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Assessment of microorganism presence on instruments used in the periodontics area of a university pre-professional practice clinic

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ABSTRACT

Objective: To assess the presence of microorganisms on the surfaces of sterile instruments in the periodontics area of a university pre-professional practice clinic. **Materials and methods:** A descriptive and observational study, in which 100 randomly selected samples of periodontics instruments were selected. The samples collected were analyzed using cultures on specific media, including blood agar, Sabouraud agar, and eosin methylene blue agar, which enabled the primary identification of four classes of microorganisms: Gram-positive cocci, Gram-positive bacilli, Gram-negative bacilli, and yeasts. **Results:** Of the instruments analyzed, 19% (n = 19) were contaminated. The highest contamination rate was observed in Gracey 3/4 periodontal curettes, with 30% (n = 12), in which only gram-positive cocci were identified, with no positive results for gram-positive bacilli, gram-negative bacilli, or yeasts. **Conclusions:** Sterilization was not completely effective in all instruments, as some showed contamination with Gram-positive cocci.

Keywords: sterilization; periodontics; biological risk containment; associated dental practice.

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INTRODUCTION

During dental practice, adequate infection control is essential due to the high risk of cross-contamination during patient care. Cross-contamination is the transmission of microorganisms between patients and clinical personnel via contaminated instruments, equipment, or surfaces. It most commonly when instruments are reused between patients without proper disinfection and autoclaving, or when instruments become contaminated before intraoral use—during unpacking, opening, or contact with other materials or surfaces (1-3). According to universal guidelines, any instrument that has come into contact with blood, tissues, or saliva must be decontaminated and subsequently sterilized. The use of contaminated items can lead to disease not only in patients but also in dentists and dental staff, who may be exposed to occupational infections such as hepatitis B and C, acquired immunodeficiency syndrome (AIDS), or tuberculosis (2, 4, 5).

The U.S. Centers for Disease Control and Prevention (CDC) emphasizes that all dental personnel exposed to biological fluids, such as blood or saliva, must adhere to the principle of biosafety universality, implemented through asepsis, antisepsis, and sterilization. Instruments should first be manually cleaned and immersed in a disinfectant solution or, in some cases, subjected to ultrasonic cleaning; they are then sealed and sterilized in a vacuum autoclave; and, finally, stored in decontaminated areas, thereby minimizing the risk of cross- or nosocomial infection. This process is especially taken with critical-range instruments, those that penetrate soft tissues or alveolar bone, such as periodontal probes and curettes for scaling and root planning (6-8).

In a study by Chávez-Fermín et al. (9), biological residues were detected on instruments used in periodontics even after dry-heat sterilization. This finding was mainly attributed to retentive surfaces on the working end of the instruments, which hinder adequate cleaning and decontamination and can lead to protein residues that contribute to bacterial resistance across subsequent sterilization cycles. Consequently, instruments showed mild to moderate contamination, representing a potential cross-infection risk, particularly for vulnerable patients.

Periodontitis is a chronic infectious-inflammatory disease of multifactorial origin that affects 30-35% of the global population. Its main etiology lies in the host immune response to bacterial infections, whereby microorganisms invade the periodontium and elicit inflammation. The condition may worsen with exposure to external pathogens, even contributing to extra-oral diseases. In this context, the risk of cross-contamination from contaminated instruments depends on microbial load, pathogenicity, and host resistance; therefore, this risk must be minimized through effective sterilization—i.e., elimination of all bacterial spores—without interference from human, mechanical, or microbial factors (3, 10-12).

Ensuring proper sterilization of dental instruments is fundamental to eliminating disease-causing microorganisms while safeguarding both healthcare personnel and patients. It also informs improvements in the sterilization workflows, instrument design, and professional training (13).

Based on the above, the aim of this study was to evaluate the presence of microorganisms on the surfaces of sterilized instruments in the periodontics area of a university pre-professional practice clinic.

MATERIALS AND METHODS

A cross-sectional study was conducted. The study population consisted of the instruments from the periodontics area of a university preprofessional practice clinic used by seventh- and ninth-semester students. Non-probabilistic convenience sampling was applied, obtaining 100 samples of dental instruments.

