MICROBIOLOGICAL CONTAMINATION IN DIGITAL RADIOGRAPHY: EVALUATION AT THE RADIOLOGY CLINIC OF AN EDUCATIONAL INSTITUTION

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ABSTRACT
The aim of this study was to evaluate the contamination rate of intra- and extraoral digital X-ray equipment in a dental radiology clinic at a public educational institution. Samples were collected on three different days, at two times in the day: in the morning, before attending patients, and at the end of the day, after appointment hours and before cleaning and disinfection procedures. Samples were collected from the periapical X-ray machine (tube head, positioning device, control panel and activator button), the panoramic X-ray machine (temporal support, bite block, control panel and activator button), the intraoral digital system (sensor), and the digital system computers (keyboard and mouse). The samples were seeded in different culture media, incubated, and colony-forming units (CFU/mL) counted. Biochemical tests were performed for suspected colonies of Staphylococcus, Streptococcus and Gram-negative bacilli (GNB). Fungi were visually differentiated into filamentous fungi and yeasts. The results indicated the growth of fungi and Staphylococcus from all sampling locations. GNB growth was observed from all sites sampled from the intraoral X-ray equipment. On the panoramic unit, GNB growth was observed in samples from activator button, keyboard and mouse. In general, a higher number of CFU/mL was present before use. It can be concluded that more stringent protocols are needed to control infection and prevent X-ray exams from acting as vehicle for cross contamination.

Key words: Radiology; digital radiography; infection control; microorganisms; bacteria; fungi.

INTRODUCTION
Radiographic exams area complementary tool for diagnosing major diseases of the oral cavity. Their increasing popularity has encouraged private colleges and clinics to adopt them¹. The advantages of digital radiography include reduced radiation exposure, rapid image acquisition, easy digital storage, and electronic image transmission, as well
as elimination of darkroom requirements and the possibility of image quality enhancement, such as changes in contrast and density.

In digital radiography, image receptors can be of two types: 1) solid electronic sensors such as charge-coupled devices (CCD) and complementary metal oxide semiconductor (CMOS) sensors, which produce images directly; and 2) photostimulable storage phosphor (PSP) plates that must be scanned to convert latent images into visible images.

Digital radiography has many advantages over conventional techniques that use radiographic film as the image receiver. However, since the receptor is reused a number of times, as opposed to the single use of radiographic film, digital systems clearly increase problems associated with infection control. The sensors or phosphor plates cannot be sterilized; thus, it is important to use effective physical barriers to protect them. At dental schools, the sensors are handled by a large number of operators and used on a great many patients, further challenging the effectiveness of infection control. In addition to the sensors, care should be taken with the other equipment involved with digital systems, such as the computer, particularly the keyboard and the mouse, and the actual X-ray machine, whether intraoral or extraoral.

Given the need to evaluate the infection control protocol followed at the Radiology Clinic (School of Dentistry), the aim of this study was to evaluate microbial contamination on the surfaces of the intraoral and extraoral digital radiology equipment used at the Radiology Clinic to identify the different groups of microorganisms present on them, and to compare contamination rates before and after clinic office hours.

MATERIALS AND METHODS

Samples were collected at the Radiology Clinic over three non-consecutive random days at two different times: in the morning, before attending patients, and at the end of the day, after appointment hours and before cleaning and disinfection procedures.

Samples were collected from the following surfaces of the radiological equipment and accessories: 1) digital intraoral system: periapical X-ray machine (tube head, positioning device, control panel and activator button), digital system (sensor), and computer (mouse and keyboard); 2) digital extraoral system: panoramic X-ray machine (temporal support, bite block, control panel and activator button) and computer (mouse and keyboard).

Material was collected using a sterile swab soaked in a test tube containing 10 mL sterile saline solution (0.85% NaCl). After using the swab, it was stored in the same tube until samples were processed. Sterilized 5x5 cm² templates were used to standardize the sampling area. The total area sampled per device was 125 cm². For the activator button, sensor and bite block, where the surface is extremely small, the entire area was used for collection. Throughout the collection process, personal protective equipment was used to avoid cross contamination.

