

# Microbiological analysis of orthodontic pliers

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## Abstract

**Objective:** To evaluate bacterial contamination of orthodontic pliers used in an academic setting. **Methods:** Thirty-four pliers were selected — 17 band remover pliers and 17 bird beak pliers. The control group was composed of 3 previously autoclaved pliers of each model. After use, the pliers in the experimental group were immersed in 10 ml of brain-heart infusion (BHI) culture medium for 2 minutes, incubated at 37° C for 24 to 48 h and seeded in duplicates in different agar-based solid culture media to detect and identify microbial agents. **Results:** Microbiological analyses revealed that there was contamination in both types of orthodontic pliers. Several bacteria were detected, predominantly staphylococcus and isolated Gram-positive (G+) cocci. The band remover pliers had a greater contamination rate and mean values of  $2.83 \times 10^9$  and  $6.25 \times 10^9$  CFU/ml, with variations according to the type of culture medium. The 139 pliers also had all types of bacteria from the oral microbiota at values that ranged from  $1.33 \times 10^8$  to  $6.93 \times 10^9$  CFU/ml. The highest mean value was found in the medium to grow staphylococci, which confirmed, in certain cases, the presence of *Staphylococcus aureus*, which are not part of the normal oral microbiota but are usually found in the nasal cavity and on the skin. **Conclusion:** Orthodontic pliers were contaminated as any other dental instrument after use in clinical situations. Therefore, they should undergo sterilization after each use in patients.

**Keywords:** Dental instruments. Orthodontics. Infection control. Contamination. Microbiology.

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## INTRODUCTION

The oral cavity has a large variety of microorganisms that form a complex environment and a diverse and often pathogenic microbiota.<sup>28</sup> Therefore, special attention should be paid to infection control and biosafety in dentistry, and procedures should be adopted to prevent and significantly reduce the chances of cross infection between patients as well as between patient and dentist.<sup>14</sup>

Infections may be transmitted by direct contact with blood and oral fluids, or, indirectly, by contact with contaminated instruments or surfaces. Some of the potentially transmissible pathogens are hepatitis B and C (HBV and HCV), herpes simplex and human immunodeficiency (HIV) viruses, *Mycobacterium tuberculosis*, different *Staphylococcus* and *Streptococcus* strains, and other microorganisms responsible for upper respiratory tract infections.<sup>2</sup> Not all individuals with important diseases can be identified before a procedure is performed; therefore, all patients, indiscriminately, should be considered potentially contaminated, and, consequently, standard precautions should be taken in all procedures with all patients.<sup>11</sup>

The terms “sterilization” and “disinfection”, although clearly different, are often confused and used incorrectly. The destruction of all forms of microbial life, including viruses, is obtained by means of sterilization. Disinfection, in turn, destroys pathogenic microorganisms but does not eliminate sporebearers and resistant microorganisms, such as the etiological agents of tuberculosis and hepatitis.<sup>3,16,10</sup>

The instruments used in medical and dental practice are classified into three categories according to the risk of infection, the need to sterilize them between uses, and their level of contamination:<sup>20,29</sup>

» Critical: They should be discarded or undergo sterilization because they penetrate soft tissue or bone.

» Semicritical: Instruments that touch oral tissues but do not penetrate hard or soft tissues. They should be sterilized after each use; if sterilization is not possible because the material is not heat resistant, the instruments should at least undergo high-level disinfection.

» Noncritical: They touch only intact skin and should only be disinfected or cleaned.

In orthodontics, concerns with infection control have intensified after the increase of cases of HIV infection, although hepatitis B and C infections, which have a high level of contamination, have been around for a long time.<sup>14</sup> Of all dental healthcare personnel (DHCP), the rate of hepatitis B infection among orthodontists is very high,<sup>10,13,27</sup> second only to oral surgery specialists,<sup>7</sup> as saliva is as infectious as blood.<sup>13</sup>

Clinical orthodontics, a specialty that usually has more patients than other dental specialties, demands planning and organization of sterilization and disinfection procedures to ensure greater protection to both patients and DHCP.<sup>18,30</sup> Disinfection does not replace sterilization and, therefore, all material that can undergo sterilization should never be only disinfected.<sup>9,10</sup> However, a common error among orthodontists is to see disinfection as an alternative to sterilization.<sup>10</sup>

This study evaluated bacterial contamination in the active tip of orthodontic pliers used in patient care by orthodontics graduate students using a microbiological method and the identification of bacterial agents.

