CONCISE REPORT

Facultative anaerobic bacteria on dentistry students' gutta-percha points: The importance of disinfection

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ABSTRACT

Background: During endodontic treatment in dentistry, if the gutta-percha points contain microorganisms that are resistant to the conditions in the root canal once it is sealed, they can lead to new infections. The purpose of this study was to determine the presence and quantification of facultative anaerobic bacteria in students' gutta-percha points.

Methods: A representative sample of dentistry students' gutta-percha points were collected, together with information on their characteristics. The points were placed in saline solution for inoculation in blood agar followed by anaerobic incubation for five days. Bacteria presence and type were determined, quantified, and identified. Following this, the X^2 test was used to verify whether there were any significant differences in the contamination found in the points between the characteristics studied.

Results: The results of the microbiological analysis revealed that 32.1% of the points appeared to be contaminated by facultative anaerobic bacteria. The most common types of microorganisms were gram-positive bacilli, followed by gram-positive coccus. *Staphylococcus epidermidis* was among the microorganisms identified. No relationship was observed between the presence of cone contamination and the characteristics, but a statistically significant difference was detected within the group defined by package opening date. Significant differences were also found in terms of the presence of spore-forming bacilli within the group defined by package opening and expiry date.

Conclusions: The presence of facultative anaerobic bacteria of clinical interest in the gutta-percha points used by dentistry students was identified and quantified. Among the bacteria identified, some are of clinical importance, such as *Staphylococcus epidermidis* and *Streptococcus mitis*. The establishment of disinfection protocols for such materials is recommended.

KEYWORDS

Gutta-percha; root canal obturation; contamination; bacteria

INTRODUCTION

During endodontic therapy, the dentist seeks to remove the microorganisms present in the root canal and prevent new microorganisms from accessing the area by establishing inhospitable conditions [1], including the insufficient or almost null quantity of nutrients, limited space, the alteration of the redox potential (physiological state based on oxidation-reduction reactions in biological systems), low oxygen concentration, and the presence of antimicrobial substances [2]. However, it has been revealed that some of the microorganisms involved in endodontic infectious processes are able to adapt to such conditions [2, 3]. Thus, if the gutta-percha points contain microorganisms that are resistant to the conditions in the root canal once it is sealed (obturated) (Figure 1), and if they possess virulence factors and the appropriate infective dose [4], they can lead to new infections.

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Conflicts of interest: None.

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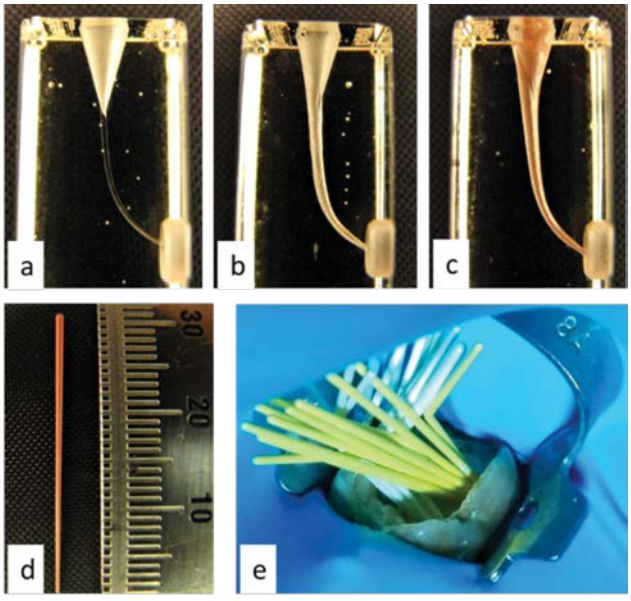
FIGURE 1: Gutta-percha points.



Legend:
(a-c) Acrylic representation of root canal preparation.
a) Untreated root canal.
b) Cleaned and shaped root canal.
c) Obturated root canal with gutta-percha points.
d) Gutta-percha point No. 25.
e) Obturation of root canal with gutta-percha points No. 15 and No. 20.

