Journal of Hospital Infection 103 (2019) e53–e60

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Complex design of surgical instruments as barrier for cleaning effectiveness, favouring biofilm formation

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ARTICLE INFO

Article history:
Received 17 August 2018
Accepted 2 November 2018
Available online 10 November 2018

Keywords:
Biofilm
Surgical instrument
Microbial
Colony count
Cleaning
Sterilization

SUMMARY

Background: Inadequately reprocessed reusable surgical instruments (RSIs) may harbour infectious agents which may then be transferred to a suitable site for replication.
Aim: To determine the cumulative effect of 20 cycles of contamination, cleaning (manual or manual followed by automated) and steam sterilization on high-complex-design RSIs used for orthopaedic surgery.
Methods: New flexible medullary reamers and depth gauges were contaminated by soaking in tryptone soya broth, containing 5% sheep blood and 10^9 cfu/mL of *Staphylococcus aureus* (ATCC 25923), for 5 min. To mimic a worse-case scenario, RSIs were dried 7 h and subjected to either (a) rinsing in distilled water, (b) manual cleaning or (c) manual plus automated cleaning (reference standard), and steam sterilization. The contamination, cleaning, and sterilization cycle was repeated 20 times. Adenosine triphosphate (ATP) was measured after cleaning procedures; microbial load and residual protein were measured following the 10^{th} and 20^{th} reprocessing, in triplicate. Scanning electron microscopy (SEM) was used to confirm soil and biofilm presence on the RSIs after the 20^{th} reprocessing.
Findings: Manual and manual plus automated cleaning significantly reduced the amount of ATP and protein residues for all RSIs. Viable bacteria were not detected following sterilization. However, SEM detected soil after automated cleaning, and soil, including biofilms, after manual cleaning.
Conclusion: Soil and/or biofilms were evident on complex-design RSIs following 20 cycles of contamination and reprocessing, even using the reference standard method of cleaning. Although the depth gauges could be disassembled, biological residues and biofilm accumulated in its lumen. The current design of these RSIs prevents removal of all biological soil and this may have an adverse effect on patient outcome.

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https://doi.org/10.1016/j.jhin.2018.11.001
0195-6701/

Introduction

Surgical site infection (SSI) is a severe postoperative complication that is estimated to be preventable in 55% of cases [1,2]. *Staphylococcus aureus* is a micro-organism frequently isolated from SSIs. These bacterial species are known for their ability to develop antibiotic resistance and to form biofilms [3–6]. A biofilm is a community of micro-organisms irreversibly attached to a surface and embedded in a matrix of extracellular polymeric substances [3,4]. Biofilm infections involving tissue or medical implants are difficult to treat due to biofilms' tolerance to antimicrobials and they often result in persistent infection [7,8].

The majority of reusable surgical instruments (RSIs) become contaminated during surgical procedures and, depending on the type and site of the surgery, microbial loads can reach values of 1500 cfu per instrument [9]. The complex design of some RSIs is one factor that may negatively affect proper reprocessing, due to areas that are difficult or impossible to brush during cleaning. Cleaning is considered the most important step of reprocessing and, if not adequate, may contribute to biofilm formation [10]. Even though most of the micro-organisms recovered after orthopaedic surgical procedures are vegetative bacteria, which are inactivated at 80°C, micro-organisms that are sheltered within biofilm are more difficult to kill [11]. Autoclaving at 121°C for up to 30 min failed to kill *S. aureus* biofilm grown under repeated cycles of hydration and dehydration [12]. RSIs are subjected to cycles of nutrition and hydration during use and cleaning, followed by periods of dehydration during storage and, therefore, to some extent resemble the *S. aureus* biofilm that was tolerant to autoclaving. *Bacillus cereus* was recovered from a flexible medullary reamer (FMR) previously contaminated and subjected to steam sterilization [13].

Inadequately reprocessed RSIs may harbour infectious agents which may be transferred to a suitable site for replication [6,11,13]. Outbreaks of healthcare-associated infections (HCAIs) occur regularly with heat-sensitive devices subjected to high-level disinfection, whereas surgical instruments subjected to moist heat sterilization are considered safe [14]. However, residual human tissue was thought to contribute to HCAI following arthroscopic procedures, despite use of a steam-sterilized arthroscope [15]. Multiple inappropriate reprocessing cycles could lead to a build-up of residue and micro-organisms on RSI surfaces, promoting biofilm formation [16,17].

