Endoscope reprocessing and patient safety: evaluation of endoscopes and quality control.

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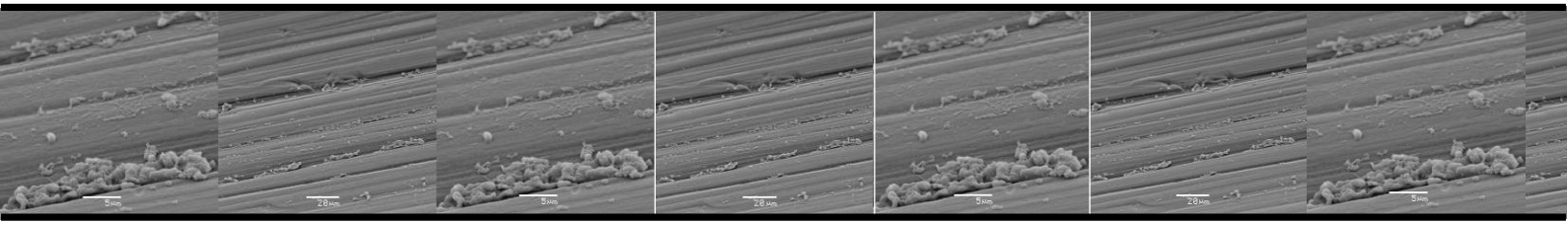

Declaration of originality

I hereby declare that the work presented in this thesis is originated from a cotutelle PhD realized at Macquarie University, Australia and at University of Sao Paulo, Brazil. Therefore, the Portuguese version of this thesis has been submitted to University of Sao Paulo and approved in 28/07/2017. To be best of my knowledge this submission contains no material previously published or written by another person, except where due reference is stated otherwise. Any contribution made by others to the research is explicitly acknowledged.

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2. Microbiological and physicochemical indicators on flexible endoscope reprocessing: manual cleaning procedure.

3. Evaluation of soil and contamination level of reprocessed gastrointestinal endoscopes.

4. Identifying contaminated areas on endoscopy unit´s surfaces: a brief report.

5. Microbial contamination on flexible endoscope channels

6. Evaluation of surface damage and biofilm formation in endoscope channels

"The Mighty One has done great things for me, and holy is His name.
His mercy is from age to age to those who fear Him". Lk 1, 50-51.

To Jesus Christ,

The one who loved me.

"eu, miserável, em Ti! *Tu, Misericórdia em mim*!"

To my parents, Luiz Júnior and Maria Rita

and my beloved siblings Larissa and Rodrigo,

we are better together!

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LIST OF ABBREVIATION

A	Ångström
AER	Automatic Endoscope Reprocessors
ATP	Adenosine Triphosphate
CDC	Centers for Disease Control and Prevention
CRE	Carbapenem-resistant Enterobacteriaceae
EO	Ethylene oxide
EPS	Extracellular Polymeric Substances
ERCP	Endoscopic retrograde cholangiopancreatography
GCS	Global Citation Score
H_2O_2	Hydrogen peroxide
HAI	Healthcare Associated Infections
HBA	Horse Blood Agar
ISI	Institute for Scientific Information
KPC-Kp	Carbapenem-resistant Klebsiella pneumoniae
LCS	Local Citation Score
MRO	Multidrug-resistant Microorganisms
O3	Ozone
OPA	Orthophthalaldehyde
PCR	Polymerase Chain Reaction
QS	Quorum-sensing
RLU	Relative Light Unit
SCI	Science Citation Indexes
SEM	Scanning Electron Microscopy
TSB	Tryptic Soy Broth
WoK	Web of Knowledge
WoS	Web of Science
WHO	World Health Organization

Abstract

The difficulties in reprocessing gastrointestinal endoscopes and risk of patient to patient transmission of infectious organisms are recognized challenges in medicine. This thesis investigated gastrointestinal endoscope reprocessing both in the clinic and in the laboratory. Analysis of clinical data was performed for assessing soil level, contamination level and biofilm formation in four different settings: before and after manual cleaning, directly after endoscope reprocessing (12 to 48 hours after disinfection), following internal channels extraction for repair (clinically used endoscope from Australia and Brazil) and environmental sampling of endoscopy unit surfaces. Experimental analysis involved investigation of endoscope internal channels for surface damage and its relationship to frequency of use.

The tests used for the analysis were adenosine triphosphate (ATP) bioluminescence for presence of soil, polymerase chain reaction (PCR) for bacterial load, microbial culture for viable bacterial numbers, scanning electron microscopy (SEM) for biofilm presence and stylus profilometer for surface roughness. Statistical analysis was performed by descriptive analysis, Mann-Whitney, Wilcoxon and Spearman tests, $p<0.05$, using IBM SPSS Statistics version 23.0.

Before and after cleaning analysis of 99 endoscopes showed cleaning effectively reducing soil ($p<0.001$) and microbial contamination level ($p=0.03$).

After complete reprocessing, all 75 endoscopes tested showed reduced soil contamination (all samples < 50RLU, from internal and external area); however the median microbial load was 3 Log_{10} and 10% of samples were culture positive. *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis,*

Staphylococcus capitis, *Roseomonas gilardii* and *Micrococcus luteus* were isolated after endoscope reprocessing.

Environmental samples were obtained from the nursing station and procedure room. Nine of the 24 (38%) samples had ATP values > 100 RLU and were then samples for microbial load by culturing. The areas with more contamination were the procedure room´s pipe and nursing station chair´s lift. Some potential pathogenic microorganisms were isolated (i.e. *Shigella spp.* and *E.coli*).