The collected samples were subjected to serial dilution in which 1 mL aliquots were transferred to tubes containing 9 mL sterile saline solution (0.85% NaCl), and so on, until 10⁻² dilution. After homogenization, 100 µL aliquots were dispensed using a pipette and seeded with a Drigalski loop on the surfaces of plates, in duplicate. The following culture media were used: mannitol agar (HiMedia, Mumbai, India), which is selective for the isolation of staphylococci; MacConkey agar (HiMedia, Mumbai, India), which is selective for the isolation of enterobacteria and other Gram-negative non-fermenting bacilli; Sabouraud dextrose agar (HiMedia, Mumbai, India), which is used for the isolation of molds and yeasts; and sheep blood agar (HiMedia, Mumbai, India), which is used for the recovery of streptococci/enterococci, staphylococci and Gram-negative bacilli.

The seeded plates with mannitol agar and MacConkey agar culture media were incubated at 35 ± 2°C in a bacteriological incubator for 24 to 48 hours. The plates containing the blood agar medium were subjected to microaerophilic conditions with a candle in an augmented atmosphere of 5% CO₂ at 35 ± 2°C for 24 to 72 hours. The Sabouraud agar plates were incubated at 27 ± 2°C for up to seven days. After the incubation period, the colonies were counted. The dilution formula was applied to calculate colony-forming units per milliliter (CFU/mL) of each sampling site and averaged, considering the duplication.

Colonies suggestive of staphylococci that were identified in mannitol agar and MacConkey agar culture media were incubated at 35 ± 2°C in a bacteriological incubator for 24 to 48 hours. The plates containing the blood agar medium were subjected to microaerophilic conditions with a candle in an augmented atmosphere of 5% CO₂ at 35 ± 2°C for 24 to 72 hours. The Sabouraud agar plates were incubated at 27 ± 2°C for up to seven days. After the incubation period, the colonies were counted. The dilution formula was applied to calculate colony-forming units per milliliter (CFU/mL) of each sampling site and averaged, considering the duplication.

Colonies suggestive of staphylococci that were identified in mannitol agar and blood agar were subcultured in tryptone soya agar (TSA; HiMedia, Mumbai, India) to remove selective agent interference for Gram stain test confirmation and for biochemical analyses. In the Gram stain test, the...
observation of grouped Gram-positive cocci is confirmatory of the group. The catalase test was used to differentiate staphylococci from streptococci/enterococci because staphylococci behave as catalase-positive bacteria. The coagulase and DNase tests served to differentiate Staphylococcus aureus from other species. At all stages, reference cultures were used as positive controls (Staphylococcus aureus ATCC 33591 and Staphylococcus epidermidis ATCC 12228). Novobiocin antimicrobial susceptibility testing was used to differentiate between Staphylococcus epidermidis and Staphylococcus saprophyticus; the positive control for sensitivity in this test was the presence of a halo equal to or greater than 15 mm. To avoid overestimating the results, the count of GNB colonies on blood agar was disregarded for the locations that presented growth in both mannitol agar and blood agar in the same collection.

Colonies suggestive of streptococci/enterococci in the blood agar medium were subcultured in TSA for confirmation by the Gram stain test, in which Gram-positive cocci are observed in long chains, while for colonies suggestive of enterococci, Gram-positive cocci are observed in short chains or in pairs. To differentiate staphylococci from streptococci/enterococci, the colonies subcultured in TSA were subjected to the catalase test, where catalase-negative colonies were selected. These colonies were further subcultured in blood agar and incubated under microaerophilic conditions for 24 hours to observe the hemolysis pattern.

Colonies suggestive of Gram-negative bacilli (GNB) in MacConkey agar and blood agar were subcultured in TSA and submitted to the Gram stain test. To differentiate between glucose-fermenting and non-fermenting bacilli, the fermentation test was conducted in a glucose broth. Oxidase test strips were used to differentiate between oxidase-positive and oxidase-negative colonies. Tests were also run using the Bactray system (Laborclin, Pinhais, Paraná, Brazil) for the biochemical identification of GNB via pH changes, substrate hydrolysis, and metabolic production. To avoid overestimating the results, the count of GNB colonies in blood agar was disregarded for the locations that presented growth in both MacConkey agar and blood agar in the same collection.

Fungi growing in Sabouraud agar were visually differentiated into filamentous fungi and yeasts. No biochemical test was performed.

The Kolmogorov-Smirnov test was used to verify that the values were normally distributed. The Wilcoxon test allowed a comparison of the CFU counts before and after the clinic’s activities. We used SPSS version 13.0 (SPSS Inc, Chicago, USA). The significance level was 5% (p≤0.05).