The aim of this study was to determine the cumulative effect over 20 cycles of contamination, cleaning (manual or manual followed by automated) and steam sterilization on high-complex-designed surgical instruments, an orthopaedic FMR and a depth gauge (DG). For this study, *S. aureus* was used for contamination as it is a major aetiological agent in SSI, can form biofilms, and is one of the organisms most frequently found contaminating RSIs post use [11,18].

Methods

Test RSIs were 8 mm × 26 cm FRM (Implantec™; Implante Materiais Médicos & Hospitalares Ltda, São Paulo, Brazil) and DG (Aptus™; Medartis, Basileia, Switzerland) (Supplementary Figure 1). The FMR was three spiral layers of stainless steel superimposed one upon another and did not allow disassembly. The DG was composed by three parts that could be dissembled, one section was lumened, with a diameter at the distal end of 1.2 mm and a proximal diameter of 2.3 mm.

Surgical instrument contamination

The RSIs were contaminated by immersion for 5 min, which is the average time RSIs are in contact with the patient (orthopaedic surgeons, personal communication) in 250 mL of tryptone soya broth (TSB), containing 10^9 cfu/mL of *S. aureus* (ATCC 25923) and 5% sheep blood (Edwards Group, Narellan, NSW, Australia). They were then dried at room temperature for 7 h, to mimic the worse-case scenario time between the use of an RSI and commencement of cleaning.

Surgical instrument reprocessing

The RSIs were contaminated and divided into three groups for reprocessing (cleaning plus sterilization). Three different cleaning protocols were used:

- Group 1. Rinsing; positive control: the RSIs were rinsed with distilled water for 30 s and dried with a sterile non-woven fabric.
- Group 2. Manual cleaning: RSIs were rinsed in distilled water for 30 s, immersed in enzymatic detergent (3E-Zyme triple enzyme bacteriostatic cleaner; Medisafe, Bishop's Stortford, UK), according to the manufacturer instructions for use (4 mL/L, at 30–40°C for 5 min). They were then scrubbed on each surface, five times, using a soft bristle brush and an appropriately sized brush for lumens (Spectrum Surgical Instruments, Birmingham, AL, USA) and rinsed in filtered water. The lumen was rinsed using a water gun which provided 90 kPa water pressure. The surgical instrument's external surface was dried using a sterile non-woven fabric, and the lumen dried using filtered medicinal air, using a compressed medical air gun (AguaJet Water Jet Pistol, Gebruder Martin GmbH & Co., KLS Martin Group, Tuttlingen, Germany).
- Group 3. Manual plus automated cleaning: RSIs were rinsed and manually cleaned as described in group 2. The lumened section of the DG and the FMR were subjected to flushing sonication (40 Hz; PCF™ System, Medisafe Sonic Irrigator, Medisafe), using 3E-Zyme, for 42 min. Afterwards, the instruments were placed in a washer-disinfector (Steris, Beauport, Quebec, Canada) and subjected to the following cycle: Pre-wash, Wash phase 1 (Prolystica™ ultra concentrate HP enzymatic cleaner; Steris), Wash phase 2 (Prolystica).

Following cleaning, three surgical instruments from each cleaning protocol were randomized and tested for residual ATP contamination, as detailed below. All instruments were then packed into medical surgical grade peel pouches (Universal Choice Wholesaler, Turrella, NSW, Australia) and sterilized at 134°C for 3 min and 30 s (Getinge Sterilization AB, Getinge, Sweden). Instruments were contaminated and subjected to reprocessing 20 times.

Detection of residual soil following reprocessing

ATP detection

Three DGs and three FMRs were tested for residual ATP on external and internal surfaces after each cleaning protocol.

The amount of ATP was determined using ATP LuciPan Pen™ – Lumitester PD-20 ATP surface (Kikkoman, Tokyo, Japan), using a benchmark of 115 RLU, classified as 'very strong evidence of cleanliness' [19]. AquaSnap total™ ATP detection device for water (Hygiene, Toronto, Canada) was used to determine the amount of ATP contaminating lumens, using a benchmark of 25 RLU, classified as 'very strong evidence of cleanliness' [19]. Hygiene luminometers measure on a scale different from that of Kikkoman [19].