Endoscope biopsy channel analysis showed that samples from Brazil had higher contamination levels than Australian samples ($p<0.001$). All channels analyzed were contaminated with soil and biofilm and presented damaged surfaces, however Brazilian samples were also contaminated with blood cells, neutrophils and fungus.

Clinically used endoscope channels (median=526.82 A) were significantly rougher than new (median=357.8 A) endoscope channels ($p=0.03$). The increased roughness in used endoscope channels could provide a good environment for bacteria and patients' soil attachment, making cleaning and disinfection harder and contributing for biofilm formation. On in vitro model, tubing damage was consistent and higher than new after 500 passages of biopsy forceps and bacteria attach to internal surface of the tube with 30 minutes of flow contact.

We conclude that even with low microbial load on endoscopes there is a potential risk of cross-infection associated with biofilm formation, frequency and quantity of use and time for reprocessing, which can easily compromise the accomplishment of endoscope reprocessing and therefore, patient safety.

Keywords: Endoscopes. Disinfection. Contamination. Biofilm.

CHAPTHER 1. LITERATURE REVIEW

1. INTRODUCTION

1.1 Healthcare Infections and Patient Safety

Worldwide healthcare associated infections (HAI) are one of the leading public health problems that can lead to severe human and economic repercussions. Although HAI are a significant threat for patient safety, they can be prevented[1] as their occurrence is influenced by a combination of patient related conditions and healthcare workers, structural and organizational matters[2].

HAI are defined by the Centers for Disease Control and Prevention (CDC)[3] as a local or systemic condition caused by an infectious agent or its toxin, following admission to an acute care setting, providing there was no evidence of infection and/or incubation at admission.

In high-income countries, the rate of HAI is approximately 5 to 10%, however, in medium or low-income countries more than 20% of acute care patients are estimated to develop HAI[4].

Due to the social and economic repercussions, the World Alliance for Patient Safety program, has intensified the effort of implementing effective measures for HAI control and prevention by mobilizing healthcare professionals, researchers, professional associations and regulatory agencies, as part of its Global Patient Safety Challenge action area[5].

The use of invasive medical devices, including reusable devices that require sterilization and failure to decontaminate these properly on, is a significant factor in the development of HAI[26], and therefore, may compromising patient safety.

Safety in patient care is considered an important quality indicator for healthcare establishments[5] and the prevention of HAI requires a multifactorial approach, such as promotion of HAI prevention education, motivating adherence to preventative recommendations, surveillance and epidemiological data collection, resource management and conducting research to ensure that strategies are based on scientific evidence[7].

1.1.1 Endoscope Contamination and transmission of Infection

Compared to surgical instruments, which mostly come into contact with sterile sites and thus become contaminated with few bacteria[8] endoscopes come into contact with the gastrointestinal system, and thus become heavily contaminated. Contamination levels of up to 6 $\log_{10}$ colony-forming units make endoscope reprocessing even more challenging as there is little margin for error[9].

Endoscope contamination assessed by microbial culture has been frequently reported in the worldwide literature[10-14] including in Australia[15] and Brazil[16,17]. International surveys indicate that the percentages of positive cultures for microbiological contamination of flexible endoscopes vary from 1.9 to 31%[15]. In Brazil, these values appear to be discrepant with 70-71% of positive endoscope cultures, referent a multicenter study involving 37 endoscopy services[16].

Evaluation of patient ready endoscopes have demonstrated that despite cleaning and high level disinfection, biofilm develops on many of the channels[18,19]. The presence of any soil compromises disinfectant action[20] and this may partly explain the why transmission of infection can occur despite high level disinfection or sterilization.

Traditionally the risk of obtaining an infection following endoscopy was thought to be low[21], however, there have been numerous papers detailing outbreaks of infection, including multidrug resistant organisms, associated with gastrointestinal endoscopy[22-27]. Using molecular and genetic techniques, endoscopes have been shown become contaminated and to transmit infectious organisms between patients[28].

Spach et al (1993) [29] reviewed scientific articles published between 1966 and 1992 and found 281 episodes of nosocomial transmission of pathogens attributable to endoscopy. In 1993, Struelens et al. reported an outbreak of post endoscopic retrograde cholangiopancreatography (ERCP) bacteremia, which was related to failure of endoscope reprocessing in an automated disinfector. Argeton et al. (1997) [30] reported an outbreak of infectious organism transmission related to endoscopy by confirming the transmission of *Mycobacterium tuberculosis* via contact with a contaminated bronchoscope. Since then, many studies have reported outbreaks of patient infection following endoscopy[31,32]. In the United States, transmission of multiresistant microorganisms associated with Endoscopic retrograde cholangiopancreatography (ERCP) have been reported with increasing frequency[33-34]. Also, in Italy an outbreak of *Klebsiella pneumoniae* related to the use of gastroscopes and bronchoscopes was reported[31].

Recently, Epstein et al. (2014) [12] reported an ERCP associated outbreak of *Escherichia coli* resistant to Carbapenem. Using culture and DNA fingerprinting, by pulsed-field gel electrophoresis (PFGE), the authors confirmed that this multiresistant microorganism persistently contaminated the reprocessed duodenoscopes, and was transmitted to patients.

The carbapenem-resistant enterobacteria (CRE), are genetically highly resistant to antibiotics and cause difficult-to-treat infections with high mortality[1].

There has been constant growth in the number of publications addressing CRE organisms. Gastmeier and Vonberg (2014)[26] reviewed the literature of cases of *Klebsiella* spp. associated with endoscopy. They found the first report of endoscopy related *Klebsiella* infection was in 1988 involving inadequately processed duodenoscopes resulting in bloodstream infections[35]. After this slow start there were 4 reports of endoscopy related patient colonization and/or infection with *Klebsiella* in 2008-2009, two from France and two from the USA. The specific source of these ERCP/duodenoscope outbreaks was not discovered but the authors discussed insufficient compliance with endoscope reprocessing guidelines[31], insufficient cleaning and delayed cleaning[36]. Up to 2013, three further outbreaks occurred and were most probably associated with instrument or washer- disinfector defects resulting in incomplete cleaning[26].

