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Viability changes: Microbiological analysis of dental casts

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: This study evaluated the survival of the most prevalent oral bacteria and fungi (*Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli,* and *Candida albicans*) in dental casts, and compared changes in the amounts of these microorganisms at different time intervals to determine how long dental casts may pose threat to the health of dental personnel and patients.

Material/Methods: When manufacturing the casts, regular water was replaced with sterile distilled water, where suspensions of the studied bacteria or the fungus at certain concentrations were prepared. When the dental casts were fully set (solidified), plaster shavings were examined immediately after the contact of the studied microorganism with the plaster, as well as after 1, 2, 24, 48, 72, 96, and 120 hours. Following that, we measured how the amount of the studied bacteria and fungi in 1 gram of the plaster changed within the studied period of time.

Results: *Klebsiella pneumoniae* survived in plaster for up to 4 days, and the reduction in the number of these bacteria became statistically significant after 1 day ($p<0.05$). *Staphylococcus aureus* remained viable in plaster for up to 4 days, and the number of these bacteria dropped after 1 day ($p<0.05$). *Escherichia coli* disappeared after 2 days, and a reduction was already observed after 2 hours ($p<0.05$). *Candida albicans* in plaster models died within 2 days, and a reduction in their number was observed after 1 day ($p<0.05$).

Conclusions: The microorganisms did not multiply in the gypsum casts and their number significantly dropped instead of increasing.

MeSH Keywords: **Dental Casting Technique • Infection Control • Laboratories, Dental**

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Background

Oral and nasopharyngeal microflora are highly abundant. Anaerobic and facultative aerobic bacteria dominant the variety and amount of types of microflora. The amount of microorganisms in the oral fluid (saliva) ranges from 4 millions to 5 billions per 1 milliliter, and in dental plaque between 10 and 1000 billions per 1 gram [1]. The biological characteristics of the oral microflora and its colonizing microorganisms are highly variable. Their resistance to non-specific oral protective factors and environmental factors (e.g., antiseptic solutions) depends on the cellular membrane, a thick layer of mucus, a capsule, or other biological characteristics. For this reason, standard bacterial cultures with differing biological characteristics were selected for this study: *Staphylococcus aureus* are gram-positive cocci found in the nostrils of up to 60% of humans; they form microcapsules, and are resistant to high temperatures (up to 70°C), disinfectant, and antiseptic solutions, and cause suppurative inflammatory processes [2]. *Staphylococcus aureus* is an important pathogen because of its virulence, antimicrobial resistance, and association with various diseases, including fatal systemic infections, intoxication, cutaneous infectious, and opportunistic diseases [3]. *Klebsiella pneumoniae* are capsule-forming bacteria found in the respiratory tract and are capable of causing atypical pneumonia. *Candida albicans* is a fungus and is part of the normal microflora of the oral cavity, but it can cause an opportunistic infection in immuno-depressed subjects. *Escherichia coli* is part of the normal microflora of the large intestine; however, due to poor hygiene it can enter the oral cavity and cause suppurative inflammatory processes. The procedures that a prosthodontist performs may cause damage to the patient's oral mucosa and gingiva. The saliva mixes with blood. During the manufacturing of the dental impressions, blood and saliva enter the setting impression material, and it becomes contaminated with bacteria and viruses [4–7]. For this reason, according to general hygiene norms, such impressions should be disinfected; yet disinfection does not always yield the desirable results [8]. When producing gypsum casts using non-disinfected or incompletely disinfected dental impressions, the microorganisms travel from the surface of the impression material into the cast [9]. The infected material then is taken to the manufacturing facilities and may pose threat to dental technicians. Dental casts are subject to several forms of contamination, ranging from direct contact with a patient's saliva, to procedures involving measurement, planning, manufacture, laboratory shipping, and storage [10]. Dental technicians touch the impressions or the casts with their hands, and their skin is frequently damaged due to their work. When cutting the dental casts, bacteria, fungi, or particles of the disinfectant may travel with the plaster dust from the material to the respiratory tract of personnel, causing infections, and may also accumulate on the clothing or open surfaces in the room. Prolonged exposure to residual disinfectant particles may trigger allergic reactions.