## MATERIAL AND METHODS

### Sample selection

Instruments ready for clinical use were collected to analyze the potential of microbial contamination of orthodontic pliers. Sample selection was random and took the students by surprise during their clinical practice classes. Therefore, they had not time to perform procedures that might change statistical data or

microscopic findings. The sample comprised 17 samples of bird beak pliers, type 139 and 17 of band remover pliers, type 347. The control group had 3 samples of 139 pliers and 3 of the 347 pliers, at a total of 6 previously sterilized pliers (autoclave) not used in any clinical procedure. These instruments were chosen because they are widely used in everyday orthodontic procedures: The 139 plier because it is made of metal only, and the band remover pliers (347), because they have a plastic component in its structure that, when pliers are used, is directly in contact with oral tissues.

### Culture media

The brain-heart infusion (BHI) medium used for the immersion of pliers is a liquid medium for the enrichment and proliferation of microbial cells to increase the number of bacteria in the sample. After dilution, cultures were seeded in duplicates in the following solid culture media with 2% agar: blood agar (BA) and nutrient agar (NA) for total count of grown colonies; eosin methylene blue agar (EMB) for the selection of gram-negative bacteria; mitis-salivarius agar (MS), for the selection of Streptococci; and mannitol salt agar (Chapman), for the selection of Staphylococci. Culture media used in this study were produced by Vetec Química Fina Ltda (Duque de Caxias, Brazil).

### Microbiological analysis

The orthodontic pliers under analysis, as well as the control instruments, had their active tips immersed for 2 minutes in 10 ml BHI. Immediately after that, the samples were incubated for 24 to 48 hours at 37°C. The samples containing BHI inoculated by the pliers underwent successive dilutions in inert saline solution (0.9% NaCl) to obtain different concentrations for each sample until a dilution of  $10^{-5}$  was obtained. The purpose of dilution was to reduce bacterial cell concentration in liquid medium for later counting.

Immediately after dilution, the samples were seeded in the different solid media described before and later incubated for 24 to 48 hours at 37°C. After that, colony forming units (CFU) in the Petri dishes were counted for comparisons and statistical analyses. Dishes with very high bacterial growth, which made counting impossible, were classified as “uncountable” ( $>10^{10}$  CFU/ml). The few cultures where no colonies were found were called “null”. A final mean number of bacterial cells per BHI milliliter was calculated using the two counts for each dilution, as long as there was no significant differences between the duplicates. Counts for the same dilution and medium that had great differences in the number of colonies were excluded from the study. Therefore, only the duplicates whose scores were equivalent were kept in the study, which ensured the reliability of results. Using the individual mean CFU/ml for each dilution, the general mean for each culture medium was calculated according to the type of pliers.

The shape and color of colonies for each culture medium were analyzed; bacteria in those colonies were examined under light microscopy and classified using Gram staining.

### Result analysis

Results of total number of grown colonies for each instrument were recorded and compared with results of the different pliers under study and between the different culture media. For those purposes, the Student t test and analysis of variance (ANOVA) were used. The level of significance was set at 5%. The SPSS 15.0 software was used for data analysis.

## RESULTS

### Growth in enrichment and seeding medium

After the pliers were immersed in 10 ml of BHI (enrichment medium), stored and incubated at 37°C for 24 to 48 hours, the liquid medium was turbid and microbial cells were deposited

on the bottom of the test tubes in 32 of the 34 samples. This indicated that there was proliferation of the microorganisms collected from the instrument surfaces and that they had microbial contamination. The fact that BHI remained clear and clean, as in the control group, in two samples, one of the 139 plier and one of the 347 plier, indicated that the instruments had been previously sterilized. After dilution, the BHI samples were seeded in duplicates in the Petri dishes containing agar. After the 24 to 48 h incubation time at 37°C, colonies were found in most cultures.

### Number of CFU per milliliter

For statistical and comparative analyses, CFU were counted whenever possible.

