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Microbial Contamination of the Outer Surface of X-ray Films

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Objectives Infection control is one of the most important aspects of dentistry. Since intraoral radiographic films are directly in contact with the oral environment, microbial contamination may transmit infectious diseases. The purpose of this study was to investigate the frequency of microbial contamination of intraoral radiographic films and compare the probable microbial contamination of two intraoral radiographic film brands available in the Iranian market.
Methods in this in vitro, experimental study, 900 radiographic films of two commercial brands, i.e. Intra X-ray and Carestream films were placed in aerobic, anaerobic, and fungal culture media immediately after removal from the packaging in sterile conditions. The samples were transferred to the respective culture media after incubation. The cultured bacteria were Gram-stained, and microscopically observed. The percentage of the contaminated intraoral radiographic films and the type of microbial contamination were reported. Data were analyzed using the Chi-square test.
Results Of all, 32.6% of the Carestream films and 44.6% of Intra X-ray films were infected by aerobic microorganisms, mostly Bacillus. In the anaerobic culture, the turbidity of the medium indicated the possible presence of microorganisms. In the fungal culture, no fungal hyphae were observed microscopically.
Conclusion The results of this study showed that intraoral films cannot be considered sterile. Intra X-ray radiographic films were significantly more contaminated than Care stream radiographic films.
Keywords X-Ray Film; Microbiology; Infection Control; Radiography

Introduction

Infection control is one of the most important aspects of dentistry. Intraoral radiographic films are directly in contact with the patients' oral environment. Since radiographic examination is used for the diagnosis and treatment of most dental problems, contamination of the outer surface of radiographic films can be concerning. Breaching the protective barriers in the oral cavity including the mucosa, dental pulp, or periodontium may lead to bacterial infections. Risk of such infections is higher in patients with oral infections or blood-borne diseases, such as hepatitis or human immunodeficiency virus.[1, 2]

The primary purpose of infection control is to prevent the transmission of diseases and cross-contamination between the patients and the staff or other patients.[3] Infection control in intraoral X-ray imaging includes the following steps: disinfection and covering the X-ray machines and counters, preparation and transfer of the films and other equipment to the radiography room, placing the film in the patient's mouth and exposing the film, transferring the bottles containing films to the darkroom, and film processing in the darkroom.[4] Radiographic films are contaminated with the saliva and sometimes blood. It is recommended to use a sealable plastic cover to avoid film contamination with oral fluids. After performing a radiographic examination, the film should be placed in a standard disinfectant solution. Afterward, the plastic cover should be washed and dried, and then removed.[3] Infection control techniques such as the use of sterile forceps and the method of two gloves and multiple gloves (over gloves) are currently used for radiographic films.[5]

The manufacturers take extreme precautions to ensure that the facilities remain clean.[6] Radiographic film manufacturing companies do not claim that these films are sterile in their available packages. Sterilization is defined as the process of elimination of all living microorganisms, including bacterial spores, while disinfecting techniques refer to reducing the number of microorganisms and their transmission. The disinfecting techniques are suitable for some clinical settings where patients are not at high risk of infection.[7]

In this study, we attempted to estimate the percentage of unused contaminated intraoral radiographic films and their type of microbial contamination. We also compared the probable microbial contamination of the outer surface of intraoral radiographic film brands available in the Iranian market. Finally, we attempted to examine whether the use of radiographic films in the oral cavity is completely safe or not.

Methods and Materials

The study protocol was approved by the institutional review board of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1395.858).

The study was conducted on two brands of intraoral radiographic films namely Intra X-ray (FL series, China)

and Carestream (E-speed, Carestream, Kodak™, USA). All the radiographic films were adult-size (size 2).

The number of samples was calculated to be 75 considering alpha=0.05 and beta=0.1.

In each medium, 150 samples of each film brand were analyzed, with control samples included. Due to the use of three types of primary culture media, a total of 900 radiographic films were studied (450 Carestream and 450 Intra X-ray radiographic films).

The cleanliness of intraoral X-ray films was examined immediately after removal from the packaging. Microbial evaluation of intraoral radiographic films as a non-sterile material was performed according to the guidelines specified by the European pharmacopoeia for microbial evaluation of non-sterile products as follows.[8]

Initially, three types of culture media, tryptic soy broth without dextrose (TSB), thioglycolate fluid medium with USP indicator (Thio) and Sabouraud dextrose broth (SDB; Quelab, Canada) were used. A total of 450 standard wide-mouth sample bottles with a polypropylene cap that contained the above-mentioned media were prepared (150 TSB bottles, 150 Thio bottles, and 150 SDB bottles). Then, the culture media were autoclave-sterilized. Using sterile forceps, gloves, and masks, the samples were placed in the bottles in sterile conditions under a class II laminar flow hood. The same was done for all 900 radiographic films.