Protein detection

The amount of protein contaminating three random surgical instruments from each cleaning protocol was determined after the 10th and 20th reprocessing cycles, using the Bicinchoninic Acid Assay Kit (BCA assay; Pierce Chemical Co., Dallas, TX, USA), according to the manufacturer's instructions. This kit measures the protein that is solubilized by detergent only; thus our results may underestimate the quantity of protein left on the instruments. Briefly, the instruments were immersed in reactant solution and incubated at 60°C for 35 min, cooled, and the optical density measured at a wavelength of 562 nm. Protein concentration was calculated from a prepared standard curve according to the manufacturer's instructions.

Microbial load

Microbial load was determined on three surgical instruments per cleaning protocol after the 10th and 20th sterilization cycles, by immersing RSIs in 10 mL of phosphate-buffered saline and sonicating for 10 min (Soniclean™; JMR Australian, Stepney, SA, Australia), using a frequency of 42–47 kHz followed by serial dilution. Each dilution was plated on to horse blood agar and incubated at 37°C for up to 48 h.

Visual assessment of surgical instruments by scanning electron microscopy

Reusable surgical instruments were aseptically sectioned into smaller pieces to enable scanning electron microscopy (SEM) to be conducted. Sectioning was performed in a biological safety cabinet covered by sterile non-woven fabric, using a sterile blade attached to a rotary tool (Dremel™ 3000, Robert Bosch Tool Corp., Racine, WI, USA). Multiple cross-sections were taken along the length of FMR, and the inner layer (second layer) removed and analysed. The DG hook part was removed and the disassembled outer part and the lumen cross-sectioned.

The following surgical instruments were analysed: one new FMR subjected to reprocessing (manual plus automated cleaning and steam sterilization: negative control), one FMR and one DG contaminated with TSB and 5% sheep blood, and one FMR and one DG from each of the cleaning protocols (groups 1, 2, and 3 described above).

Fragments were fixed in 3% paraformaldehyde, dehydrated through increasing concentrations of ethanol (50%, 70%, 80%, 90%, 100%), rinsed in 1:1 volume of ethanol/hexamethyldisilazane (HMDS), followed by immersion in 100% HMDS, air-dried, and then coated with 20 nm of gold [24]. Samples were examined using a JSM-6480 LA SEM system (Jeol, Tokyo, Japan). Biofilms may be defined as a community of bacteria attached to a surface and each other and surrounded by extracellular polymeric substances (EPSs) that are produced by themselves. Samples appearing to have bacteria surrounded by EPSs were classified as biofilm positive.

Data analysis

To test for significant differences for ATP readings between multiple treatments, a Kruskal–Wallis one-way analysis of variance combined with the Tukey all pairwise multiple comparison was used. To test for significant differences for protein contamination between treatments, a Student's *t*-test was used. SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA) and IBM SPSS Statistic View version 21 statistical programs were used.

Results

Detection of soil

A total of 180 ATP readings were obtained (60 per cleaning regime) from surface and lumens of DGs and FMRs. The positive control (rinsed only) instruments were all contaminated with high levels of ATP on the external surface, benchmark 115 RLU, mean 4680 (range: 490–11,485) for DG, and mean 15,784 RLU (range: 829–47,443) for FMR, respectively. Manual cleaning significantly reduced the amount of surface ATP residue for both the DG and FMR by between 2 and 2.5 $\log_{10}$ (>99%) ($P < 0.001$); however, 2/60 and 1/60 were >115 RLU. For the DG, no further reduction in surface ATP was observed with

