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Major Article

Potential testing of reprocessing procedures by real-time polymerase chain reaction: A multicenter study of colonoscopy devices

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Background: Reprocessing of endoscopes is key to preventing cross-infection after colonoscopy. Culture-based methods are recommended for monitoring, but alternative and rapid approaches are needed to improve surveillance and reduce turnover times. A molecular strategy based on detection of residual traces from gut microbiota was developed and tested using a multicenter survey.

Methods: A simplified sampling and DNA extraction protocol using nylon-tipped flocked swabs was optimized. A multiplex real-time polymerase chain reaction (PCR) test was developed that targeted 6 bacteria genes that were amplified in 3 mixes. The method was validated by interlaboratory tests involving 5 reference laboratories. Colonoscopy devices (n = 111) were sampled in 10 Italian hospitals. Culture-based microbiology and metagenomic tests were performed to verify PCR data.

Results: The sampling method was easily applied in all 10 endoscopy units and the optimized DNA extraction and amplification protocol was successfully performed by all of the involved laboratories. This PCR-based method allowed identification of both contaminated (n = 59) and fully reprocessed endoscopes (n = 52) with high sensibility (98%) and specificity (98%), within 3-4 hours, in contrast to the 24-72 hours needed for a classic microbiology test. Results were confirmed by next-generation sequencing and classic microbiology.

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Conclusions: A novel approach for monitoring reprocessing of colonoscopy devices was developed and successfully applied in a multicenter survey. The general principle of tracing biological fluids through microflora DNA amplification was successfully applied and may represent a promising approach for hospital hygiene.

Endoscopy plays an essential role in diagnosis and therapy of several diseases.[1] This costly and sophisticated approach is based on reusable tools that need appropriate reprocessing to avoid cross-infections.[2] Because of the type of endoscopy, the cleaning treatments used, and patient clinical features, the disinfection or sterilization levels may vary in their efficacy requirements.[3,4] Failures during reprocessing steps may occur, and contaminated endoscopes have been associated with outbreaks of health care-associated infections more frequently than any other medical devices.[2,5] Remarkably, endoscope-transmitted infections can occur even when reprocessing is performed following professional and manufacturer guidelines.[5-7] Flexible endoscopes are significantly contaminated by biological fluids, including blood or secretions.[6-8] Radical cleaning is a critical step due to the complex structure of the devices, which are characterized by narrow lumens and multiple internal channels. Endoscope reprocessing is a challenging task involving cleaning and high-level disinfection (HLD) treatments, followed by rinsing and drying before appropriate storage.[8-10] Reprocessing failures, as well as the ability of bacteria to form biofilm on the inner channels and surface roughness, increase the likelihood of health care-acquired infections.[2,11-13] Education-based actions and technical advancements can certainly improve reprocessing effectiveness and ensure proper safety levels through multidisciplinary teamwork.[2,12,14,15] Microbiologic surveillance by culture-based methods represents an established and easy-to-use approach to assess the effectiveness of reprocessing procedures, but relevant limitations should be considered, such as long response time, low specificity, and poor sensitivity, in detecting resistant microorganisms not cultivable on standard media such as viruses, protozoa, prions, or viable but not cultivable bacteria.[6,13,15-17]

Recently, rapid biochemical methods, based on the evaluation of ATP, protein, carbohydrate, or hemoglobin levels, have been proposed to assess removal of organic residues from endoscopes.[7,18,19] However, several shortcomings, such as aspecific output, low sensitivity, and interference with disinfectants, suggests further improvements are needed.[18,19] DNA-based techniques, including real-time polyerase chain reaction (PCR), may indeed show several advantages in comparison with traditional culture-based methods in that they are less time-consuming, highly specific and sensitive, affordable, and can detect viable but not cultivable bacteria.[20,21] The potential application of molecular techniques represents a very promising and challenging opportunity to further improve monitoring of reprocessed devices. Here, we report the use of a novel method to monitor the effectiveness of reprocessing by an optimized real-time PCR approach that was evaluated on colonoscopy devices using a multicenter survey. The general principle of the method was based on the observation that residual traces of biological fluid microflora (mf) on reprocessed devices represent a potential indicator of sanitation failure, when tested by an mfDNA-based approach.[22] The identification and characterization of biological fluid by mfDNA analysis were initially applied in forensics and then hospital hygiene in dental settings.[22,23] In this study, sampling and DNA extraction were developed and validated within interlaboratory tests to achieve a simple and rapid protocol for a routine monitoring. Next-generation sequencing (NGS) analysis on selected mfDNA samples was carried out to confirm the molecular data. The general principle of tracing biological fluids through mfDNA amplification was applied in a multicenter study, suggesting promising perspectives for surveillance.

MATERIALS AND METHODS

Setting and study design

This study was conducted in the main hospitals of different Italian regions (Campania, Emilia Romagna, Lazio, Liguria, Marche, Molise, Tuscany, Veneto, Sardinia, and Sicily) involving 10 endoscopy units that reprocess approximately 50-100 endoscopes per business day. Data were collected by the Coordinating Laboratory Unit in Rome (Lazio, Foro Italico). Following a checklist, each participant unit (PU) provided information on endoscopy devices and reprocessing procedures or locally available guidelines, department and referent identification, and documentation on the reusable instruments subjected to sanitization. Each PU received a kit containing nylon-tipped flocked swabs with drying active agent (4N6FLOQSwabs; Genetics, Copan Italia, Brescia, Italy) and information on sampling and storage procedures. Sampled specimens were anonymously coded and sent to 1 of 5 reference laboratories (RLs) located in different Italian regions (Tuscany, Sardinia, Sicily, Molise, and Lazio). Colonoscopy devices included in this study were Olympus (Lake Success, NY) or Pentax (Montvale, NJ) and all underwent precleaning and manual cleaning procedures according to the endoscope manufacturer's instructions.[24,25] Briefly, the HLD was performed by automated endoscope reprocessors: CISA (ERS, Milan, Italy), Olympus, Medivators (Minntech, MN), and Pentax. After HLD, all endoscope channels were rinsed and forced air was used to dry the channels. Each RL performed DNA extraction and amplification as well as sampling and processing of their own samples. In each RL, 1 operator was responsible for all DNA extractions and real-time PCR. The RL received all supplementary materials for processing the samples, including DNA extraction kits (QIAmp DNA Mini Kit and DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany), NAO (nucleic acids optimizer) basket (Copan Italia), lysozyme solution and glass beads, 1 spiked sample as an internal positive control, the Microsan-F Kit (MDD University Spin Off, Viterbo, Italy) containing standardized amplification mix with aliquots of positive and negative controls, and the protocol. All kits and reagents were from the same batches.

Sampling

Each PU identified and sampled at least 10 colonoscopy devices: 5 dirty and 5 clean. For sampling, each nylon-tipped flocked swab was wiped on a surface area of 5 cm^2 (initially, inner channel sampling was considered, showing similar results, but in this preliminary phase of the study the simplest and most reproducible approach was selected, applied, and reported). Samples were collected using aseptic techniques in a dedicated room. In addition to routine monitoring, randomly selected additional samples ($n = 40$) were also collected to be analyzed by classic microbiology, following Centers for Disease Control and Prevention and European Society of Gastrointestinal Endoscopy and European Society of Gastroenterology and Endoscopy Nurses and Associates (ESGE-ESGENA) protocols.[16,26]