

Possible Microbial Contamination During the Development of Intra-Oral Films

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Abstract: The level of possible microbial cross-contamination in an automatic dental radiograph processor was investigated. Pure cultures of *Staphylococcus aureus* ATCC 6538 P, *Escherichia coli* ATCC 1103 and *Candida utilis* ATCC 18804 and a mixed culture of these three microorganisms were prepared. In the first five days of the week, 90 films contaminated with one of these pure cultures were developed. This was followed by the processing of 36 uncontaminated films that were developed in the last two days of the week. The same procedure was repeated for the remaining two pure and mixed microbial cultures. During the study, microbial samples were taken at the end of every day, from the

inlet and outlet rollers of the automatic film processor, from the first and second developing solutions and from the intraoral films. Microbiologic counts were made using the pour plate method. The maximum contamination level was observed on the films immersed in the mixed culture; however, no or minimal contamination was established with the films kept in pure cultures. Microorganisms were found on uncontaminated films that were developed with the processing solution of the mixed culture group.

Key Words: Cross-contamination, intra-oral films, microorganisms

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Introduction

In everyday dental practice, clinicians come into contact with various microorganisms that cause many serious diseases such as herpes, pneumonia, tuberculosis, hepatitis B and AIDS. The dentists and radiology technicians may also play a role in cross-contamination between patients, by transporting the microorganisms via radiographic equipment and solutions. Therefore, the contamination by these microorganisms may be observed not only during the dental treatment and surgery, but also during the exposure and development of intraoral films.

Strict infection control procedures have been presented by the ADA (1-3) and by several other researchers (4-7) to prevent this cross-contamination. However, the lack of a generally accepted protocol necessitates further application of studies on the issue. Therefore, the purpose of this study was to examine the microbial contamination during the development of previously contaminated dental intraoral radiographic films.

Materials and Methods

Dental radiographic film processor

An automatic dental radiograph processor (Dürr XR,

Dürr Dental GmHu Co KG, Germany) was used. It was cleaned and disinfected (Sacti-med Steril, Lever Industriel) prior to use, and after each 7-day test period.

Radiographic films

The intraoral radiographic films (Minmax TRX-S, The Minimax Company, Chicago, IL 60660) were exposed with a standard dental radiography unit.

Microbial cultures

In order to contaminate the radiographic equipment, pure cultures of *Staphylococcus aureus* ATCC 6538 P, *Escherichia coli* ATCC 1103 and *Candida utilis* ATCC 18804 and a mixed culture of the above-mentioned type strains were used. Cultures were grown in Nutrient Broth (Oxoid) at 37°C for 24 hours. Cultures of these microorganisms and the mixed culture were freshly prepared every day.

Procedure

The fresh cultures in nutrient broth were centrifuged at 5000 rpm for 5 minutes. After centrifugation supernatant was decanted and harvested cells were resuspended in saline. This procedure was repeated twice, and then the culture solution was standardized to 1 absorbance at

420 nm by a spectrophotometer (Jenway 6105 UV/VIS). 50 ml culture solutions containing 5×10^{10} CFU/ml *S. aureus* ATCC 6538 P, 1.3×10^9 CFU/ml *E. coli* ATCC 1103 and 3×10^8 CFU/ml *C. utilis* ATCC 18804 were prepared. In order to simulate the clinical practice and contaminate the films, the film package was held with gloved fingers, and was immersed in culture solution for 3-5 seconds. Then, the film package was opened and the films were placed in the automatic processor. Eighteen periapical films were developed each day. During the first 5 days of the week, the films which were contaminated with the cultures were developed. In the last two days, films were developed without prior contamination. After developing, six films were randomly selected, and three of them were picked up by sterile holders, and three were contaminated by gloved fingers. Then, each was plated to petri dishes containing proper media by pressing both sides of the film, and were inoculated.