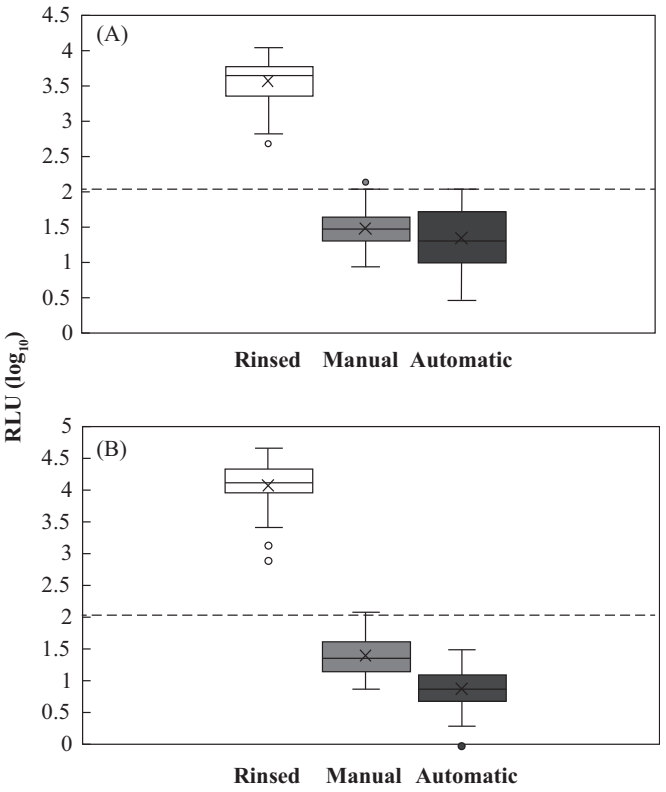

Figure 1. Amount of residual soil (adenosine triphosphate) on the surface of reusable surgical instruments over 20 processing cycles. Surface adenosine triphosphate levels on depth gauges (A), and flexible medullary reamer (B) subjected to different cleaning regimes: rinsed (positive control, $N = 60$; manual cleaning, $N = 60$; manual plus automated cleaning, $N = 60$). Dashed line: 2.06 $\log_{10}$ = 115 RLU (relative light units), classified as 'very strong evidence of cleanliness' [19].

automated cleaning (cleaning protocol 3) (Figure 1A), and 1/60 remained above the benchmark (115 RLU). For FMR, automated cleaning resulted in an additional non-significant reduction in ATP level of 0.5 $\log_{10}$ (Figure 1B). There was no significant difference in the amount of ATP contamination between manual and automatically cleaned instruments.

The mean of ATP reading, benchmark 25 RLU, from control (rinsed only) DG lumens was 56 RLU (range: 5–255), whereas the lumens of all control FMR (rinsed only) were highly contaminated with ATP (mean: 1116 RLU; range: 76–6965). The ATP readings after manual cleaning significantly decreased for both DG (mean: 1.48 RLU; range: 0–41) and FMR (mean: 3.68 RLU; range: 0–54) ($P < 0.001$), although one DG and one FMR ATP reading were >25 RLU. ATP was not detected (0 RLU) from lumens of instruments subjected to automated cleaning ($P < 0.001$) (Figure 2).

A significant amount of protein accumulated on positive controls (rinsing only) both for DG (mean: 1380 μg; range: 420–2341) and FMR (mean: 2191 μg; range: 1576–2807) by the 10th cycle ($P \leq 0.04$). Significantly more protein accumulated between the 10th and the 20th cycles in FMR (mean: 3891 μg; range: 2645–5137) ($P = 0.03$), but there was no increase in the amount of protein contaminating the DG with increasing cycles (mean: 1212 μg; range: 685–1739). Both manual and manual plus automated cleaning reduced the protein contamination to below the detection of the assay (equivalent to 5 μg of bovine serum albumin), irrespective of the number of cycles. No viable organisms were isolated from DGs and FMRs following autoclaving for all the test groups.

Visual assessment of surgical instruments

New FMRs were confirmed as being free from organic debris (Figure 3A). By contrast, new RSIs subjected to five cycles of immersion in TSB containing sheep blood (Figure 3B) for 5 min, rinsed and subjected to steam sterilization, clearly showed the presence of organic matter on their surfaces.

DG of the positive control (rinsed only) showed the presence of organic matter and biofilm, with coccoid micro-organisms embedded in an amorphous matrix of extracellular polymeric substances (Figure 4A, B). Large patches of biofilm were also present on lumenal (Figure 4C) and continuous biofilm on the slim surfaces of DGs subjected to manual cleaning (Figure 4D). DG subjected to manual plus automated cleaning had biological deposits (Figure 4E, F), although there was no evidence of biofilm.