Aerobic cultivation: TSB-containing bottles were incubated at 37°C for 24-48 h. After incubation, the bottles were examined for turbidity. Subsequently, a set of opaque bottles was used for Gram-staining. The passage was completed in differential media for the isolation of putative bacteria.

Isolation of bacilli and cocci: After Gram-staining and preparation of microscopic slides, microscopic observation was performed to assess the presence of bacilli and/or cocci.

Isolation of Staphylococcus aureus (S. aureus): The samples that showed presence of Gram-positive cocci in microscopic observation underwent catalase testing to differentiate Staphylococcus from Streptococcus. Mannitol salt agar (Chapman) medium was used to differentiate S. aureus from Staphylococcus epidermidis (S. epidermidis) and Staphylococcus saprophyticus (S. saprophyticus). Then, coagulase testing was performed by the tubular method. This test is positive for S. aureus, and negative for S. epidermidis. (Figure 1)

Figure 1- TSB and Thio containing bottles showing turbidity after incubation

Isolation of S. epidermidis: Novobiocin antibiotic susceptibility testing in blood agar medium was performed to screen coagulase-negative staphylococci, such as S. epidermidis and S. saprophyticus.

Isolation of Micrococcus: Bacitracin is an antibiotic that inhibits the growth of micrococci but causes no inhibitory effect on the growth of staphylococci. In presence of Micrococcus, bacitracin antibiotic susceptibility testing revealed the formation of growth inhibition zone in the blood agar medium.

Anaerobic cultivation: Bottles containing Thio were incubated at 37°C for 24-48 h. The bottles were then examined for turbidity. Positive Thio cultures were placed in an anaerobic jar next to A-type GasPak (Merck™, Anaerocult™, Germany). The passage was made for opaque bottles, and samples were observed through the Gram-staining technique. Because of the lack of facilities for the detection of anaerobic bacteria at the genus and species levels, the experiments were finished at this stage.

Fungal cultivation: Bottles containing SDB were stored at room temperature (25°C) for one week. After this time, the bottles were checked for turbidity. Opaque samples were Gram-stained. If yeast was observed microscopically, fungal contamination was recorded.

It should be noted that standard microbial strains were also cultured with these methods simultaneously to validate the culture media and methods. Bottles containing media without any X-ray film were also used as negative control at the same time.

The percentage of contaminated intraoral radiographic films and the types of microbial contamination were recorded as descriptive data. In case of positive microbial finding, the Pearson Chi-Square test was used to assess the significance of the association between the two commercial radiographic film brands. Data were analyzed by SPSS® version 21. $P \leq 0.05$ was considered statistically significant.

Results

Aerobic culture findings: Gram-staining and microscopic observation showed the presence of Gram-positive bacilli and cocci in our samples. Positive catalase testing confirmed the presence of staphylococci. Growth on mannitol salt agar and positive coagulase testing showed the presence of S. aureus. Novobiocin antibiotic susceptibility testing in blood agar medium revealed the presence of S. epidermidis due to the formation of growth inhibition zone. Finally, bacitracin antibiotic susceptibility testing revealed the formation of growth inhibition zone in the blood agar medium, indicating the presence of Micrococcus. Totally, 116 out of 300 samples (38.6%) were contaminated with aerobic microorganisms. A total of 49 out of 150 Carestream films (32.6%) and 67 out of 150 Intra X-ray films (44.6%) were contaminated. The Pearson Chi-Square test showed a statistically significant difference between the two film brands (P=0.033). The aerobic culture results are presented in detail in Table 1. Most

contamination in both brands was related to bacillus bacteria.

Anaerobic culture findings: The turbidity seen in the Thio medium indicated the presence of anaerobic bacteria. (Figure 2) The result of Gram-staining of the positive samples was then compared with aerobic culture findings. These bacteria were similar to the detected bacteria in aerobic culture which are also classified as facultative anaerobes; thus, we could not prove the presence of obligate anaerobic microorganisms.

Fungal culture findings: No fungal hyphae were observed microscopically in the samples obtained from opaque SDB culture media.

Table 1- Infected films with aerobic culture bacteria

Films brand	Genus				No Bacteria	Total
	Bacillus	***S. aureus***	***S. epidermidis***	***Micrococcus***		
Carestream N (%)	42 (28%)	101 (67.3 %)	3 (2%)	2 (1.3%)	101 (67.3%)	150 (100%)
Intra X-ray N (%)	60 (40%)	83 (55.3 %)	4 (2.6%)	2 (1.3%)	83 (55.3%)	150 (100%)

Figure 2- Coagulase testing. The presence of *S. aureus* was revealed by coagulation found in the left test tube.