Kim et al. (2016)[27] retrospectively investigated patients who underwent procedures with contaminated duodenoscopes and found a 7.6% colonization rate with CRE, thus increasing these patients' chances of developing disease.

During an outbreak of carbapenem-resistant *K. pneumoniae* (KPC-Kp), Naas et al. (2010)[11] found that six patients became colonized following endoscopy with the same contaminated device previously used on the index patient, and that two of these patients developed clinical infection due to the microorganism. The endoscopy procedure was described as significantly related to the isolation of KPC-Kp strains[25].

The confirmation of the transmission chain between patient and contaminated endoscope can be verified by studying the strains isolated in both by molecular and genetic techniques. Marsh et al. (2015)[28] determined that the microorganisms present in

the endoscope and the patient after an outbreak of KPC-Kp not only belonged to the same family, they were genetically related, thus confirming its transmission. The phylogenetics of seven genomes of *K. pneumoniae* isolated from patients and devices confirmed the endoscope as a source of transmission of the microorganism.

However, KPC-Kp is not the only microorganism associated with outbreaks following endoscopic procedures, but transmission and infection can be due to other bacteria and even viruses[42]. Recently, Robertson et al. (2017)[24] reported an outbreak of HAI with *Salmonella enteritidis* after endoscopic procedures. As early as 1995, gastrointestinal endoscopy with biopsy, was recognized as a risk factor for acquisition of hepatitis C virus[36] and Delarocque-Astagneau et al. (2007)[37], confirmed the relationship between digestive endoscopy and hepatitis C seroconversion, in Tunisia.

1.2 Bacterial Biofilms: brief considerations

Biofilms are structured communities of microorganisms that adhere to one another and generally to an organic (biotic) or inorganic (abiotic) surface. Bacteria preferentially grow as biofilm rather than as planktonic single cells. Bacterial attachment to abiotic surfaces such as medical devices can lead to biofilm formation and result in patient colonization and infection[38-41].

Biofilms consist of small colonies of bacteria surrounded by a matrix of Extracellular Polymeric Substances (EPS). Utilising a process of quorum sensing, the colonies develop into a three-dimensional mature biofilm[42,43]. During quorum-sensing biofilm bacteria produce and release chemical signals are into the surrounding environment. Once these signals reach a threshold, they regulate gene function and thus coordination of activities such as biofilm formation and virulence factor production[43,44].

Biofilm structure is influenced by environmental conditions. These include chemical and physical conditions, such as, temperature, humidity, pH, and proprieties of the surface the bacteria adhere to [45,46].

Any device, regardless of their composition, is susceptible to microbial colonization, biofilm formation. When used clinically, medical devices quickly become covered with patient secretions such as blood, mucous and urine. Deposition of these macromolecules onto devices results in a thin layer of host material or conditioning film. The conditioning film increases the ease of bacterial adhesion to the device as many bacteria have receptors on their surface for host macromolecules. Following initial attachment, the microorganisms are reversibly adhered and can easily be removed by surface cleaning[40,41, 44]. However, if the microorganisms remain in contact with the surface, the bacteria become irreversibly attached. A mature biofilm then develops with microbial multiplication and excretion of extracellular matrix by the attached cells. Finally, the biofilm releases planktonic cells that can adhere to new surfaces[41,44].

The matrix, formed by EPS, is the larger component of the biofilm biomass, being responsible for its structure, metabolism and protection. This high-density structure of microbial cells is organized in a way to optimize nutrients availability to their cells, contributing to the formation of this more resistant community than individual planktonic cells. Furthermore, EPS composition can also vary according to the type of microorganism forming the biofilm structure, however, the basic constituents are proteins, polysaccharides, nucleic acid and water[38,40].

Biofilms can be formed by same species (monomicrobial) or different species microorganism (polymicrobial). The formation of polymicrobial (mixed) biofilms may vary according to the availability of microorganisms in the environment. Interaction

between different species in the biofilm can be competitive when different species compete for nutrients or the different species coexist and collaborate for mutual growth[46].

Because they are arranged in this complex structure, biofilms have reduced susceptibility to inactivation by chemical agents, such as antimicrobials or disinfectants, and immune response when compared to planktonic cells[41,45,48]. The EPS of biofilms acts as a physical barrier against biocides, and when combined with the reduced metabolic rate of the sessile biofilm cells make biofilms up to 1000 times more tolerant to antimicrobials than planktonic cells. This makes biofilm formation an important bacterial survival strategy[49,41, 50].

1.3 Flexible Gastrointestinal Endoscopes

Annually, around 18 million gastrointestinal endoscopies are performed in USA, at an estimated cost of 32.4 billion dollars[51]. However, as endoscopy procedures are often performed outside hospitals, in specialized clinics in both the public and private sector, estimating the number of procedures worldwide is difficult.

Although an invasive procedure, gastrointestinal endoscopy is clearly beneficial for diagnosis and treatment of gastrointestinal conditions whilst simultaneously decreasing the necessity for major surgical interventions[52]. However, immediate complications, (i.e bleeding and perforation) and late complications such as infection[53] can occur.

The design and specifications of a flexible endoscope varies with endoscope type and are related to procedural requirement, however, all endoscopes have a complex basic structure of internal and external components[7] (Figure 1).