Each day, after the films were developed, specimens from the inlet and outlet rollers of the processor were obtained by sterile swabs. Additionally, 1 ml solutions from the developer and fixer were collected daily by sterile pipettes. The swabs smeared on the rollers were placed in 10 ml sterile saline, and serial dilutions were prepared. The specimens from the developer and fixer were diluted, and microorganism counts were determined by the pour plate method (8).

Mannitol Salt Agar (Oxoid) for *S. aureus* ATCC 6538 P, Endo Agar (Oxoid) for *E. coli* ATCC 1103, Biggy Agar

(Oxoid) for *C. utilis* ATCC 18804 and Plate Count Agar (Oxoid) for mixed culture were used. The petri dishes were inoculated by keeping them under optimum conditions for suitable periods, and the microorganism counts were determined. The results were analyzed by analysis of correlation in Ege University Computer Research Center to investigate the effect of radiographic processing on the CFUs. The statistical confidence interval was 95%.

Results

After contaminating the film packages with pure cultures of *S. aureus* ATCC 6538 P, *E. coli* ATCC 1103, *C. utilis* ATCC 18804 and the mixed culture of these microorganisms, the results revealed that the inlet and outlet rollers of the automatic radiographic processor were contaminated, but no contamination in the processing solutions (developer and fixer) was observed (Tables 1,2).

Microbial contamination was observed on some of the processed films which were held by sterile holders; but a very high level of contamination was observed in the films which were held by the gloved fingers that were immersed in culture solution.

With *S. aureus* ATCC 6538 P, decreasing amounts of microorganisms were observed on the inlet rollers in five days. On the other hand, the contamination levels of the outlet rollers fluctuated during the test period. On the films which were held with sterile holders, contamination

Table 1. Microbial contamination during the development of radiographic films which were previously contaminated with *S. aureus* ATCC 6538 P and *E. coli* ATCC 1103.

DAYS	<i>S. aureus</i> ATCC 6538 P (CFU)					<i>E. coli</i> ATCC 1103 (CFU)				
	Inlet Rollers	Developer	Fixer	Outlet Rollers	*Film surface	Inlet Rollers	Developer	Fixer	Outlet Rollers	*Film surface
1	2.8×10^7	-	-	50	-	6.0×10^5	-	-	-	-
2	2.5×10^7	-	-	10	-	1.0×10^5	-	-	-	-
3	4.8×10^6	-	-	80	-	7.25×10^3	-	-	-	-
4	6.9×10^6	-	-	10	1	3.0×10^3	-	-	-	-
5	2.0×10^5	-	-	10	6	90	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-

- no growth

* films held with sterile holder

Table 2. Microbial contamination during the development of radiographic films which were previously contaminated with *C. utilis* ATCC 18804 and mixed culture.

DAYS	<i>C. utilis</i> ATCC 18804 (CFU)					Mixed Culture (CFU)				
	Inlet Rollers	Developer	Fixer	Outlet Rollers	*Film surface	Inlet Rollers	Developer	Fixer	Outlet Rollers	*Film surface
1	1.16 x 10 ⁵	-	-	-	2	2.11 x 10 ⁶	-	-	20	4
2	2.5 x 10 ⁷	-	-	-	-	9.2 x 10 ⁶	-	-	61	56
3	4.8 x 10 ⁶	-	-	-	-	1.13 x 10 ⁷	-	-	60	114
4	6.9 x 10 ⁶	-	-	-	3	2.75 x 10 ⁷	-	-	56	118
5	2.0 x 10 ⁵	-	-	-	16	4.58 x 10 ⁷	-	-	55	142
6	-	-	-	-	-	3.23 x 10 ⁷	-	-	10	22
7	-	-	-	-	-	840	-	-	-	-

- no growth

* films held with sterile holder

was observed on the film surfaces on days 4 and 5, but there was no contamination on days 6 and 7 (Table 1). The correlation analysis of these results revealed that there was a negative and statistically significant relation between the microbial contamination on the inlet rollers and the time period (days) ($r = -0.884$, $p < 0.05$). On the outlet rollers, a negative but statistically insignificant relation was observed ($r = -0.609$, $p > 0.05$).