Table 1 shows that the greatest discrepancy of mean CFU/ml values between instruments was found in NA, a nonselective and nondifferential medium. Band remover pliers had a mean contamination rate 10 times greater than that of 139 pliers, and the differences between the two types of pliers were statistically significant ( $p=0.008$ ).

In MS and Chapman media, differences were also found in mean values between the pliers, and these findings may be correlated with practical activities. The significantly greater mean ( $p=0.009$ ) number of colonies in Chapman culture, a selective medium for *Staphylococcus* sp and a differential medium for *S. aureus*, obtained in the 139 plier group suggests a greater contact of this type of pliers with the skin. These bacteria colonize the surface of human skin and the nasal cavity mucosa<sup>15</sup>. Such findings may suggest that the pliers were used to produce other orthodontic devices, that is, in laboratory. Moreover, in the 139 group, ANOVA results revealed that the Chapman medium was the only one that had a significant difference from the NA medium, which had the lowest number of CFU/ml.

In contrast, the MS medium had a high, but not significantly different, mean CFU/ml value for the band remover pliers, which indicates a greater trend towards contamination of this type of pliers. As this medium is selective for *Streptococcus* and differential for *S. mutans*, this result may be explained by the direct contact of the instrument with the surfaces of

TABLE 1 - Mean CFU/ml in samples collected from 139 and 347 pliers, grown in BHI and seeded in different solid culture media.

Culture media	Brief description	Number of samples		CFU/ml		t Test
		model 139	model 347	model 139	model 347	
Nutrient agar (NA)	Rich medium	21	19	$1.33 \times 10^9$	$2.83 \times 10^9$	0.008*
Blood agar (BA)	Rich medium	26	22	$3.66 \times 10^9$	$4.65 \times 10^9$	0.492
Eosin methylene blue agar (EMB)	Gram-negative selective medium	24	22	$3.00 \times 10^9$	$2.99 \times 10^9$	0.992
Mannitol salt agar (Chapman)	Gram-positive selective medium ( <i>Staphylococcus</i> sp.)	26	22	$6.93 \times 10^9$	$3.19 \times 10^9$	0.009*
Mitis salivarius agar (MS)	Gram-positive selective medium ( <i>Streptococcus</i> sp.)	6	8	$3.34 \times 10^9$	$6.25 \times 10^9$	0.317

\* Statistically significant results of comparisons between 139 and 347 models of pliers using t test (level of significance = 5%).

teeth, gingiva and mucosa in the posterior region of the oral cavity, where bacterial plaque often accumulates. These bacteria are part of the oral microbiota and are classified as substantially more carcinogenic.<sup>6</sup>

Finally, in the EMB, a selective medium for Gram-negative bacteria, and the BS cultures, a rich medium, mean number of CFU/ml in BHI was similar for 139 pliers and band remover pliers.

### General morphological characteristics of colonies and microorganisms grown in each culture medium

The microbial colonies had variable shapes, sizes and colors. For the analysis under light microscopy, 41 Petri dishes of all types of media were selected to include the greatest variety of samples of grown colonies. The Gram method was used for slide staining.

Table 2 and Figure 1 describe the most frequent shape of the colonies and the type of

bacteria in them. In the dishes with NA, yellow colonies were predominant. According to microscopic analysis, they were primarily composed of staphylococci or Gram-positive bacilli. In BA, the most common bacterial types were staphylococci and G+ streptobacilli, found in white and light yellow colonies with smooth or rough surfaces. Moreover, some colonies had microorganisms that could destroy the blood cells found in the BA cultures. The translucent or greenish halos around the different colonies seen in dishes with that agar confirmed the presence of hemolytic bacteria. Streptococci found in the oropharynx, in pharyngeal inflammations and in skin infections are examples of hemolytic microorganisms.<sup>19</sup> In EMB medium, several types of bacteria were visualized, and there was a predominance of isolated cocci and G+ streptobacilli. Isolated G+ and G- bacilli were also found; they formed purplish colonies with an irregular surface and

TABLE 2 - Colonies, shape organization and classification of most frequent bacteria in different culture media according to Gram staining.