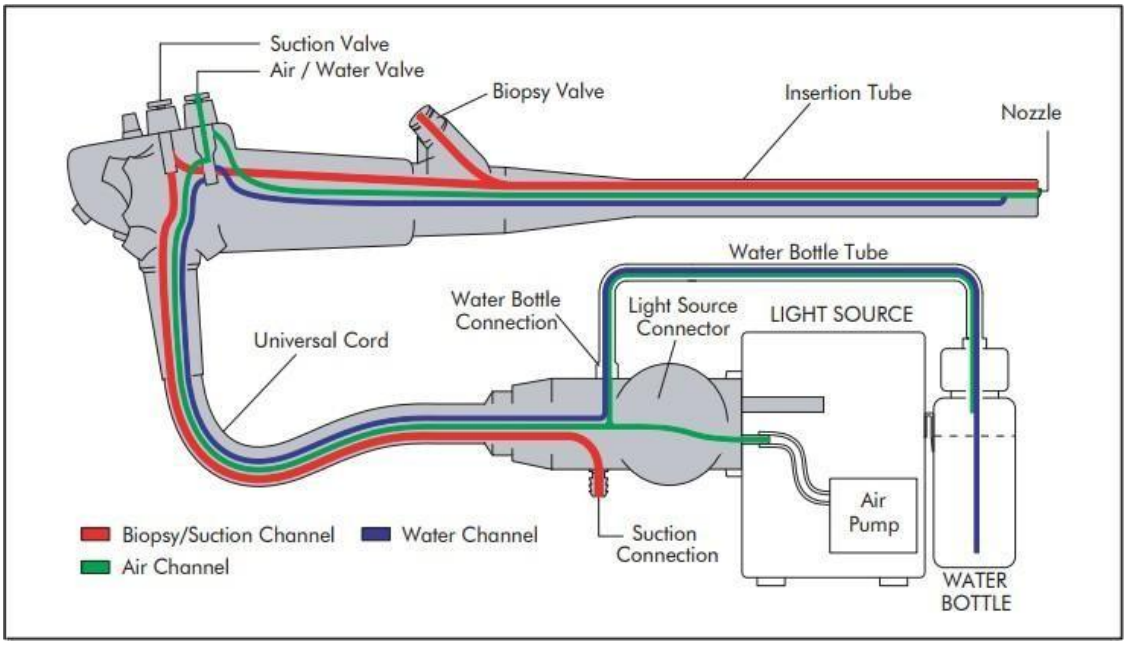

Figure 1. Representation of gastrointestinal flexible endoscope. Source: Olympus. Endoscope channel guide (2003).

External basic components are: light guide plug, to be connected to the light source at the time of endoscopy procedure; universal cord, which connect the light wires to the body of the endoscope; control head, containing controls for angulation, air jets, water and suction, and biopsy channel access valve; and insertion tube, the portion of the endoscope that is inserted into the patient during the procedure[54,2].

Internally, the basic components are: objective lens and imaging sensor; light guides, which contain the optic fibers which carry the light from the light source to the tip of the instrument (Figure 2). The instrument (biopsy) channel outlet, which allows both suction and insertion of endo-therapy accessories (i.e. biopsy, haemostasis); and port for air and water supply (Olympus, 2015).

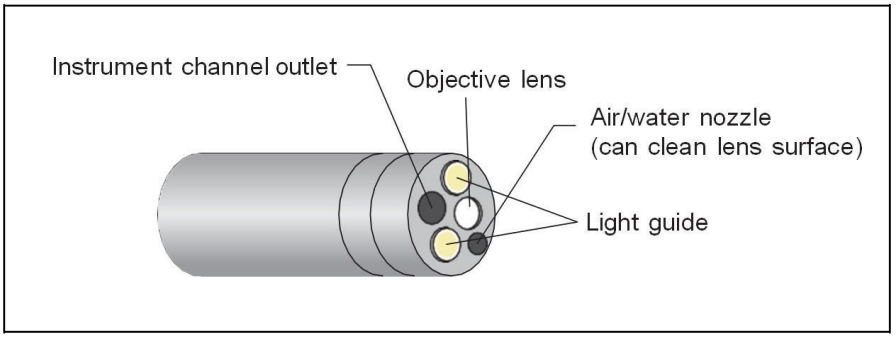

Figure 2. Representation of a gastrointestinal flexible endoscope insertion tube tip showing internal components by a cross-sectional view. Source: Olympus Medical Business (2015).

Internal channel length and caliber can vary according to each flexible endoscope specifications. Additionally, some flexible endoscopes may have a few structural variations, such as, elevator wire and accessories. An overview of the types of endoscopy procedures, endoscopes and required decontamination level is presented in table 1.

Type of Endoscope	**Rigid endoscope example**	**Flexible endoscope example**	**Level of Decontamination**
Invasive - passed into normally sterile body cavities or introduced into the body through a break in the skin or mucous membrane	Arthroscope Laparoscope Cystoscope	Nephroscope Angioscope Choledochoscope	Sterilization by steam or a low temperature method e.g. gas plasma
Non-invasive - in contact with intact mucous membrane, but does not enter sterile cavities	Bronchoscope	Gastroscope Colonoscope Bronchoscope	High-level disinfection, e.g. immersion in glutaraldehyde, peracetic acid, chlorine dioxide

Table 1. Types of endoscopic procedures. Modified from WHO (2016)[2].

When compared to flexible endoscopes, reprocessing of rigid endoscopes is relativity easy, because of their simpler structure[2]. In addition, the presence of optic fiber guides, makes flexible endoscopes heat sensitive devices. Similarly, the presence of structures such as elevator-wire devices makes flexible endoscopes much more complex increasing cleaning difficulty and, therefore, increasing the risk of process failure. Recent endoscopy HAI outbreaks have frequently involved the use of duodenoscopes containing elevator-wire devices[55]. These outbreaks have resulted in additional duodenoscope cleaning and process recommendations by endoscope manufactures and statutory bodies[1,56].