The aim of study was to evaluate the survival of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* bacteria, and *Candida albicans* fungus in non-disinfected dental casts. The results of the study could be used to determine how long dental casts may pose threat to the health of the personnel and the patients. Perhaps disinfection of dental impressions would be sufficient, skipping additional disinfection of the dental casts in order to reduce exposure of the personnel to possible allergens – the residues of disinfectant solutions in the plaster. Although extensive research has been carried out, there are no published studies in which similar issues were addressed.

Material and Methods

During this experimental study we investigated the survival of the selected bacteria and fungi in dental casts. The study was conducted using standard bacterial (*Staphylococcus aureus* ATCC 25928, *Klebsiella pneumoniae* ATCC 13883, and *Escherichia coli* ATCC 25922) and fungal (*Candida albicans* ATCC 60193) cultures. Saline suspensions (McFarland turbidity standard 0.5) were prepared using standard bacterial cultures cultivated in trypticase soy agar for 24 hours at 35–37°C, and *Candida albicans* culture cultivated in Sabouraud agar for 3 days. We evaluated and compared changes in the number of the microorganisms in gypsum casts at different time intervals.

Gypsum casts (an equivalent to dental cast) were manufactured in a sterile laminar-flow hood under sterile conditions, using standard alabaster plaster class 2 "Moldabaster" (Heraeus-Kulzer, Germany) for dental casts. The plaster was sterilized; therefore, there was no need to form a control group. The dental casts were prepared by pouring 65 g sterile plaster powder, 5 ml sterile distilled water, and 1 ml of a specific bacterial or fungal suspension (McFarland turbidity standard 0.5) into a sterile mixing container. A sterile spatula was used to stir the contents until an integral mass was formed, which was subsequently placed into a sterile closed container. During the test period the closed containers with the samples were placed on a dental lab shelf to simulate casual cast storage conditions. The number of the aforementioned standard microorganisms per 1 g of the substance was evaluated after 1, 2, 24, 48, 72, 96, and 120 hours. Sterile clamps were used in the making of each sample. The sample – a piece of the gypsum cast of a known weight (ca. 1.0 g) – was ground to powder in a sterile mortar, and then placed into a sterile glass with 5 ml of sterile saline solution. The suspension of plaster powder was diluted at the ratio of 1:5, 1:25, 1:125, and 1:625. One ml of the suspension from each test tube with different dilution ratios was poured into 10 ml of 45°C trypticase soy agar (or Sabouraud agar, in the case of *Candida albicans*) in 2 Petri dishes that were then stored at 35–37°C (in case of *Candida albicans,* Sabouraud agar was stored at 20–25°C). After the cultivation of the indicated duration, colonies of the

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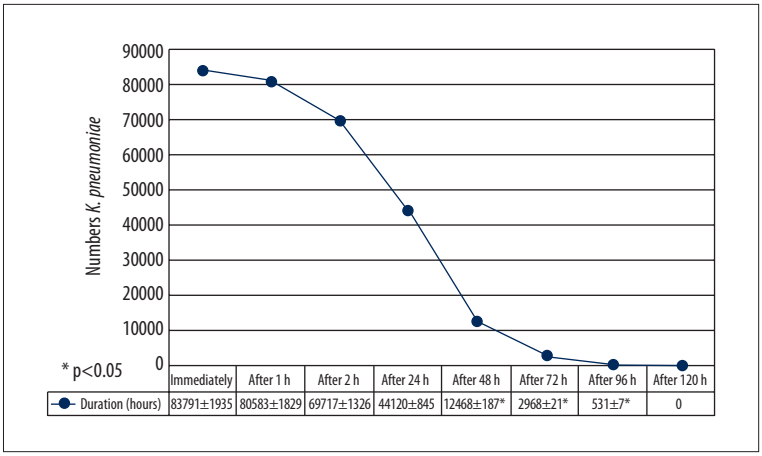

Figure 1. Changes in the number of *Klebsiella pneumoniae* ATCC 13883 in 1g of the gypsum cast.