RESULTS

Altogether, 78 samples were collected from the Radiology Clinic, with half the samples (three per site) collected before the clinical procedures and the other half (three per site) collected after clinic office hours and before cleaning and disinfection procedures.

Tables 1 and 2 present the data describing the quantitative distribution of the different microorganisms from the collection sites for intraoral and extraoral digital systems, respectively. Fungi were present at all collection sites, both before and after clinic office hours. Staphylococci were also highly prevalent, being absent only in a few specific collection periods. In contrast, GNB were found less frequently, although they were present at all the collection sites before the use of the intraoral equipment.

In assessing the growth of Staphylococcus/ Streptococcus in the intraoral digital X-ray system, all the colonies tested positive for catalase, i.e., no growth of Streptococcus/enterobacteria was observed. After coagulase and DNase testing, Staphylococcus aureus was not isolated. Regarding the novobiocin susceptibility profile, 23.81% were resistant and were identified as Staphylococcus saprophyticus, whereas 76.19% were sensitive and characterized as Staphylococcus epidermidis. In the extraoral digital X-ray system, only one sample, which was collected after the use of the control panel, had a negative result for catalase and was visible as Gram-positive cocci in long chains, which are representative of Streptococcus. This colony was subcultured in blood agar and incubated under microaerophilic conditions for 24 hours, at which point a partial hemolysis pattern was observed that was alpha-hemolytic. The rest of the colonies, which were catalase-positive bacteria, were subjected to coagulase and DNase testing. Only one colony, which was obtained from the activator button before use, showed positive results for both tests and was identified as S. aureus. Regarding the novobiocin susceptibility profile, 31.34% were
resistant and identified as *S. saprophyticus*, whereas 64.93% were sensitive and characterized as *S. epidermidis* (Fig. 1).

In the evaluation of GNB growth on the intraoral digital X-ray system, all samples were negative for the glucose fermentation test. Approximately 30% of the samples were oxidase-positive GNB, and 70% were oxidase-negative GNB. Tests were conducted using the Bactray system, which identified *Proteus mirabilis* on the positioning device, control panel, tube head and keyboard before use and on the sensor and keyboard after use in 52.94% of the samples, with a probability of 90.55%. *Pseudomonas pseudoalcaligenes* was identified on the tube head, positioning device, control panel, button and mouse before use in 29.41% of the samples, with a probability of 83.18%. *Acinetobacter baumannii* / *calcoaceticus* was identified on the sensor, positioning device and tube head before use in 17.65% of the samples, with a probability of 65.5%. Regarding the glucose fermentation test for the colonies identified on the extraoral digital X-ray system, 23.08% of the samples were fermentation-positive bacteria, and 76.92% were fermentation-negative bacteria. Only one oxidase-positive sample was identified. The Bactray system identified *Acinetobacter baumannii* / *calcoaceticus* on the keyboard before use and on the keyboard and activator button after use in 53.85% of the samples, with a probability of 65.5%. *Serratia plymuthica* was identified on the keyboard after use in 15.38% of the samples, with a probability of 62.84%.

*Burkholderia pseudomallei* was identified on the keyboard after use in 7.69% of the samples, with a probability of 48.78% (Fig. 1).

In the evaluation of fungal growth from the intraoral digital X-ray system, 3.64% filamentous fungi and 242 Cristiana P. Malta, et al.

Table 1: Descriptive data (mean, median, minimum, and maximum) of the quantitative distribution of the various microorganisms in the respective collection locations in the intraoral digital system. Data are presented in CFU/mL.

<table>
<thead>
<tr>
<th></th>
<th>BEFORE USE</th>
<th></th>
<th>AFTER USE</th>
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<td>Cocci*</td>
<td>GNB</td>
<td>Fungi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Med</td>
<td>Min</td>
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<td>0.25</td>
</tr>
</tbody>
</table>

*Cocci: Staphylococcus*
### Table 2: Descriptive data (mean, median, minimum, and maximum) of the quantitative distribution of the various microorganisms in the respective collection locations in the extraoral digital system. Data are presented in CFU/mL.

<table>
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<tr>
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<th>Coccì*</th>
<th></th>
<th></th>
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<tr>
<td></td>
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<td>Min</td>
<td>Max</td>
<td>Mean</td>
<td>Med</td>
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</tbody>
</table>

* Coccì: Staphylococcus e Streptococcus

### Table 3: Comparison of CFU/mL counts (median) before and after the clinical procedures, for the intraoral digital X-ray system.