Discussion

Infection control is a major concern in dentistry. Since intraoral radiography is applied in the diagnosis and treatment of most dental problems, contamination of the outer surface of radiographic films can be one of the challenging issues.[1] However, little research has been done in this respect.

The results of this study showed that X-ray films could not be considered germ-free. Although contamination alone does not necessarily indicate development of a disease, presence of bacteria on the surface of radiographic films should not be overlooked. Besides, contamination of the outer surface of Chinese Intra X-ray radiographic films was higher than that of Carestream radiographic films.

Staphylococcus was among the isolated bacteria. Gram-positive cocci include Staphylococcus, Aerococcus, Micrococcus and Peptococcus and only one of them is pathogenic, which is S. aureus. It is the cause of various superficial and deep infections. There is an enzyme called coagulase on the surface of this bacterium known as the cell wall attached coagulase or clumping factor, which causes resistance and stability of bacteria in the tissues. S. aureus induces rapid accumulation and forms a visible clot in the culture medium by coagulase. But S. epidermidis and S. saprophyticus do not produce any clot. S. epidermidis is a commensal on the skin. it is not pathogenic or has very little pathogenicity. In some cases, however, S. epidermidis and Micococcus can cause severe infections and bacteremia as opportunistic pathogens.

Bacillus is a rod-shaped, Gram-positive bacterium that grows in aerobic conditions. These bacteria produce heat-resistant spores. They are abundant in the dust in the form of saprophyte and therefore, a high proportion of bacteria contaminating the culture media belong to this genus. Bacillus anthracis is the cause of anthrax, and the main pathogen in this genus i.e. Bacillus cereus causes food poisoning. Bacillus subtilis has also been involved in some infections of the human body.[9]

Ranjbari et al. carried out a similar study on surface contamination of dental anesthesia cartridges at Shahid Beheshti University of Medical Sciences.[10] They examined cartridges made by 4 different domestic and foreign manufacturers. Three media including TSB, Thio and SDB were used, which were similar to our study. They showed that 6.3% of aerobic cultures, 1.8% of anaerobic cultures and 0.7% of fungal cultures were contaminated with microorganisms. They concluded that the contamination of cartridges was not negligible, and placing them in a sterile surgical set should be avoided. European cartridges were completely germ-free. Aerobe contamination was higher than anaerobe and fungal contamination in domestic cartridges, which corroborate our results.

The type of bacteria found in this study was more or less similar to that in a study by Fox et al. They investigated X-ray cassettes as a possible source of pathogens that could

cause nosocomial infections. Forty cassettes in a diagnostic imaging department in England were swabbed to examine bacterial contamination, specifically for presence/absence of methicillin-resistant S. aureus. Their results demonstrated a large amount of growth in samples taken from the cassettes. Coagulase-negative Staphylococcus, micrococci, diphtheroids and species of Bacillus were identified. They showed that X-ray cassettes and imaging plates were often exposed to pathogens and should be considered as potential sources of cross-infection. Furthermore, the patient's skin is usually in direct contact with the X-ray cassette or imaging plate.[11]

Kalathingal et al. examined the contamination of photostimulable phosphor (PSP) plates which were routinely used in the clinics. PSP plates were placed in blood agar medium; 56% of the culture media exhibited growth of bacterial colonies. Some of those bacterial colonies were cultured on mannitol salt agar; 76.47% of them indicated growth and 69% were Gram-positive [12]. In a study by Souza et al, microbial contamination of 50 PSP plates in dental radiology services was evaluated. They used moist sterile swabs that were rubbed on the PSP plates and then cultured them on Mueller Hinton agar plates; 73.3% of the samples were contaminated, predominantly with bacteria of Staphylococcus genus.[13] The main bacteria responsible for contamination were of different genus and the rate of PSP plate contamination was higher than the rate we found in our study. The difference is mainly because of the fact that they studied the PSP plates which are used multiple times. In all digital systems, the image receptor is reused several times in contrast with the single use of conventional radiographic films.[14] Intraoral digital image acquisition increases the probability of cross-infection and needs stricter requirements for infection control.[15]The standard precautions are a wise strategy regardless of the type of image receptor used.[16]

Freitas et al. assessed the rate of cross-infection in dental X-ray devices. They investigated the presence of pathogenic microorganisms in high-touch areas of dental X-ray systems. The results showed that 70% of the surfaces had microbial contamination. The most frequent microorganisms were from the Staphylococcus genus[17] which agreed with our results. In the former studies, samples were obtained from surfaces that are used several times, as opposed to our study which revealed the presence of hazardous microorganisms on the surface of unused intraoral films. However, in order to prevent cross-contamination and minimize the risk of disease, it is extremely important that the practitioners be aware of effective safety measures in all stages of oral radiography and follow them.[18]

This study had some limitations. It was not possible to study other brands of intraoral radiographic films due to large number of samples, increased workload, high cost, and prolongation of the research process. In this study, the possible presence of anaerobic contamination of X-ray films was indicated, but it was not feasible to detect bacteria at the genus and species levels due to the lack of specialized laboratory facilities.