Microbiological analyses of endodontic failure reveal that the microorganisms present in the secondary infection differ from those found in the initial infection [5]. The secondary infection reveals a predominance of facultative anaerobic and gram-positive bacteria [6, 7] given the entrance of microorganisms during or after the treatment [8]. Facultative anaerobic bacteria are versatile in adapting to conditions with or without oxygen, they are able to occupy a variety of environments, and many of the species in the group can lead to infection [9]. These factors make them interesting to analyze in terms of their presence on gutta-percha points.

Given the fact that gutta-percha points (Figure 1) are made with a component that prevents the proliferation of microorganisms (zinc oxide), that some are sold in sterile conditions, and that their quality can be affected by sterilization or disinfection, there is no firm requirement to subject them to high-level disinfection or sterilization [10, 11]. However, many studies have revealed that the points become contaminated during storage and manipulation even in recently opened packages [10, 12, 13].

The aim of this study was to determine the presence and quantity of facultative anaerobic bacteria on dentistry students' gutta-percha points and to determine whether there is a relationship between the presence of the bacteria and the students' use of the instruments.

METHODS

The study complied with ethical principles and voluntary and confidential participation through the completion of informed consent forms by the participating students. This study was approved by the ethics subcommittee of the Universidad Cooperativa de Colombia.

Points collection

A representative sample of students' gutta-percha points at the Universidad Cooperativa de Colombia's Faculty of Dentistry, Villavicencio with a confidence interval of 95% (n = 81), was collected. As well as the point samples, information was gathered on the type of storage, point diameter, point brand, package opening date, and expiry date. The points were taken directly from their packages under aseptic conditions.

Microbiological analysis

For this analysis, the points were placed inside Eppendorf tubes with sterile saline solution and were shaken by hand for a minute in order to release the bacteria present in the materials. Subsequently, 200 μl of the solution was plated in duplicate in blood agar and incubated for five days at 35° C in anaerobiosis jars with AnaeroGen Thermo Scientific sachets. After the incubation process, the presence of bacteria colonies in the points was determined and the colony-forming units (CFU) were quantified. The morphology of the colonies was described, and a gram stain along with observation of the cell morphology was carried out using culturing in aerobic conditions to confirm whether the colonies were of facultative anaerobic organisms. Finally, a catalase test was carried out and colonies to be identified were selected in a microbiology-certified laboratory using the VITEK system.

Statistical analysis

The SPSS program (version 22.0) was used to determine the median value and interquartile range of the total CFUs present in the points. The X^2 test was used to verify whether there were any significant differences in the contamination found in the points between the groups defined by the characteristics studied.