Culture media	Most common colony configuration	Shape, organization and classification of bacteria according to Gram staining
Nutrient agar (NA)	Yellow, smooth	G+ staphylococci *, isolated G+ bacilli
	White, smooth	Isolated G+ cocci, G+ coccobacilli, G- streptobacilli **
	Orange, smooth	G+ staphylococci, G+ coccobacilli
Blood agar (BA)	Yellow, smooth	G+ staphylococci, G- sarcinae, isolated G+ bacilli, G+ coccobacilli
	White, smooth	G+ staphylococci, G+ streptobacilli, isolated G+ cocci, G+ streptococci
	Rough, white	G+ streptobacilli, isolated G+ bacilli, isolated G+ cocci, G+ streptococci, G+ coccobacilli, G+ diplococci
Eosin methylene blue agar (EMB)	Purple, rough	Isolated G+ cocci, isolated G+ and G- bacilli, G+ streptobacilli
	Pinkish, smooth	Isolated G+ cocci, isolated G+ bacilli, G+ diplococci, G+ diplobacilli
Mannitol salt agar (Chapman)	Yellow, smooth	G+ staphylococci, isolated G+ cocci, G+ streptobacilli, isolated G+ bacilli, G+ tetrad-forming organisms
	Pinkish, smooth	G+ staphylococci *, isolated G+ cocci
Mitis salivarius agar (MS)	Blue, smooth	Isolated G+ cocci, isolated G+ and G- bacilli, G- sarcinae
	Clear, smooth	Isolated G+ cocci *, G+ streptobacilli

\* G+ = Gram positive; \*\* G- = Gram negative.