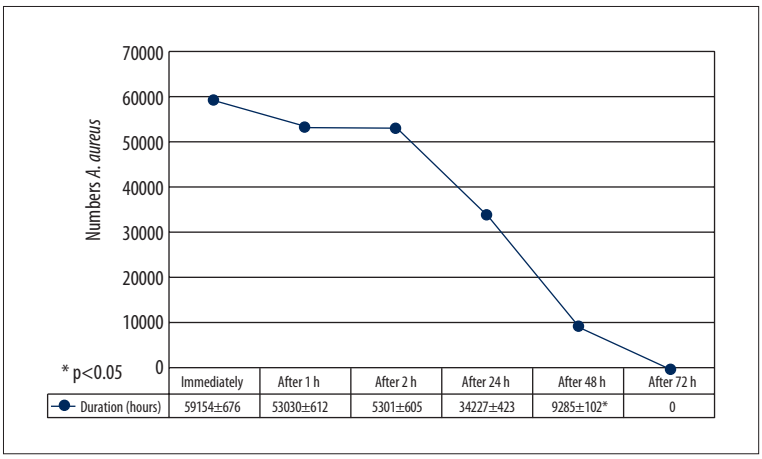

Figure 2. Changes in the number of *Staphylococcus aureus* ATCC 25928 in 1g of the gypsum cast.

respective bacterium or fungus in the 2 Petri dishes were evaluated, calculating the mean number of the colonies; multiplying this number by the degree of dilution revealed the number of bacteria or fungus per 1 g of plaster. Each experiment was conducted 10 times. Statistical analysis was performed by applying the χ^2 criterion. The data were considered to be statistically significant if the probability of an error was $p<0.05$.

Results

The results of the study are presented in Figures 1–5. The study showed that *K. pneumoniae* survived the longest in gypsum casts – up to 4 days – compared to the other studied microorganisms (Figure 1).

A statistically significant reduction in the number of *K. pneumoniae* (from 83791±1935 to 44120±845) in gypsum casts was observed after 1 day ($p<0.05$). The number of *K. pneumoniae* colonies was also the highest, compared to other bacteria.

S. aureus survived in gypsum casts for up to 3 days. After the first and the second hour in the gypsum cast, the number of bacteria remained nearly unchanged. It was only after 2 days that a statistically significant difference was detected (a reduction from 59154±676 to 9285±102, $p<0.05$). Gram-positive bacteria (staphylococci) remained viable for up to 3 days in the gypsum casts, presumably due to their cell membrane composed of a thick peptidoglycan layer, a microcapsule (a thin layer of mucus over the cell membrane), and high resistance to environmental factors (Figure 2).

No gram-negative bacteria (e.g., *E. coli)* were found in gypsum casts after 2 days, and a statistically significant reduction in their numbers was observed just after 2 hours (from 19483±336 to 4705±74, $p<0.05$) (Figure 3).

Microorganisms with a eukaryote structure (e.g., the fungus *C. albicans)* died within 2 days, and a statistically significant reduction was observed after 1 day (from 31 366±2759 to 12 291±1234, $p<0.05$) (Figure 4).

Figure 5 presents the comparison of the standard microorganisms amounts (the bacteria and the fungi) at different time intervals, and the quantitative reduction in their numbers.

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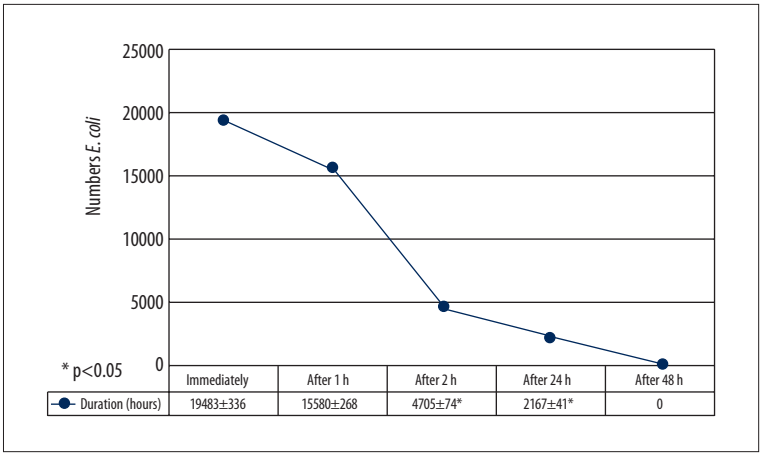

Figure 3. Changes in the number of *Escherichia coli* ATCC 25922 in 1g of the gypsum cast.