RESULTS

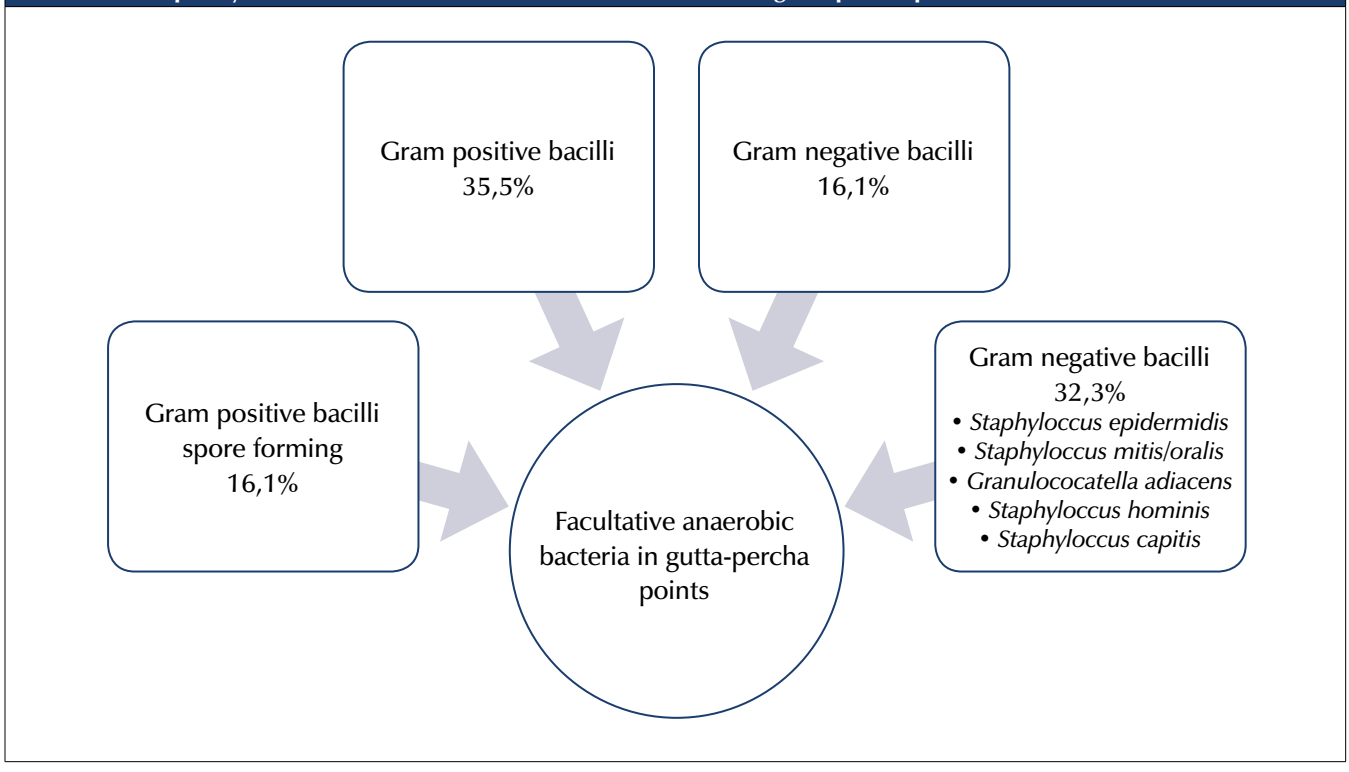
Microbiological analysis

The microbiological analysis revealed that 32.1% (n = 26) of the points were contaminated by facultative anaerobic bacteria, with a median value of 5 CFU/ml (interquartile range [IQR] 5-15). The CFU/ml range found in the contaminated points was 5 CFU/ml to 40 CFU/ml.

The study revealed different types of colony morphology and bacterial cells. It confirmed that all the isolates were of facultative anaerobic bacteria. Among the types of bacteria found in the contaminated points, gram-positive bacilli presented the highest percentage, with a median of 5 CFU/ml (IQR 5-5); followed by gram-positive coccus, with a median of 8.8 CFU/ml (IQR 5-16.9); gram-negative bacilli, with a median of 5 CFU/ml (IQR 5-7.5); and gram-positive spore-forming bacilli, with a median of 5 CFU/ml (IQR 5-22.5) (Figure 2).

Return to TABLE OF CONTENTS

FIGURE 2: Frequency of facultative anaerobic bacteria in contaminated gutta-percha points.



The influence of point characteristics on the presence of facultative anaerobic bacteria

This study found that most of the points were of the same brand (87.7%), that 36% of the points were stored in locations outside the clinic (students' houses or lockers), and that 21% of the points were past their expiry date. It was also detected that 24.6% of the points came from packages that had been opened over 12 months prior, and 1.2% were not packaged at the time of collection.

In the microbiological analysis, a greater frequency of contaminated points was observed in the groups defined by characteristics such as the type of storage (clinic), point brand (No. 3), package opening date (six to 12 months), expiry date (for over 12 months), and point diameter (No. 80). The only statistically significant difference found ($p < 0.05$) was related to the package opening date, although not in a linear relationship. With respect to the different types of microorganisms detected in the points, statistically significant differences were found ($p < 0.05$) insofar as spore-forming gram-positive bacilli were present within the groups defined by package opening date and expiry date.