With *E. coli* ATCC 1103, a significant decrease in the microbial contamination of the inlet rollers was observed during the first five days, but on days 6 and 7, there was no contamination. Likewise, on the outlet rollers and on the film surfaces, no microbial contamination was recovered (Table 1). The relation between the microbial contamination on the inlet rollers and the time period was statistically insignificant ($r = -0.695$, $p > 0.05$).

With *C. utilis* ATCC 18804, microbial contamination on the inlet rollers was constant in 5 days, but no contamination was observed on days 6 and 7. On the outlet rollers, no *C. utilis* was recovered. On days 1, 4 and 5, microbial contamination was revealed on the film surfaces. The relation between the microbial contamination of the film surfaces and the time period was statistically insignificant ($r = -0.624$, $p > 0.05$).

With mixed culture, an increasing amount of microbial contamination was observed during the first 5 days. On days 6 and 7, this amount decreased. On the outlet rollers and film surfaces, microbial contamination was recovered for 6 days, but none was observed on day 7. The relation

between the microbial contamination on the inlet rollers and the film surfaces was statistically significant ($r = 0.871$, $p < 0.05$). The same relation between the outlet rollers and film surfaces was again statistically significant ($r = 0.844$, $p < 0.05$).

Discussion

In this study, the experimental microorganisms were used because of their possible presence in the oral cavity, and their potential pathogenicity. *S. aureus* ATCC 6538 P is a species of Gram (+), opportunistic pathogen (9) commonly observed in the oral flora (10). In this experiment, this bacteria was resistant to the radiographic processing of the films. *E. coli* ATCC 1103, a species of Gram (-) flora found in the intestinal tract is also an opportunistic pathogen (11) and was the most fragile microorganism tested. *C. utilis* ATCC 18804 is a yeast found in the oral cavity and is observed to be the most resistant to radiographic processing (12). The overall results obtained by application of these 3 microorganisms are in accordance with those of Bachman et al. (11).

In this study, a mixed culture prepared with these 3 organisms was also used. As seen in Tables 1 and 2, the highest contamination was observed with this mixed culture group. We think that these results are very important, since the mixed culture group resembles the mixed microbial oral flora (8). The reason for the highest contamination observed in this group may be the effect of synergism between these three microorganisms (13).

The number of microorganisms in the culture solutions which were used to contaminate the paper-covered film packages was 3×10^8 - 5×10^{10} CFU/ml. The concentrations of the solutions were intentionally prepared high, because the contamination of the film packages was intended rather than the films themselves.

An uncountably high number of microorganisms were observed on the films that were held with gloved fingers. On some of the films that were held with sterile holders, no contamination was found, but on some others, only randomly localized microorganisms were found. This observation is found to be important in order to show the cross-contamination within the radiographic processor.

While some degree of contamination was observed on the surfaces of the rollers, none was found in the developer and fixer. This may be due to the pH and various chemical components of the processing solutions. This finding is in agreement with the results of Katz et al. (14), but Stanczyk et al. (15) and Bachman et al. (11) have observed minimal contamination in the processing solutions.

In our study, the contamination from the atmosphere of the darkroom, which is accepted as an important contamination source (16), was prevented by selective media. Also, the contamination which may occur during the microbial processes was eliminated. Therefore, only the cross-contamination between the dentist, radiology technician and the patient was established. In conclusion, in our study, the initial inoculum of the cultures was 3×10^5 - 5×10^{10} CFU/ml, and this level decreased to 1.7×10^5 - 1.9×10^7 CFU/ml in 5 days on the inlet rollers, and to 0-87 CFU on the surfaces of the films which were held by sterile holders after the processing was completed. This is a significant decrease. However, since the probability of infection is related to virulence and time, in addition to the number of microorganisms, the risk of cross-contamination should not be overlooked. Therefore, in order to prevent this cross-contamination, strict infection control procedures should be applied not only in dental operating rooms and laboratories, but also in the radiographic environment.

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