<table>
<thead>
<tr>
<th></th>
<th>Coccì*</th>
<th></th>
<th></th>
<th>GNB</th>
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<th></th>
<th>Fungi</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>p</td>
<td>Before</td>
<td>After</td>
<td>p</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
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<td>0.05</td>
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<td>0.10</td>
<td>0</td>
</tr>
<tr>
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<td>0.46</td>
<td>0.10</td>
<td>0</td>
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<td>1.00</td>
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<td>Keyboard</td>
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<td>0</td>
<td>0.17</td>
<td>0.20</td>
<td>0.10</td>
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</table>

*Coccì: Staphylococcus
p: significance level
p≤0.05: statistically significant difference by Wilcoxon test.

96.36% yeasts were distinguished visually. For the extraoral system, 68.74% filamentous fungi and 31.26% yeasts were distinguished (Fig. 1).

The results of the comparisons between CFU/mL counts before and after the clinical procedures are shown in Tables 3 and 4. For the intraoral digital system, the Wilcoxon test indicated significant differences only for the fungi collected from the tube head, control panel, mouse and keyboard. For the extraoral digital system, the CFU/mL counts...
before and after clinical procedures showed a significant difference only for the fungi collected from the keyboard. Notably, the number of colonies was always higher before clinical procedures.

**DISCUSSION**

Digital X-ray is a major advance in radiographic imaging and is increasingly being adopted by dental professionals. It is already used even in undergraduate courses in dentistry and has great potential to aid diagnosis and treatment procedures and to facilitate image storage, transfer and retrieval. However, the substitution of films by sensors or phosphor plates does not free the digital equipment from cross-contamination; on the contrary, the reuse of the image receptors increases the importance of infection control.

Bacterial, viral, and fungal infections pose a significant hazard in dental practice, and biosafety principles must be followed to prevent contamination of equipment, operators and patients. Dental radiography, which is normally overlooked because it is not routinely associated with needles, sharp instruments and waste blood, has recently become a concern because infectious

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**Table 4: Comparison of CFU/mL counts (median) before and after the clinical procedures, for the extraoral digital X-ray system.**

<table>
<thead>
<tr>
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<th>Fungi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
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*Cocci: Staphylococcus e Streptococcus

p ≤ 0.05: statistically significant difference by Wilcoxon test.
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Many microorganisms are related to dental practice. Even though some of them belong the normal microbiota, they should be considered opportunistic pathogens which can cause human infections constituting a disease when introduced into unprotected sites or in situations of immune system deficiency, depending on their virulence factors. The upper respiratory tract and oral cavity are colonized by numerous microorganisms such as Staphylococcus, Streptococcus, Porphyromonas, Prevotella, Haemophilus, Eubacterium, Enterobacteriacea, Actinomyces, Acinetobacter and Candida.

The genus Staphylococcus is composed of diverse species that can be found in human clinical samples. These microorganisms are important pathogens and, in general, involved in various diseases mediated by toxins, such as skin diseases, bacte­remia, endocarditis, pneumonia, osteomyelitis, septic arthritis, urinary tract infections and opportunistic infections. The species most commonly associated with human diseases are Staphylococcus aureus – the most virulent and best-known member of the genus– and Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis and Staphylococcus saprophyticus. The results of the present study indicated growth of Staphylococcus from all sampling sites, and Staphylococcus aureus, Staphylococcus saprophyticus and Staphylococcus epidermidis were also identified. The rate of contamination with Staphylococcus is of concern because, although these bacteria are members of the normal microbiota of the skin and mucous membranes of humans, they also cause suppuration, abscess formation, various pyogenic infections, and even fatal septicemia.

Streptococci and enterococci are Gram-positive, catalase-negative, and oxidase-negative bacteria that usually grow into pairs and in chains. Among the important streptococci is Streptococcus pyogenes, which is responsible for suppurative afflictions such as pharyngitis, soft tissue infections, and streptococcal toxic shock syndrome and for non-suppurative disorders such as rheumatic fever and glomerulonephritis. Streptococcus agalactiae is responsible for diseases in newborns and infections in pregnant women. Several β-hemolytic streptococci...
are important. \textit{Streptococcus viridans} is responsible for abscess formation in deep tissue; \textit{Streptococcus anginus}, for septicemia in neutropenic patients; \textit{Streptococcus mitis} and \textit{Streptococcus salivarius}, for subacute endocarditis; \textit{Streptococcus mutans}, for tooth decay; \textit{Streptococcus bovis}, for cancer of the gastrointestinal tract; \textit{Streptococcus pneumoniae}, for pneumonia, meningitis, and bacteremia\cite{22}. According to Jorge\cite{23}, only four groups are considered oral streptococci, i.e., the \textit{mutans}, \textit{anginosus}, \textit{mitis}, and \textit{salivarius} groups. In this study, streptococcal contamination of the extraoral X-ray device control panel proved the existence of oral cavity microorganisms on the radiographic equipment; these microorganisms are often present in oral mucosa and in saliva\cite{22}.