Based on the results of this study, investigations on other brands of radiographic films and also identification of anaerobic contaminants by the genus and species are suggested. There are other bacteria in the environment that play a role in pathogenicity and should be investigated as well.

Conclusion

Based on the results of this study, we can conclude that X-ray films are not sterile. They may be infected with some pathogens, such as aerobic microorganisms. Intra X-ray radiographic films were significantly more contaminated than Carestream radiographic films.

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Conflict of Interest

No Conflict of Interest Declared■

References

1. Sebastiani FR, Dym H, Kirpalani T. Infection control in the dental office. Dent Clin North Am. 2017;61(2):435-57.
2. Mustafa EA, Humam AK, Al-Mosuli TA. Evaluation of dental assistant awareness concerning infection control policy of blood borne diseases. J Int Dent Med Res. 2015;8(1):21-4.
3. Mallya SM. Quality Assurance and Infection Control. White and Pharoah's Oral Radiology E-Book: Principles and Interpretation. 2018; chap12:270.
4. White SC, Heslop EW, Hollender LG, Mosier KM, Ruprecht A, Shrout MK. Parameters of radiologic care: An official report of the American Academy of Oral and Maxillofacial Radiology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91(5):498-511.
5. Thomas S, Agarwal M, Mehta G. Intraoperative glove perforation—singleversus double gloving in protection against skin contamination. Postgrad Med J. 2001;77(909):458-60.
6. Bushong SC. Radiologic science for technologists-E-book: physics, biology, and protection: Elsevier Health Sciences; 2018. Chap:12:P: 208.
7. Waltz PK, Zuckerbraun BS. Surgical site infections and associated operative characteristics. Surg. Infect. 2017;18(4):447-50.
8. European Directorate for the Quality of Medicines & HealthCare: Microbiological Quality of non-sterile Pharmaceutical Preparations and Substances for Pharmaceutical use. European Pharmacopoeia 70. 2011, Strasbourg: Chap5.1.4, EDQM.

9. Carroll KC, Butel JS, Morse SA. Jawetz Melnick & Adelbergs Medical Microbiology 27 E. McGraw Hill Professional; 2015 Chap:11:P:175-8.
10. Ranjbari M, Yaghmaei M, Hakemi-Vala M, Hosseinpour S. Assessment of bacterial contamination of the external surface of anesthetic cartridges. J Dent Sch. 2015;33(4):277-81.
11. Fox M, Harvey JM. An investigation of infection control for x-ray cassettes in a diagnostic imaging department. Radiography. 2008;14(4):306-11.
12. Kalathingal S, Youngpeter A, Minton J, Shrout M, Dickinson D, Plummer K, et al. An evaluation of microbiologic contamination on a phosphor plate system: is weekly gas sterilization enough? Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(3):457-62.
13. Souza TM, Castro RD, Vasconcelos LC, Anjos Pontual A, Perez FM, Anjos Pontual ML. Microbial contamination in intraoral phosphor storage plates: the dilemma. Clin Oral Invest. 2017;21(1):301-7.
14. Negron W, Mauriello SM, Peterson CA, Arnold R. Cross-contamination of the PSP sensor in a preclinical setting. J Dent Hyg. 2005;79(3):8.
15. Gumru B, Tarcin B, Idman E. Cross-contamination and infection control in intraoral digital imaging: A comprehensive review. Oral Radiol. 2020;6:1-9.
16. Bartoloni JA, Chariton DG, Flint DJ. Infection control practices in dental radiology. Gen Dent. 2003;51(3):264-71.
17. Freitas CVS, Dias LS, Araújo CS, Silva VC, Neto VM, Souza JIL. Assessment of microbiological contamination of radiographic devices in School of Dentistry. Braz Dent Sci. 2012;15(1):39-46.
18. Fernandes LM, Zapata RO, Rubira-Bullen IR, Capelozza AL. Microbiologic cross-contamination and infection control in intraoral conventional and digital radiology. RGO. 2013;61(4):609-14.

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