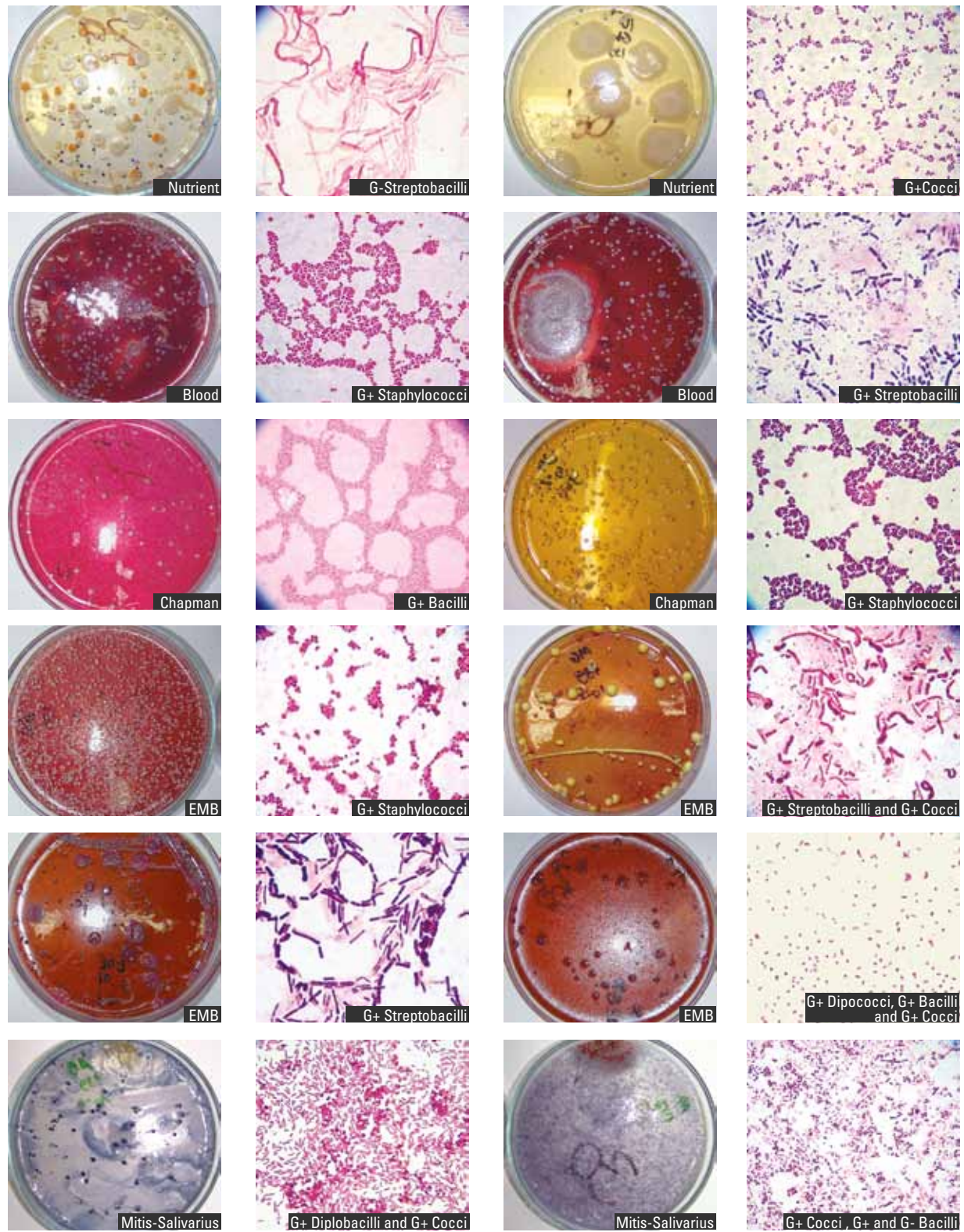


FIGURE 1 - Microbial colonies grown in different culture media and microscopic aspect (Gram staining; 1000 X magnification) of bacteria found in most frequent colonies of each medium.

outline. In the Chapman cultures, G+ staphylococci were prevalent in yellow and pinkish colonies, which indicated, in several cases, the presence of *Staphylococcus aureus*, confirmed by the change of agar color. MS had G+ cocci, and isolated G+ and G- bacilli; bluish, round and small colonies were predominant.

## DISCUSSION

Over 300 bacterial species have already been described in oral microbiota.<sup>26</sup> In healthy individuals, these microorganisms coexist in equilibrium with the host, but environmental changes and microbial imbalances may originate infections.<sup>1</sup> For example, brackets and orthodontic bands induce specific changes in the oral environment, such as a lower pH and an increase of bacterial plaque,<sup>1</sup> higher levels of *S. mutans*<sup>1,22</sup> and an increase in the number of *Lactobacilli* species.<sup>1,24</sup>

This study found that biosafety procedures adopted in academic settings are not efficient to reduce the risk of infection. The term “cross infection” refers to the transfer of microorganisms from one person or object to another person and the resulting infection. It should be distinguished from cross contamination, which refers to the transfer of microorganisms from one person or object to another person which may or may not result in infection.

Of the several types of bacteria found in this study using light microscopy, isolated G+ cocci and microorganisms arranged as staphylococci were the most frequent. Such microorganisms may belong to different bacterial species that may cause several diseases. As in several infectious diseases, immunodepression is an important factor in an individual's susceptibility to infection.<sup>19</sup> Both types of pliers under analysis presented bacterial contamination. Band remover pliers had the most contamination, and most bacteria were those that are found in the oral microbiota. This may be assigned to the

direct contact of this instrument with intraoral structures and to the presence of plastic material in its tip, which may favor the retention of microorganisms. The 139 pliers, in addition to contamination by microorganisms found in the oral cavity, had a high rate of contamination by staphylococci, which are bacteria that colonize the nasal mucosa and the skin. This finding may be explained by the use of this instrument during the manufacture of orthodontic appliances, because, in theory, these pliers are not supposed to be placed directly in the mouth.

The *Staphylococcus* genus has more than fifteen different species, and *S. aureus*, *S. epidermidis* and *S. saprophyticus* are the most important in healthcare settings.<sup>25</sup> These microorganisms, responsible for nosocomial infections, are some of the most resistant pathogenic bacteria and may survive for months in dry surfaces at temperatures higher than 60°C.<sup>29</sup> Some of the diseases caused by staphylococcal enzymes and toxins are superficial infections, such as furuncles, carbuncles, pustules, abscesses, conjunctivitis and angular cheilitis, as well as more severe diseases, such as toxic shock syndrome, osteomyelitis, pneumonia,<sup>25</sup> bacterial endocarditis and septicemia.<sup>25,29</sup> Some of the important diseases caused by *Streptococcus* species are respiratory tract infections, such as pharyngitis and tonsillitis, which may be accompanied by scarlet and rheumatic fever.<sup>25</sup> One of the complications of acute pharyngitis may be the dissemination of infection into the ear (otitis media), the mastoids, the base of the tongue or the floor of the mouth.<sup>25</sup> Other diseases caused by streptococci are infections of soft tissues in the oral cavity or the skin, as well as caries, primarily caused by mutans microorganisms.<sup>25</sup>