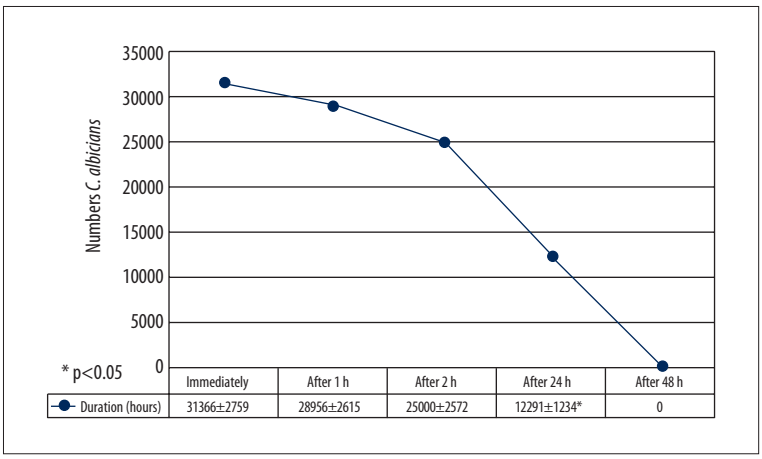

Figure 4. Changes in the number of *Candida albicans* ATCC 60193 in 1g of the gypsum cast.

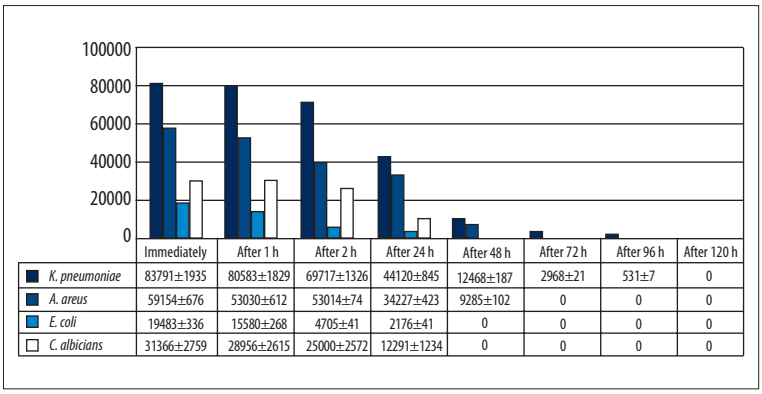

Figure 5. Comparative characteristics of the changes in the number of bacteria and fungi in 1 g of the gypsum cast.

The quantitative evaluation of the viability of standard microorganisms in gypsum casts showed that physical-chemical characteristics of gypsum casts have different effects on the survival of the standard microorganisms. Structural and physiological characteristics of *K. pneumoniae* and *S. aureus* affect their survival (their quantity) with respect to that of *E. coli* and *C. albicans*. The findings of the study showed that the microorganisms did not multiply in the gypsum casts; instead, their numbers significantly dropped.

Discussion

The study was conducted using the most common oral bacteria and fungus: *Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans,* and *Escherichia coli*. Studies performed in Japan showed that all the microorganisms used in our study – *Staphylococcus aureus, Klebsiella pneumoniae*, and *Escherichia coli* – were detected in the oral cavities of 13.6% of patients with no health complaints [11]. According to other authors, *Staphylococcus aureus* is detected in the oral cavities of 15.8%