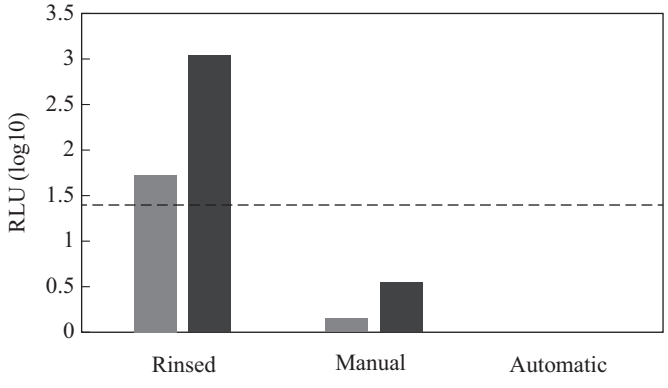

Figure 2. Amount of residual soil (adenosine triphosphate) in the lumen of depth gauge (grey bars) and flexible medullary reamer (black bars) following the 20th processing cycle through different cleaning regimens: rinsed (positive control, $N = 60$; manual cleaning, $N = 60$; manual plus automated cleaning, $N = 60$). Dashed line: 1.39 $\log_{10}$ = 25 RLU (relative light units), classified as 'very strong evidence of cleanliness' [19].

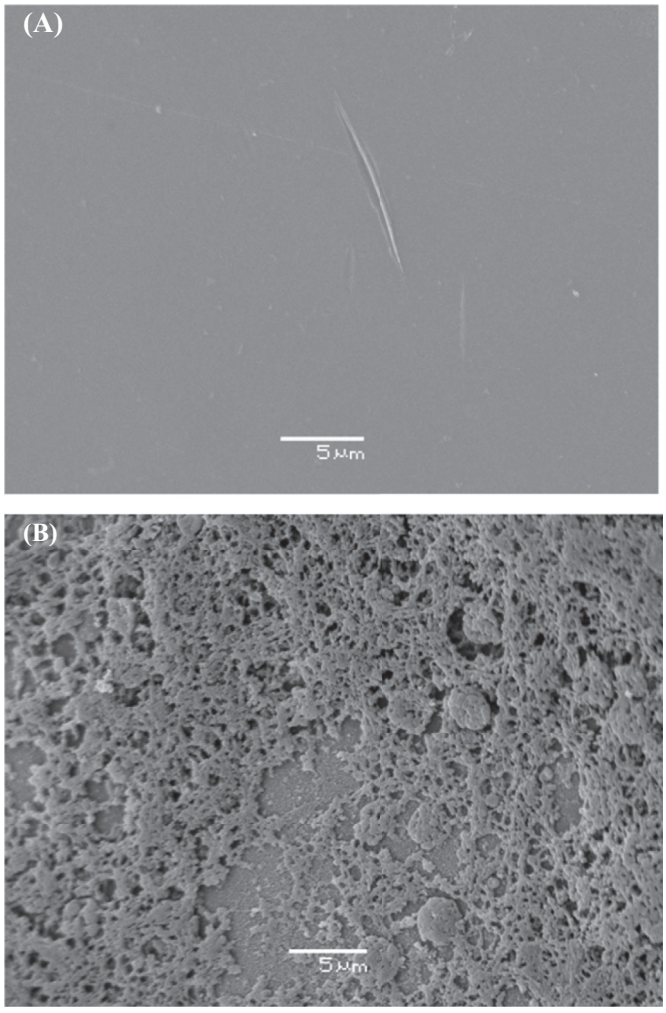

Figure 3. Micrographs of new flexible medullary reamer (FMR) without contamination (A). Micrographs of FMR contaminated with tryptic soya broth and sheep blood (B). Supplementary Figure S1 shows the FMR.

Micrographs of the inner layer (second layer) of FMR subjected to different cleaning regimens showed the presence of soil (Figure 5). On the positive control FMR (rinse only), large patches of biofilm were easily detected (Figure 5A). Soil and a clump of attached cells were evident on the manually cleaned FMR (Figure 5B) and the FMR subjected to automated cleaning was contaminated with biological deposits (Figure 5C).