Pathogens may be transmitted from one patient to another by direct or indirect contact with reused instruments inadequately prepared, and with contaminated surfaces or hands.<sup>21</sup>

Several studies found contamination after inadequate disinfection of instruments used in patients, which stresses the need to follow adequate disinfection procedures.<sup>14,23</sup> Sterilization or high-level disinfection is the recommended procedure against HBV and HIV. However, disinfection efficacy is affected by factors such as the nature of the object (type of slots and hinges) and by duration of exposure to disinfecting products.<sup>14</sup> All materials that can be sterilized should never be only disinfected. According to some authors, infection control methods currently adopted in some orthodontic offices are not satisfactory, maybe because it is believed that this specialty has a low risk of contamination.<sup>8,18</sup>

A survey conducted with a group of orthodontists found that 49% sterilized their pliers, whereas 49% disinfected them. One reason for the high usage of disinfection methods may be the cost of sterilization, as the orthodontist should have several pliers if each instrument is to be sterilized. Other reasons mentioned are the fact that sterilization shortens the useful life of materials, the large number of patients per day, and the shorter duration of appointments. Moreover, orthodontists may be more flexible in terms of infection control than dentists in other specialties because they may believe that their young population is less likely to be infected with HIV or HBV.<sup>30</sup> However, recent studies showed that there has been an increase in HIV infection among individuals younger than 20 years.<sup>17</sup> Woo et al<sup>30</sup> reported that, of the total number of patients seen in orthodontic clinics, 21% were children, 52% were teenagers, and 27%, adults. Adolescents or adults account for the largest percentage of patients receiving orthodontic treatment. In addition, all patients should be treated as if they were potentially infective. Because most patients with HBV and HIV infection are asymptomatic, they may disseminate the virus in offices.<sup>10</sup>

Of the many viral diseases that may be acquired in a dental office, the most often mentioned are hepatitis (B, C and D), herpetic conjunctivitis, herpes simplex, herpes zoster, measles, chickenpox, rubella, mumps and AIDS. The most important infections caused by bacteria, according to the literature, are tuberculosis, syphilis, pneumonia, infections by streptococci and staphylococci.<sup>12</sup>

The incidence of hepatitis B after accidental exposure to contaminated materials or due to lesions caused by sharp instruments used in patients that have HBsAg antigens is about 20%. In the same circumstances, the risk of HIV transmission is between 0 and 0.5%.<sup>19</sup> An aggravating factor in HBV transmissibility is its high resistance and its high infectious capacity, as it has been shown to remain infective up to six months at room temperature and up to seven days when exposed to surfaces.<sup>4,10</sup> In less than 0.00000001 ml of blood, hepatitis B virus is potentially infective for 7 days after the surface is dried.<sup>10</sup>

This study showed orthodontic pliers have great contamination rates and that, by means of contaminated instruments, several types of microorganisms may be transmitted between individuals. This is a truly relevant fact because of the immense number of bacteria and, particularly, viral particles that are secreted in oral fluids, and a small amount of saliva has the potential to cause severe diseases, such as hepatitis B. Therefore, virus dissemination should not be overlooked, although this study focused on the identification of contaminating bacteria.

The prevention and control of cross infection in the dental office are current patient demands and rights. Therefore, all dental health-care personnel should be aware of these facts. Such knowledge will help them to change their procedures and adopt correct biosafety measures for all patients as a way to stop the propagation of infections.



## CONCLUSION

This study found high rates of bacterial contamination in the two types of orthodontic pliers selected for investigation. Data showed that band remover pliers had greater contamination rates, probably because of their direct contact with intraoral structures and tissues. The 139 pliers also showed high contamination by agents found in the oral microbiota, but mean CFU/ml was relatively greater in the Chapman

agar cultures, a medium to grow staphylococci, which are microorganisms found not in the oral cavity, but, rather, on the surfaces of human skin and in the nasal mucosa.

The disinfection procedures adopted did not seem to be effective to reduce contamination. More efficient measures should be adopted to control infection, so that microorganisms are not transmitted to patients or between patients and the members of the orthodontic team.

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