Overall, given the complexity of flexible endoscope structure with long and narrow channels, endoscope reprocessing should be carefully performed to avoid equipment damage and/or process failures resulting in contaminated endoscopes being used clinically and possible transmission of infectious organisms to patients[20,57].

1.4 Endoscope Reprocessing

Reprocessing of a medical device consists of using a validated process for removal of contamination[56]. The reprocessing of flexible endoscopes must also include high level disinfection or sterilization to kill any remaining microorganism contamination[57,58].

Spaulding's classification is used to determine what level of decontamination equipment should be subjected to. This is based on a risk analysis of the possibility of infection developing and the consequence of that infection. There are three broad categories: critical devices entering sterile areas require sterilization; semi-critical devices contacting mucous membranes or non-intact skin require a minimum of high-

level disinfection; and non-critical devices contacting intact skin require disinfection[56,60]. By Spaulding´s classification flexible endoscopes are considered semi-critical items, and so require high-level disinfection[66].

Sterilization is the process of eliminating or destroying all microbial forms, using either chemical or physical methods. The main sterilizing agents used in healthcare services are steam under pressure, dry heat, ethylene oxide gas, hydrogen peroxide gas plasma and liquid chemicals[61]. For heat-stable equipment e.g. stainless steel surgical instruments and some rigid endoscopes, sterilization by steam under pressure is recommended. For heat sensitive equipment low temperature options including hydrogen peroxide sterilization, ozone sterilization, ethylene oxide sterilization and liquid chemical sterilization can be used[56]. Sterilization is also used for decontaminating endoscope accessories[61].

As some endoscopes are used to access sterile areas, these instruments are categorized as critical items, and therefore, should be sterilized. However, there is no evidence that flexible endoscope sterilization reduces the risk of transmission of infection and improves patient safety[2,62].

Chemical sterilants can be used for both sterilization or high-level disinfection, by varying the period of exposure to the chemical. If exposed for long periods of time, i.e. 3 to 12 hours, some disinfectants can achieve sterilization with destruction of all types of microorganism[1].

Disinfection of endoscopes is usually conducted using liquid chemical agents. Increasing the temperature at which disinfection occurs, can increase its efficacy resulting in the elimination of most microorganisms, excluding spores[61]. Disinfectant efficacy can be affected by device structure as it is essential to ensure contact with the disinfectant and device surface. This can be difficult if blind channels are present. Other

factors that affect disinfectant efficacy include temperature, pH, disinfectant concentration, contact period with disinfectant agent and the effectiveness of cleaning process[63].

In general, global recommendations for endoscope reprocessing follow the same steps, including, cleaning, disinfection/sterilization, rinsing, drying and storage as illustrated in figure 3[64,20,21,65.]

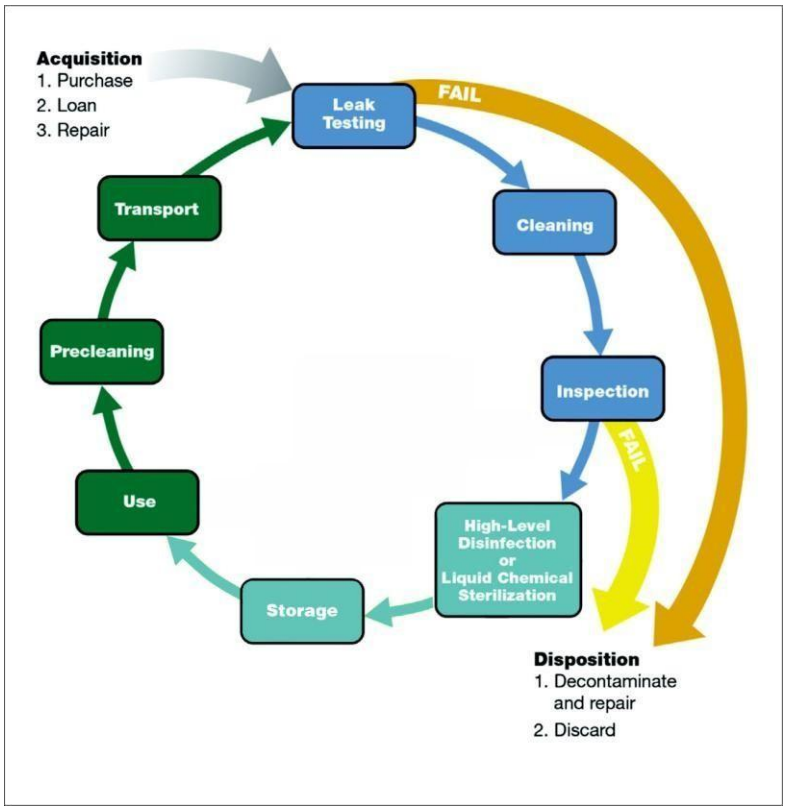

Figure 3. Flexible endoscope reprocessing cycle. Adapted from Bashaw M.A. (2016)[65].

As illustrated above, flexible endoscope reprocessing is necessary either after acquisition of an endoscope, which includes purchase, acquiring an endoscope on loan from a third party or following endoscope repair and after clinical use. After clinical use the endoscope is subjected to a leak test according the individual manufacturer's instructions. Leak test failure is an indicative of endoscope damage which can hinder

the reprocessing process and cause permanent damage to sensitive endoscope components, such as cameras and light guides by contact with water or chemicals. However, passing a leak test does not guarantee that the internal features of the endoscope are undamaged[20].

