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Major article

Analysis of microbial load on surgical instruments after clinical use and following manual and automated cleaning

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Background: We aimed to monitor the microbial load and identify the microorganisms recovered from surgical instruments after clinical use and following manual and automated cleaning.
Methods: This experimental study was carried out in the Laboratory of Oral Microbiology and Anaerobes at the Federal University of Minas Gerais in Brazil. Microbial samples were taken from 125 surgical instruments used in 25 types of gastrointestinal surgeries.
Results: The average microbial load was 93.1 CFU/100 mL after clinical use and 41 CFU/100 mL and 8.24 CFU/100 mL on instruments following 2 sequential steps of manual cleaning, respectively, and 75 CFU/100 mL and 16.1 CFU/100 mL on instruments after automated cleaning. Surgical wound classification significantly affected the microbial load recovered on instruments. Coagulase-negative *Staphylococcus*, *Escherichia coli*, *Pseudomonas* spp, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* complex were recovered.
Conclusions: The average microbial load observed after the cleaning steps decreased, and the decrease in microbial load was more pronounced using the manual method compared with that observed using the automated method.

Some medical devices (MDs), including most surgical instruments, are manufactured to allow reuse until the limit of their effectiveness and functionality is reached.[1] This practice can lead to a reduction in both the costs and the amount of waste generated from single-use items. However, it is necessary to ensure that MDs remain safe for reuse on patients and for manipulation by medical personnel during MD reprocessing to protect them from infection hazards by avoiding microorganism transfer or other adverse events related to the use of MDs.[2,3]

MD reprocessing involves the use of the following set of standardized and interdependent actions: prewash, reception, cleaning, drying, assessment of integrity and functionality, preparation, disinfection or sterilization, storage, and distribution for reuse.[1,4] The reduction in the microbial load on MDs during cleaning is an essential step that increases the safety and reliability of the sterilization process.[1-3] Although directly associated with health care quality, the inadequacy of MD reprocessing has not always been documented as being responsible for complications resulting from the care process, and it is reported only when related to episodes of outbreak.[5,6]

MDs can be cleaned using a manual method suitable for delicate and complex products, or using automated methods in which ultrasonic and/or decontamination washers are employed; automated cleaning methods are highly recommended because they are more likely than manual methods to be reproducible and they can be validated.[1,4,7,8] Although automated cleaning typically provides superior results when compared with manual methods, Vassey et al[9-11] noted an increase in residual protein on instruments that had been cleaned in an ultrasonic bath.

Regardless of the cleaning method employed, improper maintenance of MDs can increase the bioburden during MD processing.[8] The accumulation of proteins, salts, and dirt on MDs protects microorganisms from direct contact with sterilizing agents and favors bacterial adherence and biofilm formation. To be effective, the cleaning methods used must substantially reduce the levels of infectious agents present on MDs, such as bacteria, endotoxins,

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fungi, viruses, organic and inorganic matter that allow microbial growth and survival, and potential pyrogens.[1,8]

Given the concerns surrounding the appropriate procedures used for cleaning MDs, the aim of this study was to determine the microbial load and microbiologic profile of microorganisms recovered after clinical use and during manual and automated cleaning of surgical instruments used for digestive system surgeries.

MATERIALS AND METHODS

This experimental study was conducted in the Central Sterile Services Department (CSSD) of a large hospital in partnership with the Oral and Anaerobic Microbiology Laboratory of the Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil. We analyzed 125 instruments that were used in 25 types of gastrointestinal surgical procedures performed in a large teaching hospital; the procedures were classified as clean-contaminated procedures (involving the oral cavity, esophagus, stomach, liver, and biliary tract) and contaminated procedures (involving the large intestine).[12]

The inclusion criteria for the MDs selected were the following: the surgical instruments had to exhibit similar characteristics such as presence of grooves and joints, they had to be approximately 20 cm long, and they had to harbor visible dirt (eg, blood, organic matter, and inorganic matter) to ensure that a chosen MD was used during surgical procedures. The elected instruments included clamps such as crile, hemostatic, Rochester, Foerster, Kocher, and needle-holders.

At the end of each surgical procedure, in the operating theatre, surgical instruments were moistened (for 30-60 minutes) using towels soaked in tap water to keep them wet before being transferred to CSSD for cleaning. We selected 5 instruments once the MDs had been transferred to CSSD, and collected study samples immediately after each of these sequential cleaning steps: (1) clinical use in digestive tract surgeries (representing the sample collected from instruments before cleaning); (2) manual cleaning involving soaking in an enzymatic detergent (Indazyme 6 plus, Indalabor Indaiá laboratório farmacêutico LTDA, Minas Gerais, Brazil, dilution: 2 mL/L water for 5 minutes [the time recommended by the manufacturer]) and brushing under tap water (5 times) by using brushes featuring with soft bristles and by applying firm strokes to detach any visible dirt attached to the instrument (Manual Method - Step 1); (3) manual cleaning and subsequent cleaning in a thermal washer-disinfector (regularly validated according to ISO 15883) (Manual Method - Step 2); (4) automated cleaning in an ultrasonic washer (Automated Method - Step 1); and (5) automated cleaning in an ultrasonic washer, followed by cleaning in an automated washer-disinfector, which was the same used in Step 2 of the manual method (Automated Method - Step 2). Figure 1 shows the steps followed for sample collection.