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of patients, and *Candida albicans* in 63.0% of patients [12]. The findings of our study showed that the duration of the survival of microorganisms in gypsum casts varied depending on the types of the studied cultures. This is probably due to certain biological characteristics – the structural peculiarities of the microorganisms. Among Gram-positive bacteria, staphylococci are the most resistant to both chemical and physical environmental factors. These bacteria have a thick layer of peptidoglycan and a microcapsule, which is a mucous layer of polysaccharide origin. The Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* differ from staphylococci in their cell membrane structure. However, *Klebsiella* bacteria have a thick mucous layer of polysaccharide origin – a capsule – that ensured their longest survival in gypsum casts compared to other microorganisms. *Escherichia coli* have no capsule and thus are more susceptible to unfavorable environmental conditions, thus these bacteria survived in gypsum casts for only 1–2 days. The fungus *Candida albicans,* although it has a eukaryote cell structure, survived in gypsum casts longer than *Escherichia coli* did because fungi have a chitin membrane [13]. The oral cavity may contain pathogenic as well as relatively pathogenic microorganisms that can be transferred into the environment through impressions. In patients with damaged oral mucosa, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Escherichia coli* may cause suppurative inflammatory processes. *Staphylococcus aureus* is the principal causative agent of osteomyelitis. If *Klebsiella pneumoniae* reach the lower respiratory tract, they may cause pneumonia. *Escherichia coli* may cause not only a local inflammatory process, but bacteremia as well. *Candida albicans*, as part of the normal oral microflora, is found in 40–60% of individuals; however, this fungus can cause candidiasis in immuno-compromised states or in cases of antibiotic overuse [1,13].

Most commonly, alginate and silicone dental impressions are used in clinical practice. Due to their composition, structure, and the hydrophilic setting mechanism, alginate impressions are easily infected by oral microorganisms. A more monolithic structure of the silicone impression mass and its non-hydrophilic setting mechanism significantly reduce the attachment of microorganisms to the impression surface. Selecting a suitable impression mass may reduce the spreading of the infective agents as early as the first stages of prosthesis manufacturing. Alginate impressions are more frequently contaminated with oral bacteria and fungi, compared to silicone impressions, and they are also more difficult to disinfect. Alginate impressions pose a significantly higher threat of contaminating dental casts with bacteria and fungi, compared to silicone impressions [14], thus silicone impressions are safer with respect to the transmission of the infective agents. Previous studies have shown that dental impressions delivered at dental laboratories contain bacteria irrespectively of whether they were disinfected or washed under running water [15]. This suggests that dental casts produced using well-disinfected impressions require no additional disinfection. If the dental casts are stored under normal conditions of a dental technician's office, the number of microorganisms should change more rapidly than it did during our study due to frequently changing temperature and humidity, and because of the plaster pH of 6.1. An additional disinfection of dental casts would not yield significant results, compared to the risk that the dental technicians would face because of exposure to a potential allergen – the disinfectant on the surface of the dental cast. Although studies have shown that the effect of the disinfectant on the precision of the dental casts is not significant, the risk remains that the disinfectant may alter the physical characteristics of the dental cast surface, making it more porous, brittle, and more susceptible to wear and tear. Such changes may result in reduced precision of the manufactured dental casts [16,17]. Rinsing of the oral cavity with an antiseptic solution prior to odontological procedures may reduce the presence of microorganisms in the oral cavity. The most popular oral antiseptic, chlorhexidine, is not always effective against fungi or bacterial spores. Thus, mouth rinsing with an oral antiseptic prior to taking impressions followed by full disinfection of the impressions would reliably reduce the contamination of dental casts with microorganisms [18]. As our results show, the infection risk from the cast decreases over time. If the impression is disinfected and pre-impression mouth rinsing is used consistently, the dental casts would pose less threat because of the poor survivability of bacteria and fungi in gypsum.

Conclusions

1. Gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Klebsiella pneumoniae* demonstrated the longest survival in dental casts (up to 4 days), compared to *Escherichia coli* and *Candida albicans,* which remained viable during the first 2 days).
2. Dental casts are not a suitable medium for the multiplication of the colonizing microorganisms. Individual protective measures (gloves, protective goggles, and respiratory protection measures) should be used in order to avoid possible contamination of dental impressions and casts with microorganisms, and to prevent their transmission and spread in environments such as dental laboratories.

Statement of conflicts of interest

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