DISCUSSION

The main causes of endodontic failure are attributed to microbial factors, the persistence of infectious microorganisms in the root canal, microfiltration, or to inadequate control in the aseptic chain [5, 6]. This study found that 32.1% of the dentistry students' gutta-percha points were contaminated by facultative anaerobic bacteria. Other studies have reported percentages of aerobic bacteria contamination in 5% to 40% of points from different sources (new and recently opened, stored, in use, etc.) [10, 13]. Few studies have examined the presence of facultative anaerobic bacteria only. Gomes et al. [12], for example, report contamination by this type of bacteria in 5.5% of the points.

The microflora present in endodontic failure is different from those present in the primary infection given that it is facultative anaerobic bacteria and gram-positive bacteria [6, 7] that predominate. Most of the species found belong to the Firmicutes phylum [7], which includes bacteria of the genera *Bacillus*, *Staphylococcus*, and *Streptococcus*, among others. In this study, the types of microorganisms were gram-positive bacilli, gram-positive coccus, and gram-negative bacilli. Bacteria of the genera *Staphylococcus*, such as *S. epidermidis*, *S. capitis*, and *S. hominis*, were identified. Other studies have also reported the presence of these microorganisms in the points [10, 12]. A clinically-important species frequently found in these materials is the *S. epidermidis*, which has been associated with dental abscesses [14], endocarditis [15], bacteremia [16], and other types of infection. These bacteria share certain features with *E. faecalis*, which is frequently associated with endodontic failure [17] given its ability to form biofilms [18] and for being resistant to several antimicrobials [19].

Other clinically interesting microorganisms identified are the *Streptococcus mitis*, involved in endodontic infections [8], endocarditis, septicemia, and bacteremia, among others [20]; and *Granulococatella adiacens*, associated with cases of endodontic infections [21], endocarditis [22], and bacteremia [23], among other infections.

Bacteria in the *Bacillus* genera were also identified. Some of these had spore-forming characteristics, making them resistant to physical (heat, cold, radiation, drying) and chemical (disinfectants) conditions and, as such, hard to eliminate from medical equipment. This highlights the importance of determining the most effective medium for their destruction [24]. Different *Bacillus* species have been progressively implicated in a broad range of infections, including abscesses, bacteremia, septicemia, wounds, endocarditis, and meningitis, among others. Many of these occur as secondary or mixed infections in immunosuppressed patients, but a significant proportion lead to primary infections in healthy individuals [24].

According to the scientific literature, some of the microorganisms identified in this study may lead to infection; for this to happen, however, they have to survive inhospitable conditions [1, 3], and possess the appropriate virulence factors and infective dose [4]. The microorganisms in the points analyzed were found in low doses, but susceptibility to infection in immunocompromised individuals is different [25].

With respect to the relationship between the presence and quantification of facultative anaerobic bacteria and the characteristics of the points used by the students, the only significant differences found were between the groups defined by package opening date, but there was no linear relationship. Other studies have not found significant differences between the properties studied and the contaminated points [12, 13].

CONCLUSIONS

In this study, facultative anaerobic bacteria were found in 32.1% of the points used by the dentistry students. Among the bacteria identified, some are of clinical importance, such as *Staphylococcus epidermidis* and *Streptococcus mitis*. No relationship was found between the presence of this type of bacteria and the point properties, indicating that contamination is random more than it is conditioned by opening and expiry date, brand, and diameter. As such, disinfection before use is essential.

Recommendations

We recommend the development of studies to establish appropriate disinfection protocols that do not alter the points' physical and chemical properties. The protocol must consider contamination by highly resistant bacteria structures such as spores and establish compulsory disinfection of the points before use in order to maintain the aseptic chain during treatment.

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