Discussion

The number of complex-design RSIs used in orthopaedic and other complex surgeries increases each year. We have shown that manual cleaning is incapable of removing all patient soil and that biofilm may form even with only 20 cycles of

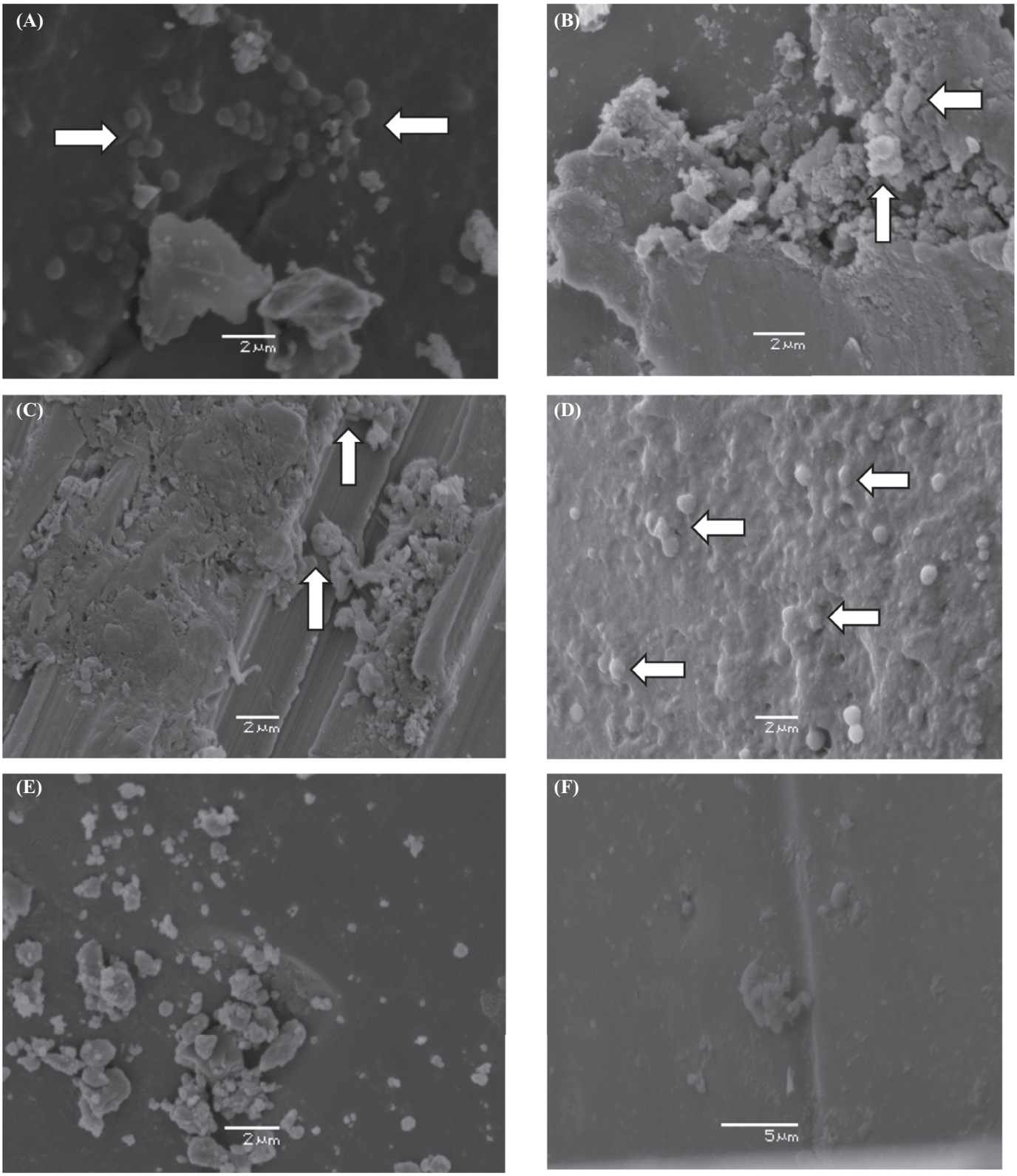

Figure 4. Micrographs of depth gauges submitted to 20 cycles of contamination, cleaning, and sterilization. Upper row: positive control depth gauges (rinsed); arrows show large patches of biofilm and bacteria are covered in extracellular polymeric substances (A, lumen; B, hook part). Middle row: manually cleaned depth gauges, showing large patches of biofilm (C, lumen) and continuous biofilm (D, slim part); arrows show where the outlines of bacteria in clumps are easier to see. Lower row: manually plus automatically cleaned depth gauges, showing presence of soil (E, lumen; F, slim part). Supplementary Figure S1 shows the depth gauge.