The bacilli or Gram-negative rods belonging to the \textit{Enterobacteriaceae} family are widely distributed in nature; found in soil, water, vegetables, and the intestinal tracts of humans and animals\cite{24}. They cause a variety of diseases in humans, including 30\% to 35\% of all bacteremia, over 70\% of urinary tract infections, and a large number of intestinal infections. The \textit{Salmonella} serotype \textit{Typhi}, and \textit{Shigella} and \textit{Yersinia pestis} species are always associated with human disease, while other species such as \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, and \textit{Proteus mirabilis} are commensal members of the normal microbiota that can cause opportunistic infections\cite{25}. \textit{Escherichia coli}, \textit{Klebsiella spp} and \textit{Yersinia} have been detected in the human oral cavity and subgingival samples\cite{23}. Gram-negative non-fermenting bacilli are a non-spor-forming aerobic group that do not use carbohydrates as an energy source or degrade them by fermentative pathways, and have special requirements for growth\cite{24}. They constitute a group of opportunistic pathogens of plants, animals and human beings, and their taxonomic classification has undergone changes in recent years. However, most of the clinically significant isolates are members of five genera: \textit{Pseudomonas}, \textit{Burkholderia}, \textit{Stenotrophomonas}, \textit{Actinobacter} and \textit{Moraxella}\cite{22}.

In the present study, GNB growth was observed from all locations sampled from the intraoral radiographic equipment. On the panoramic unit, GNB growth was observed only from the activator button, keyboard and mouse. The following species were identified: \textit{Proteus mirabilis}, \textit{Pseudomonas pseudoalcaligenes}, \textit{Acinetobacter baumannii}, \textit{calcoaceticus}, \textit{Serratia plymuthica}, \textit{Klebsiella pneumoniae} and \textit{Burkholderia pseudomallei}.

Approximately 80,000 identified species of fungi exist; however, less than 400 are medically important, and fewer than 50 species cause approximately 90\% of fungal infections. Most pathogenic fungi are exogenous, and their natural habitats are water, soil and organic waste. Candidiasis and dermatophytosis are the fungal infections (mycoses) of highest incidence, caused by fungi of the resident microbiota which are highly adapted to survival in the human host\cite{26}. In the present study, although fungi were the most prevalent microorganisms, with growth in all samples and from all sampling sites, they were only identified visually as filamentous fungi or yeasts.

Although no study in the literature has determined the maximum amount of microorganisms allowed in a clinical dental setting, the goal is to reduce this amount as much as possible to promote health and prevent disease. Importantly, we can never be certain whether we are dealing with an immunocompromised patient during a radiographic examination. For these patients, a low number of microorganisms can cause disease, or normal microbiota can cause opportunistic infections.

The results of this study also showed a higher CFU/mL count before the use of the radiographic equipment, possibly due to the timing of the sampling, because the swabbing actions during collection before the use of the equipment may have cleaned the collection sites. A second hypothesis is that the equipment and surfaces are poorly sanitized.

Because potentially infectious individuals are not always identified through information from their medical history or through physical, clinical, and laboratory exams, protective measures should be adopted to prevent or reduce the transmission of pathogenic microorganisms that can cause various types of infectious or contagious diseases. Thus, the dentist is primarily responsible for cross-infection control in the clinical workplace, and must maintain asepsis while conducting X-ray exams and verify that the necessary measures for safe and effective infection control are being followed by all team members. Methods for sterilization, disinfection, mechanical barriers and personal protective equipment should be used in all dental specialty
work, including radiology, to ensure a favorable environment for maintaining the health of staff and patients. Based on the results of this study, acquisition of intraoral and extraoral digital radiographs increases the possibility of cross-infection, creating the need for more stringent protocols for infection control in radiological practice, in order to prevent X-ray exams from being vehicles for cross contamination, particularly at educational institutions.

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