Endoscope reprocessing initially includes pre-cleaning and cleaning steps and occurs prior to the disinfection/sterilization process. Overall, the process of cleaning flexible endoscopes aims to remove secretions, and organic matter from the device, by a combination of a chemical agent (enzymatic detergent) and mechanical force (wiping and/or brushing) and assessment of the endoscope's external and internal compartments[20]. Cleaning also removes microorganisms[20], but does not kill them[66]. The cleaning step alone can reduce endoscope bacterial load by up to 4 $\log_{10}$[67,58].

It has been shown that the presence of organic matter compromises reprocessing because it acts as a physical barrier, impairing/preventing disinfectant contact with the device surface[68,58]. Thus, removal of organic and inorganic soil is an essential procedure to achieve successful reprocessing[20,64, 69] .

The use of enzymatic detergent to clean flexible endoscopes is globally recommended[20,,56,59,64,71]. An enzymatic detergent contains enzymes that degrade proteins, lipids and carbohydrates and thus digest the soil making it easier to remove[70]. Despite being able to reduce bacterial load due to soil and organic matter reduction, enzymatic detergents are not bactericidal. Thus, solutions containing enzymatic detergent should only be used for one endoscope before being discarded[59,71].

The pre-cleaning step consists in suctioning water and enzymatic detergent through endoscope internal channels followed by wiping the external area, and is performed in the procedure room immediately after endoscopy. Immediate precleaning reduces the time that organic material is in contact with the device's surface[1]. Increasing the contact time of organic matter with the endoscope surface can increase both soil and

microbial adhesion that subsequently can affect the disinfection/sterilization process[20,58,72]. Consequently, reducing the time between the end of the endoscopy and beginning of endoscope reprocessing improves the initial removal of the organic matter and so, contributes to successful reprocessing[1].

The endoscope must then be cleaned which must be performed in the endoscope unit's "dirty area". Endoscopes can be manually or automatically cleaned utilizing adequate water, detergent, correctly sized brushes and cloths. However, it should be conducted by a trained professional, familiar with the endoscope model's structure and its cleaning techniques requirements[20].

Manual cleaning refers to the immersion of the device in enzymatic detergent solution and manually cleaning both the external and internal parts of the endoscopes. This includes external brushing the control head, internal flushing of all channels with detergent solution and internal brushing of the biopsy channel, including additional channel portions when present; and rinsing the device in plenty of running water, including the internal channels, and ensuring the removal of any organic matter residue and/or detergent from the endoscope surface[20,65].

Automated cleaning can be achieved by automatic washing machines or at initial cycle of automatic endoscope reprocessors (AER). The use of automatic endoscope reprocessors is cited in international protocols[20,59,63,65] and has the advantage of providing standardization of cleaning, ensuring control of variables such as temperature, contact time/quantity of chemical agents and volume of rinse water used, hence avoiding human errors. The efficacy of AER has been demonstrated to be equal to or exceeding manual cleaning alone[73-75].

However, the use of only AER for cleaning endoscopes is controversial with many guidelines[71] still recommending manual cleaning prior to submitting the

endoscope to AER cycles. This is because the importance of friction in removing soil, either by pressure or brushing, has been previously demonstrated[59] and many believe that adequate friction is not provided in AER.

High level disinfection can also be conducted manually, by immersing endoscopes in disinfectant solution and ensuring channel perfusion, or automatically in AER machines[20,21,58]. High level disinfectants kill vegetative microorganisms on surfaces, except some bacterial spores[56,76]. Disinfectants approved internationally to be used in endoscope reprocessing are: glutaraldehyde, peracetic acid, hydrogen peroxide with peracetic acid, hydrogen peroxide, orthophthaldehyde (OPA) and chlorine based system[56] however, manufacturer's recommendations regarding material compatibility need to be considered.

Recommendations for concentrations and exposure time should be carefully followed to ensure the validity of the disinfection process. Despite their degradation over time, disinfectants are relatively stable substances, so agents such as glutaraldehyde, for example, can be used for 14 to 28 days after their preparation in the case of manual disinfection. However, to ensure the effectiveness of the process, strip testing for validation of the effective concentration of the disinfectant solution is recommended[58,64].

Following cleaning, disinfection and rinsing, endoscopes need to be dried. Many guidelines suggest injection of alcohol into the channels followed by forced air drying[20]. This can be performed manually or automatically in AER. According to several guidelines fail to recommend endoscope drying and this increases the risk of contamination and biofilm formation[54,77,78]. In fact, environmental contamination of endoscope can occur from contaminated water used for rinsing endoscopes either manually or in AER[78,79]. Additionally, in vitro testing showed that biofilm formed in

undried flexible endoscopes, even after effective disinfection, demonstrating the importance of the drying in preventing endoscope contamination[80].

Once processed, endoscopes need to be stored appropriately to prevent contamination by organisms found in the hospital environment. Storage of endoscopes in drying cabinets is recommend[1,54,57]. Drying cabinets force filtered medical air through the channels of the stored endoscope, thus providing a dry environment and preventing bacterial replication.

Inappropriate endoscope reprocessing may be related to human performance, inadequate conditions of inputs and equipment, such as structural and maintenance problems, failure to follow guidelines, organizational and environmental problems[81-83]. Therefore, the performance and success of endoscope reprocessing can vary accordingly to each healthcare service reality.

Spinzi et al. (2008)[84] reported a national study of 70 public and private endoscopy services in Italy, with the majority (84.4%) having automated endoscope reprocessors available and 67% using glutaraldehyde as a disinfectant in the reprocessing of gastrointestinal endoscopes. In a similar study conducted in 189 endoscopy units in China, Zhang et al. (2011)[85] showed that 22% of the services had automatic reprocessors and 88.5% used glutaraldehyde for the high-level disinfection of endoscopes. In addition, another study showed that most (18/20) European countries use automatic endoscope reprocessors in their endoscopy services[86].

