 and 5 min After Disinfec- tion (n=10)	Hypochlorite 10 min Before Disinfection (n=10)	Hypochlorite 5 min Before Disinfection (n=10)	Hypochlorite 10 and 5 min After Disinfec- tion (n=10)
Enterobacteria	1	1	0	0	0	0	0	0
Coagulase-Neg- ative Staphylo- cocci	5	5	0	3	0	4	3	0
<i>S. aureus</i>	1	1	0	1	0	1	1	0
<i>Streptococcus</i> spp (viridans)	9	8	10	10	0	10	9	0
Yeast	0	0	0	1	0	0	0	0

From 50 impressions made, we obtained 60 plates for analysis of microorganisms. This is explained by the bacterial growth before and after washing with sterile water. This did not occur in the disinfectant groups because there was growth only prior to disinfection. Thus, there was a predominance of Gram-positive bacteria in 58 plates (96.66%). For the most part, biochemical tests identified microorganisms that are common in the oral microbiota, such as *Streptococcus* sp of the viridians group in 37 impressions (61.66%); negative-coagulase *Staphylococcus* in 20 (33.33%); however, we also found *Staphylococcus aureus* in 5 impressions (8.33%), this is a pathogenic and unusual bacterium in the oral microbiota.

## Discussion

Some irreversible hydrocolloids feature disinfectant agents in their composition, such as chlorhexidine. According to Moreira and Cruz,<sup>10</sup> these agents are effective on antimicrobial action when present in irreversible hydrocolloid powder. However, the results showed that the chlorhexidine in the formulation used did not cause antimicrobial action, since after washing using only sterile water, over 300 CFU were found when counting the colonies (Table 1). The inefficiency of the agent in this case can be explained by its aggregation to the irreversible hydrocolloid during jellification, or due to a low concentration in the alginate formulation.<sup>11</sup>

Other studies found similar results, impressions that were washed only using water presented bacterial growth.<sup>12-15</sup> Washing the impressions using water is a usual procedure, however, using antibacterial substances is still needed for a more effective disinfection.<sup>16</sup>

*in vitro*, they observed that the substances were effective in inhibiting microbial growth. Furthermore, Linhares *et al.*,<sup>17</sup> found that 1% sodium hypochlorite was effective in disinfecting impressions, also by spraying for 10 minutes. Although the sodium hypochlorite solution is recommended for disinfection, some care is needed in its handling, since it is a hazardous material that can cause irritation on the skin, mucous membrane and eyes, as well as the corrosion of metallic materials.<sup>18</sup>

Regarding the disinfection time, we observed that it was effective for both 5 and 10 minutes (Table 1). The advantage from a reduced disinfection time is the optimization of the work routine. In addition, a longer time in this procedure can lead to some significant dimensional changes on the irreversible hydrocolloid, as stated by Nassar *et al.*,<sup>19</sup> and Anusavice<sup>8</sup> in their research. Our results corroborate with Okazaki *et al.*,<sup>20</sup> whom also found good results of disinfection using 1% sodium hypochlorite for only 5 minutes.

The oral cavity houses around 500 to 700 species of microorganisms. This occurs since several sites (habitats) with different environmental conditions that favor microbial growth exist, including the teeth, gingival sulcus, tongue, cheek, hard and soft palate and tonsils. Many of these microorganisms can be transmitted during dental procedures and may be associated with diseases.<sup>21</sup>

Similarly, Egusa *et al.*,<sup>22</sup> showed the existence of different microorganisms in alginate impressions. From 30 samples investigated in selective agar medium, we detected among the isolates 56.7% *Staphylococcus* sp., 30% *Candida albicans* sp, 26.7% Methicillin-resistant *Staphylococcus aureus* (MRSA) and 6.7% *Pseudomonas aeruginosa*. This shows that

irreversible hydrocolloid is prone to the retention of pathogenic microorganisms, which may be inside the impression and move on to the plaster during the leak.

In another research, Egusa *et al.*,<sup>23</sup> found that the frequency of isolation of *Streptococcus*, *Staphylococcus*, *Candida*, MRSA and *P. aeruginosa* were 100%, 55.6%, 25.9%, 25.9% and 5.6%, respectively.

The isolation of *S. aureus* in some of the impressions shows the presence of pathogenic bacteria that may be present on the oral mucosa transitionally. According to Saramanayake<sup>3</sup> several enzymes and toxins are produced by *Staphylococcus aureus*. The most important are the coagulase and enterotoxin, the main diseases caused by *S. aureus* are osteomyelitis, endocarditis, sepsis and pneumonia.

The importance of disinfection procedures is emphasized by the presence of this microbial diversity in the impressions (Table 2) since some bacteria can survive for long periods of time on surfaces. Žilinskas *et al.*,<sup>24</sup> investigated the survival of fungi and bacteria in plaster models that were prepared with 1 mL bacterial suspension and stored in a sterile container for evaluation between 1 hour and 120 hours. The results found that *S. aureus* remained viable for four days.

Our results show that the impressions must be systematically disinfected, so the material contaminated with blood and saliva does not become a source of cross-infection. Thus, disinfection and sterilization procedures must be adopted

for all dental procedures, aimed at reducing the possibility of cross-infections that may result in serious infectious diseases, both in the dental staff and patients.

This study presented limitations regarding the population and sample collection area. Larger population numbers and a larger collection area could show greater bacterial variability.

## Conclusion

Our results evidence the microbial contamination in the handling of these impressions, and that the microbial load was not reduced after the impression was washed only with water (control group). Thus, a treatment with disinfection agents is still required, since this procedure is not effective in eliminating the microorganisms in the impressions. Despite the predominance of oral bacteria, the presence of *S. aureus* in some impressions points to the possibility of pathogenic microorganisms being found.

The microbial load was reduced after the impressions were disinfected with 2% chlorhexidine or 1% sodium hypochlorite, proving that these disinfection procedures are effective to remove microorganisms present in the alginate impressions. Furthermore, the disinfection of the impressions for 5 minutes was as efficient as it was for 10 minutes. A reduced disinfection time can optimize the work routine in the dental office.

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### Mini Curriculum and Author's Contribution

1. Thais Dias Lemos Kaiser – DDS and MSc. Contribution: conception and design of the study; standardization of the methodology; data interpretation; preparation of the manuscript; writing the manuscript; critical review and final approval.
  2. Paula Sampaio de Melo – DDS and MSc. Contribution: preparation of the manuscript; critical review and statistic.
  3. Laís Falcão Borgo – Undergraduate student. Contribution: conception of the study; preparation of culture media; sample collection; inoculation and Peak; identification tests of microorganisms; analysis of results; data interpretation and preparation of the manuscript.
  4. Julieti Fernandes Flor – Undergraduate student. Contribution: preparation of culture media; sample collection; inoculation and Peak; identification tests of microorganisms; analysis of results.
  5. Francisco de Assis Bozzetti – Undergraduate student. Contribution: preparation of culture media; sample collection; inoculation and Peak; identification tests of microorganisms; analysis of results.
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