Samples were grouped into three categories: Group 1 (G1), North Carolina periodontal probe (n = 20); Group 2 (G2), Gracey 3/4 curette (n = 40); and Group 3 (G3), Gracey 11/12 curette (n = 40). Differences in sample size reflected the functional configuration of each instrument: the probe has a single active end, whereas the Gracey curettes have two.

Samples were collected before the instruments were used on patients. The inclusion criteria included sealed periodontics instruments sterilized by moist-heat autoclaving, with sampling authorized by undergraduate students. Instruments that were damaged, in poor condition, or visibly soiled biological residues were excluded, as these would indicate inadequate washing protocols or prior package opening before sampling.

Study variables were the presence of microorganisms and the instrument type. Sampling repeatability and reproducibility were assessed beforehand. Prior to sampling, the principal investigator complied with all biosafety measures, including a fluid-resistant suit, surgical gown, cap, shoe covers, mask, and gloves.

Selected instruments underwent cleaning, disinfection, packaging in sterilization pouches, and autoclaving using a Phoenix® model 39209S unit, in accordance with protocols established by the Ministry of Public Health of Ecuador (14). Instruments were then distributed to students in the periodontics pre-professional practice clinic before patient care and subsequently opened by the investigator on a sterile field.

Sampling was performed specifically on the working end of G1, G2, and G3 instruments, all used for scaling and root planing. Strict biosafety standards were observed throughout to avoid cross-contamination. A sterile Citoswab® swab packaged with Stuart transport medium was used. The package was opened from the top without contact with the swab tip. Swabbing of the sterile instrument's working end occurred inmediately after pouch opening and prior to patient use.

Samples were transported to the laboratory in Stuart medium to preserve microbial viability. In the microbiology laboratory, streak plating was performed on blood agar (BA) for Gram-positive cocci and bacilli, eosin methylene blue agar (EMB) for Gram-negative bacilli, and Sabouraud dextrose agar (SDA) for yeasts. For this process, the samples were incubated at 36 °C for 24 to 48 hours (15).

After incubation, microbial growth was evaluated for presumptive identification and colony counting. Growth on BA was Gram-stained to differentiate Gram-positive cocci or bacilli. After recording and verification, microbiological material was autoclaved and discarded in red biohazard bags, in accordance with biological waste management regulations. Data were recorded on a form specifically designed for this purpose and later processed in Microsoft Excel 2021. Descriptive analysis used absolute and relative frequencies.

The Statistical Package for Social Sciences (SPSS) was used for chi-square analysis, with a confidence level of 95% and a statistically reliable contamination interval ranging from 11.3% to 26.7%. Chi-square tests also assessed associations between the variables analyzed.

The study was approved by the Ethics Committee of Universidad Católica de Cuenca, under code CEISH-UCACUE-135.

RESULTS

Overall, 19% of instruments were contaminated. The highest rate was observed in Gracey 3/4 curettes (G2), with a contamination rate of 30% (n = 12). In contrast, Gracey 11/12 curettes (G3) showed the lowest rate, with only 8% (n = 3), while periodontal probes (G1) presented an intermediate rate of 20% (n = 4). A chi-square test ($\alpha = 0.05$) found no statistically significant differences in contamination among instrument types (Table 1).

Table 1. Microbiological evaluation of periodontal instruments using blood agar culture

	G1		G2		G3		p	Total	
	n	%	n	%	n	%		n	%
Non-contaminated	16	80.0	28	70.0	37	92.0		81	81.0
Contaminated	4	20.0	12	30.0	3	8.0	0.11	19	19.0
Total	20	100.0	40	100.0	40	100.0		100	100.0

G1: Periodontal probe; G2: Gracey 3/4 curette; G3: Gracey 11/12 curette; p: Chi-square statistical test ($p < 0.05$).

Growth of Gram-positive cocci was exclusively observed on BA. No growth of Gram-positive bacilli, Gram-negative bacilli, or yeasts was detected (Table 2).