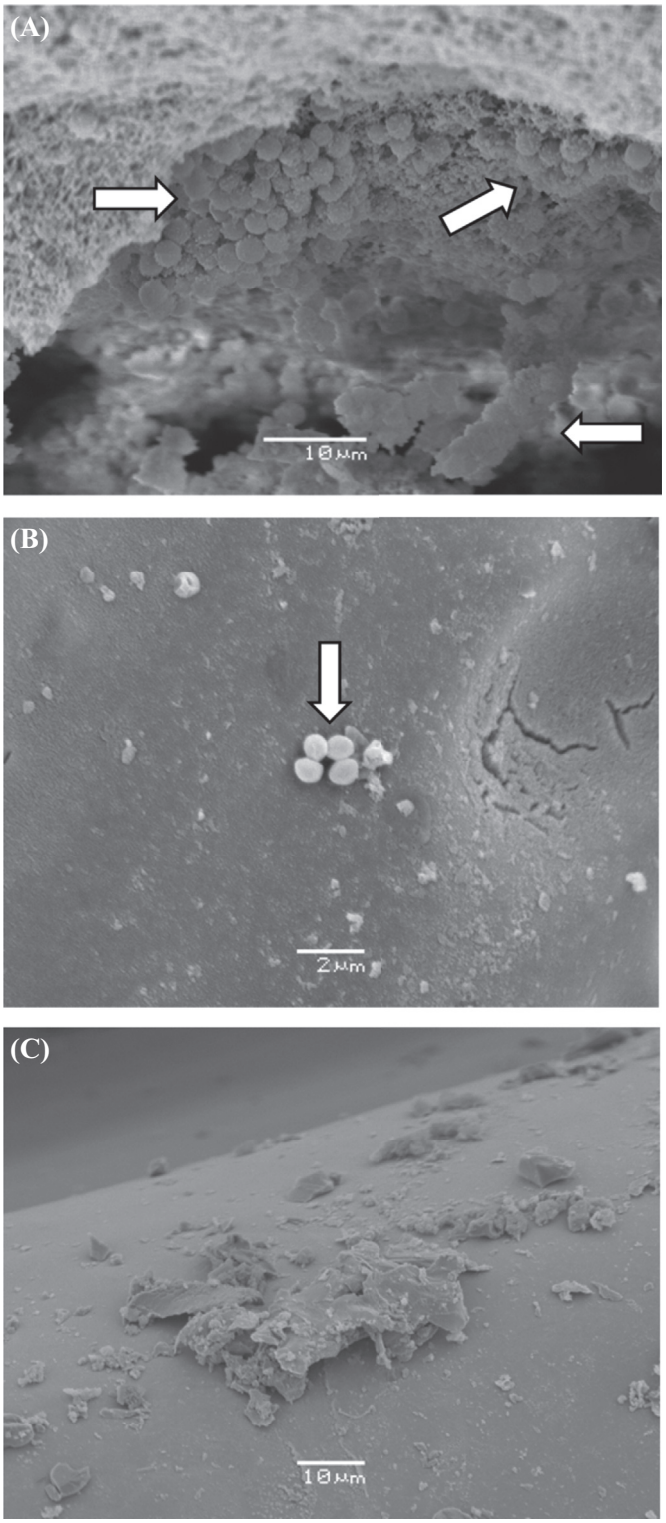

Figure 5. Micrographs of the inner layer of flexible medullary reamer submitted to 20 cycles of contamination, cleaning and sterilization. (A) Positive control flexible medullary reamer (FMR) (rinsed), showing large patches of biofilm. Surface-attached bacteria are easily seen. Arrows point to areas where the bacteria are more engulfed by extracellular polymeric substances. (B) Manually cleaned FMR; arrow shows surface-attached bacteria. (C) Manual plus automatically cleaned FMR; presence of soil. Supplementary Figure S1 shows the FMR.

contamination and reprocessing (Figure 4E, H). Clinically, manual cleaning is neither reproducible nor uniform; combined with the possibility of human error, this facilitates the accumulation of soil and may enable biofilm formation [20,21]. This is worrisome especially for those sterilizing service units that are lacking automatic washers, yet are reprocessing complex-design RSIs, as is the case in some Brazilian hospitals [22,23].

Our results showed that the RSIs subjected to manual cleaning followed by automated cleaning were positive for organic residue, as detected by surface ATP (Figure 1), and the presence of soil was visually confirmed by SEM (Figure 5C, D). The degree of contamination is open to interpretation as readings $<$90 RLU mean that ATP is detected; however, as this is below the lower level of quantification for Kikkoman luminometers, the recorded amount is not accurate [19].