1.5 Endoscope Reprocessing Monitoring Systems

Monitoring endoscope reprocessing is another important aspect of promoting patient safety, given that allows detection of failures of the process and comparison of performances to inform decision-making. However, among the international guidelines

there is inconsistency regarding the recommendations for surveillance of endoscope reprocessing, leading to a diversity of behaviors and lack of uniformity between services and institutions. Two main issues contribute to international disparities of routine surveillance; the need for strong evidence in support of the surveillance method to be recommended in guidelines, and healthcare services condition in that they need to approve the necessary funding for surveillance.

1.5.1 Visual Inspection

Inspection is an important step of general medical device cleaning process[2]. Most international guidelines include visual inspection of the endoscope as a method for evaluating the process of flexible endoscope cleaning[54,6,]. According to CDC (2017)[1] visual inspection of the endoscope is an essential step and must be performed after manual cleaning by all endoscope reprocessing services. Visual inspection not only evaluates cleaning by inspecting the endoscope for debris but also checks the endoscope for visual defects and damage, which can also interfere with the decontamination process.

However, visual inspection is not an ideal method for determining effectiveness of flexible endoscope[65] principally due to the endoscopes design. The external surface is black which easily masks soil and the presence of narrow internal channels are impossible to visualize. Visrodia et al. (2014)[87] evaluated the efficacy of visual inspection of gastrointestinal endoscopes compared to the ability of rapid tests for blood, protein and ATP tests to detect patient soil. Although no device was found to be dirty after visual inspection, 82% of the endoscopes had at least one rapid positive soil test. In addition, Martiny, Floss and Zühlsdorf (2004)[88] in an experimental study of

automated cleaning evaluation have shown that only the visual inspection of endoscopes is not enough to determine the effectiveness of the cleaning process, especially in the internal channels of the endoscope, by comparing visual inspection with microbial analysis.

1.5.2 Adenosine Triphosphate Testing

As an alternative to visual inspection, some researchers have suggested the use of Adenosine Triphosphate (ATP) testing as a method of evaluating the efficacy of the endoscope cleaning process[89-91]. The use of the ATP test to monitor the process of surface cleaning has been reported in the literature for some decades in the food industry[92] and recently in health services[93].

ATP is present in all living organism's cells and constitute their main source of energy. Luciferase is an enzyme that converts ATP into light, thus, the presence of ATP can be detected in a bioluminescence assay[94,95]. As ATP is present in all living organisms, it detects the presence of food, human cells and bacteria, therefore, it is an indicator of the level of surface contamination. ATP assays offer the following advantages: they are easy and practical to do; the result of the test is obtained quickly, taking less than 5 minutes from time of sample collection to results, this ensures that the endoscopes can be immediately recleaned at point of use[96,97].

On the other hand, some possible limitations on the use of ATP as a test for the evaluation of the cleaning procedure have been reported in literature. These limitations center around the inaccuracy and variability of ATP readings, particularly when used to measure low levels of ATP. Whiteley, Derry and Glasbey (2013)[98] evaluated 3 brands of ATP bioluminometers and found that while all brands could effectively measure the difference in ATP contamination on a $\log_{10}$ scale between 0.001 and 1mg/L of ATP,

performance outside this range was poor. There was no out of range warning given by any of the meters. Individual readings were inaccurate, and in addition, each luminometer brand quantified the amount of ATP using a different RLU scale. This compromised the comparison of ATP values obtained from different devices. Whiteley et al. (2015)[99] demonstrated in a study with four commercially available brands of ATP testing that the devices have low accuracy and reproducibility with the standard deviation of up to 50%. Similarly, in another study Omidbakhsh, Ahmadpour and Kenny (2014)[100] also showed limited sensitivity of ATP bioluminometers to detect low levels of microbial contamination.

Comparing ATP results between different brands available in the market and different situations in terms of sampling is difficult. Thus, it is necessary to prepare an institutional protocol with recommendations for interpretation of the results obtained (determination of a reference value for acceptable levels of soil) for use in clinical practice.

1.5.3 Microbial Culture Testing

Several researchers, including guidelines, describes the frequent use of microbial cultures for surveillance of gastrointestinal endoscope reprocessing[4,14,85,101]. Bajolet et al. (2013)[103] reported flaws in the process of cleaning and drying endoscopes from the investigation of multidrug resistant bacteria in patients submitted to endoscopy by means of surveillance culture. Naryzhny, Silas and Chi (2016)[104] described the implementation of routine microbial cultures in duodenoscopes after outbreak of multiresistant bacterial infection as one of the epidemiological surveillance measures.

According to Australian guidelines microbial culture of reprocessing of gastrointestinal endoscopes is recommended on a regular basis for monitoring of possible reprocessing failures [20,54]. Microbial culture also was identified as the only means of detecting damage to the internal channels of gastrointestinal endoscopes in the endoscopy unit routine practice[54].

On the other hand, some international protocols do not include surveillance culture as part of recommendations on quality control of gastrointestinal endoscopes reprocessing due to the time to obtain the results and costs involved with the technique. According Gillespie; Kotsanas; Stuart (2008)[105] microbial culture in endoscope routine surveillance is time consuming, costly and hard to sample. Additionally, challenges on interpreting the findings of microbial surveillance culture can be a problem for its implementation on clinical practice, especially in the case of isolating environmental strains[1,106].

In order to overcome the difficulties related to the sampling and interpretation of results of microbial cultures of reprocessing surveillance of endoscopes, GENCA (2003)[20] recommends the frequency that endoscopes must be tested and which microorganisms should be investigated, thus directing the procedure and interpretation of cultures. Regarding the periodicity of the surveillance of the microbial cultures in the gastrointestinal endoscopies every four weeks for duodenoscopes and bronchoscopes, and every three months for other gastrointestinal endoscopes, such as gastroscopes and colonoscopes.