Table 2. Microorganisms found in contaminated samples.

Microorganism	n	%
Gram-positive cocci	19	100.0
Gram-positive bacilli	0	0.0
Gram-negative bacilli	0	0.0
Yeasts	0	0.0
Total	19	100.0

DISCUSSION

Analysis of sterilized periodontal instruments showed a 19% contamination rate with Gram-positive cocci identified exclusively on BA. In contrast, cultures on SDA and EMB were negative, confirming the absence of yeasts and Gram-negative bacilli. These findings demonstrate the presence of microbiological contamination despite prior packaging in sterilization pouches, which should, in principle, ensure effective sterilization.

Similar results were reported by Resendiz et al. (16), who documented contamination rates of 7% to 19% in previously sterilized instruments stored in sealed packages. Likewise, Chanchareonsook et al. (17) identified contamination in autoclave-sterilized periodontal instruments from an outpatient dental care center. Those instruments were classified as Category II according to the Society for Healthcare Epidemiology of America

(SHEA) guidelines, corresponding to procedures with a theoretical but unlikely possibility of blood-borne viral transmission. In that study, contamination was attributed to a failure in activating the steam sterilization cycle, preventing achievement of adequate parameters for the process. This is consistent with the present study, in which, due to random sampling, it was not always possible to verify whether each instrument underwent an effective cycle which could explain the high bacterial load in certain samples. In contrast, the studies by Barker et al. (18) and Owusu et al. (13) reported no contamination in instruments sterilized using autoclave systems.

Contamination varied across groups after autoclaving. Anterior curettes exhibited the highest levels of contamination, possibly related to surface complexity and working-end design or variability in cleaning and sterilization effectiveness. These results are supported by the findings of Chávez-Fermín et al. (9), who demonstrated that the instruments can accumulate biological residues, mainly associated with the lack of prior washing and decontamination. The study reported contamination in 31% of the curettes, classified as mild to moderate. Comparable patterns have been reported for other critical instruments, such as endodontic tools, which may retain substantial microbial loads even after sterilization (19).

Although periodontal probes showed comparatively low contamination, the grooves in the North Carolina probe may hinder removal of biological material and thus reduce sterilization efficiency. This finding is consistent with that reported by Carrasco-Ruiz et al. (20), who conducted a study on periodontal probes and detected microbial contamination after sterilization, isolating Gram-positive cocci and Gram-negative bacilli in culture media. These authors demonstrated that the use of paper sterilization pouches ensured effective sterilization, whereas the use of cloth or plastic bags led to microbial contamination even after autoclaving. Multiple factors can impair autoclave efficacy, including inadequate drying and improper temperature control.

This study provides a basis for future research aimed at developing standardized protocols for cleaning, packaging, and sterilization of dental instruments. It also underscores the importance of monitoring sterilization procedures and designing instruments less prone to contamination, thereby contributing to the safety of both dental personnel and patients. The study further emphasizes the importance of continuous education and strengthened awareness regarding biosafety in dental practice, particularly within university learning environments.

One of the main limitations of this study was the inability to perform specific microbiological identification tests to determine the exact microorganisms present on the contaminated instruments; only a preliminary identification using basic techniques was conducted. In addition, a non-probabilistic convenience sampling method was employed, which introduces selection bias and limits the generalizability of the findings to other clinics, institutional settings, or types of dental instruments. Environmental and technical factors related to the sterilization process, such as water quality, autoclave maintenance, and storage conditions of the sterilized instruments were not evaluated, and may have influenced contamination.

CONCLUSIONS

The study revealed that part of the analyzed instruments exhibited contamination, with Gram-positive cocci identified on their surfaces. However, no statistically significant differences were observed in contamination levels among the different types of instruments.

Conflict of interest:
The authors declare no conflict of interest.

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Author contributions:
MAAA, JECR: conceptualization, methodology, research, writing – review & editing.
MNJR, MVLI: formal analysis, methodology, research, writing – original draft.
JMSO: methodology, research, writing – original draft, writing – review & editing.

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