All instruments were culture negative following steam sterilization, although micro-organisms incorporated into biofilm were detected on positive control instruments and on manually cleaned DGs by SEM. Bacteria within biofilms are not readily culturable due to their low metabolic rate, thus it is not always possible to detect them using standard plate culture, but these non-culturable micro-organisms are still infectious and periodically release cells which may result in new infections [24]. Additionally, live bacteria, as detected by viability staining and confocal laser scanning microscopy, have been detected in autoclaved *S. aureus* biofilm, and the biofilm, although immediately culture negative by plate culture post autoclaving, returned positive cultures with prolonged incubation in liquid media [12].

The FMR could not be disassembled for cleaning and the spiral design of the FMR prevented brushing or pressurized water jet access between the spirals, thus facilitating the harbouring of micro-organisms and organic debris. Furthermore, these instruments are subjected to an unrecorded number of reprocessing cycles and are often used until their functionality is lost, favouring accumulation of high amounts of soil, as has been shown on FMR obtained from Australian hospitals [25]. Once biofilm forms on surgical instruments, it is difficult to detect, hard to remove, and even automated cleaning may fail to eradicate it due to its adhesion to the surface [26,27].

The accumulation of residues and/or biofilm on DG or FMR internal layers raises the possibility of debris/live biofilm being released from instruments during their use and directly inoculated into patients, increasing the risk of infection or causing inflammation resulting in aseptic implant loss [28,29]. A concern related to inadequate reprocessing of RSIs is the presence of residual endotoxins that may inhibit initial osseointegration of implants and result in aseptic loosening [29]. Furthermore, residual biological matter, such as protein on RSIs, is of concern due to the risk of transmission of infectious prions, and due to the contribution of soil to sterilization failure [30—34].

RSIs evaluated in this study are provided by loaner companies and are transported to different hospitals, and this can contribute to the inter-institutional transmission of micro-organisms, including resistant strains [35,36]. Each country is responsible for regulating RSI reprocessing; however, ensuring effective reprocessing of FMRs, DGs and other complex-designed instruments is a difficult task [35,36—38]. Additionally, although it is important to follow the manufacturer's recommendations for decontaminating instruments, company

recommendations are usually generic, and frequently have not been validated for each specific RSI, which may also lead to inadequate cleaning. For example, Azizi *et al*. evaluated the efficacy of the automated cleaning protocol recommended by the manufacturer of a surgical aspirator tip, and concluded that the recommended method was ineffective [39]. Tosh *et al*. investigated an outbreak of SSIs following knee arthroscopy and found that the manufacturer's cleaning instructions for shaver handpiece suction channels were inadequate and that tissue was contaminating cleaned channels [15]. This led the US Food and Drug Administration to require manufacturers to validate cleaning protocols [40]. To ensure decontamination of all types of RSI, there is a need to validate cleaning protocols minimally for each group of RSIs or surgical box family [35,37,38,41]. These findings also highlight the need for validating automated cleaning equipment and training personnel [6,21,41].

In conclusion, this in-vitro model showed that inadequate cleaning (rinsing only) of *S. aureus*-contaminated complex-design RSIs led to biofilm formation. None of the cleaning protocols removed all debris from the surgical instruments. After the 20th contamination/reprocessing cycle, soil residue, including biofilm, was observed on the instruments subjected to manual cleaning. These results highlight previous findings and confirm that complex-design RSIs cannot be reprocessed multiple times without risk of residual protein and/or microbial contamination occurring [25]. This indicates the need for further regulatory policy regarding the design and manufacturer's instructions for use of RSIs — for example, approving the manufacturing and use of instruments that can be completely disassembled, or indicating single use when this is not possible, especially for FMRs, considering that many sterilizing service units do not have automated cleaning equipment available.

Conflict of interest statement
None declared.

Funding sources
This work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) (Process number: 484578/2013-8); Cotutelle International Macquarie University Research Excellence iMQRES scholarship (allocation no. 2015142), and Coordination for the Improvement of Higher Education Personnel (CAPES) Scholarship (process no. 99999.006361/2014-05 and process no. 99999.006360/2014-09); and Macquarie University VC Innovation Fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2018.11.001.

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