Samples were collected according to methods described previously.[2,13–16] On each day of sample collection, only 1 surgical procedure was selected. The elected instruments were transferred to a sterile plastic bag containing 500 mL sterile distilled water; this volume was used to allow complete immersion of all selected instruments. The bag was sealed and then placed in an ultrasonic bath (9 L capacity) for 3 treatments of 5 seconds each applied using an ultrasonic washer (USC-2800 model, Enge Solutions, São Paulo, Brazil), at a frequency of 40 KHz and a power of 30 W. Next, the bag containing the instrument was agitated for 5 minutes at 160 rpm in an orbital shaker (Kline 255-B model, Fanem LTDA, São Paulo, Brazil).[13] During each sample collection, instruments were retrieved from the plastic bag using aseptic techniques, and the collected samples were sealed, identified, and transported to the Microbiology Laboratory of the Institute of Biological Sciences, Federal University of Minas Gerais located nearby; samples were transported within 60 minutes in a cooled thermal box whose internal temperature was monitored to ensure adequate preservation of the characteristics of the samples.[17]

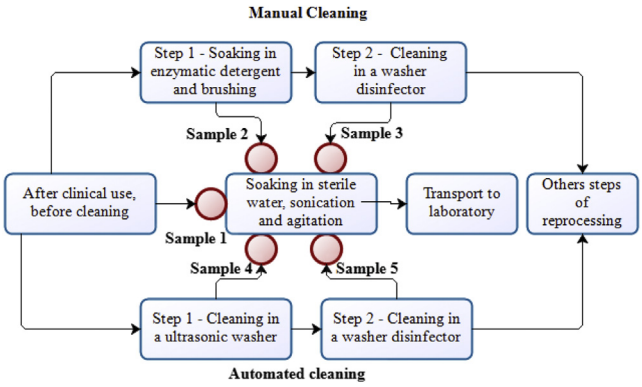

Fig 1. Sequential steps of manual and automated cleaning processes and respective times of sample collection.

The samples were again agitated in an orbital shaker for 5 minutes at 160 rpm and then filtered (using vacuum) through an autoclavable Sterifil (47-mm Sterifil Holder; Millipore indústria e comércio LTDA, Barueri, São Paulo, Brazil) containing a previously autoclaved cellulose nitrate membrane (0.45 μm; HAWP04700); we collected 100-mL aliquots under a laminar flow hood (Microbiological Biosafe I, Vecco, Campinas, São Paulo, Brazil). The filter membranes were overlaid on selective culture media, MacConkey agar (BBL, Biomérieux, Marcy-l'Étoile, France), Mannitol agar (BBL), and Sabouraud Dextrose Agar (Difco Laboratories, Detroit, Mich), which allowed the growth of specific microorganisms. Furthermore, a nonselective medium, Brain Heart Infusion (BBL) supplemented with 5% horse blood, was used for estimating the total microbial load of the samples. The culture plates were incubated in a bacteriologic incubator (Fanem CD Model 347) at 37°C for 24-48 hours, and the number of colonies formed on the plates, representing the number of microbes in the samples, was expressed in colony-forming units per 100 mL. Representative colonies of distinct morphotypes were subcultured on Brain Heart Infusion agar (BBL) to obtain pure cultures and were then stored in a freezer at −86°C in Brucella Broth (BBL) supplemented with glycerol (10%).

After the collection process Gram staining was used as the first step in microorganism identification, after which specific biochemical tests were conducted for each group. Gram-positive cocci were characterized using catalase, coagulase, and DNase tests, and gram-negative rods were further characterized by performing oxidase, citrate, malonate, sulfate agar indole and motility tests, and modified Rugai tests.

Isolates of epidemiologic importance and those considered to be causative agents of health care-associated infections were identified to the genus and species levels by using the Vitek II (Biomerieux) automation system together with Gram-Negative microbial identification test cards. Filamentous fungi that were recovered were identified according to their macroscopic and microscopic characteristics.[18]

Statistical analyses were performed using SPSS 15.0 software (IBM-SPSS Inc, Armonk, NY). The significance level for statistical difference was 5% ($P = .05$) and the confidence interval was 95%. The microbial loads in the 5 samples were compared with other variables by using the Mann-Whitney *U* test, because the required assumptions for this statistical model (normality and homoscedasticity) were not met. We also used the Wilcoxon test for paired samples.