In Multisociety guideline on reprocessing flexible endoscopes, Petersen et al. (2017)[71] explained that international recommendations for microbial cultures of endoscopes have been developed to investigate infection outbreaks and not for routine reprocessing surveillance, although they recognize that the isolation of microorganisms from the gastrointestinal tract makes it possible to identify reprocessing failures. CDC

(2017)[1] recommendations do not include microbial culture as an essential resource for evaluating the quality of reprocessing of flexible endoscopes due to controversies among professionals and researchers about the evidences that support the practice. However, microbiological monitoring has picked up lapses in endoscope reprocessing prior to any evidence of patient infection[107].

STUDY AIMS

Endoscope decontamination is a complex process and a challenge to professionals in the field because of, but not limited to, the following aspects:

- Contamination risks are multifactorial and are not totally transparent,
- The structural and human resources conditions within endoscopy units are diverse and vary between hospitals and countries,
- Most units conduct a high number of invasive procedures increasing the risks to the patient and staff,
- Endoscopy procedures are relatively brief and cost of endoscopes is high, thus reprocessing of equipment is time limited which increases the chance of failure of processing,
- Cleaning of endoscopes requires well trained health professionals, which is not always recognized by those managing human resources,
- Despite professional society guidelines being based on expert advice, scientific evidence is lacking for many of the processing steps,
- Microorganisms form biofilms on the internal surfaces of endoscope channels, and contribute to the failure of the decontamination process.
- The configuration of the endoscope is complex, with valves and several narrow and long channels, many of which are not large enough to brush, making it a challenge for reprocessing.

Based on the above the study hypothesis are:

1. The presence of biofilm can affect endoscope reprocessing, and
2. Endoscope channel damage can affect biofilm formation.

Study Objectives

- General Objective

  To investigate the reprocessing of gastrointestinal endoscopes by means of microbiological and physicochemical structural indicators, to provide a scientific basis for development of improved endoscope cleaning and disinfection processes.

- Specific Objectives

– To evaluate trends in endoscope reprocessing high impact publications by means of a bibliometric analysis.

– To evaluate the usefulness of ATP assays to detect contamination of clinical endoscopes by biological and microbiological soil post use, after manual cleaning and after reprocessing.

– To determine if neutralization of disinfectant is necessary for accurate microbial testing.

– To analyze microbial contamination of channels obtained from clinical endoscopes in Brazil and Australia.

– To analyze the condition of internal working channels obtained from clinically used and new colonoscopes.

– To develop an in vitro assay to model channel damage by endoscopic biopsy forceps.

– To investigate the biological and microbiological soil contamination of endoscopy unit environmental surfaces.

CHAPTER 2. TEMPORAL TRENDS ON ENDOSCOPE REPROCESSING

2.1 Introduction

A bibliometric analysis was performed for assessing scientific knowledge at endoscope reprocessing area and evaluating trends based on high impact publications from Web of Science database.

2.2 Temporal trends and epidemiology of Endoscope Reprocessing: a bibliometric analysis (1974-2017)

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Candidate Contribution:

- Study design

- Laboratorial experiment

- Data analysis

- Manuscript preparation

Overall contribution: 70%

Abstract

Failure of flexible endoscope reprocessing can result in contaminated endoscopes being used on patient(s) and may lead to outbreaks of infection. In order to evaluated trends in endoscope reprocessing and support decision making in the future, this study aimed to map scientific knowledge and evaluate published evidence of endoscope reprocessing failure using the Web of Science database. The search identified 595 records published from 1974 to 2017 and data was analyzed using HistCite™ software. Through mapping endoscope reprocessing scientific knowledge for analyzing high impact publication it was possible to realize that the investigation of infection transmission by flexible endoscopy has been related to failure of endoscope reprocessing for decades. The problems related with endoscope reprocessing failure were grouped into three main areas: importance of cleaning for endoscope reprocessing; importance of reprocessing recommendation compliance; and presence of contamination despite using recommended procedures.

Keywords: Endoscope. Reprocessing. Disinfection. Bibliometric analysis.

Introduction

The difficulties of gastrointestinal endoscope reprocessing are globally recognized and the challenge of ensuring safe patient-ready endoscopes mobilizes healthcare professionals, researchers and regulatory agencies attention. Endoscope decontamination is a multifactorial process and includes cleaning followed by disinfection and suitable storage. However, these processes can be impacted negatively by the complex design of endoscopes making cleaning and its validation difficult, human performance and organizational/structural requirements.

Failure to adequately process endoscopes can lead to persistence of bacterial contamination and result in patient infection[11,24,56,81].

Historically, the risk of infection transmission associated to gastrointestinal endoscopy was reported to be extremely low (1 in 1.8 million procedures)[23]. However, as later studies demonstrated that even though infections related to endoscopy procedures are rare, the actual amount of transmission is likely to be underestimated[108]. This is partly due to lack of epidemiological surveillance following endoscopy[95,108,109].

Currently, outbreaks associated with contaminated endoscopes have been widely reported in the literature[12,24,83,103,110,]including with isolation of multidrug resistant organisms[23]. The increase in identification of these outbreaks has been attributed to many of the outbreaks being associated with antibiotic resistant organisms with a high mortality rate[108]. Many of these outbreaks have been attributed to failure to follow decontamination guidelines, including insufficient cleaning, inadequate drying and storage and defective equipment[93,121]. However, other outbreaks have occurred even when endoscopes were reprocessing following published guidelines[13,122]. Over time the methods of endoscope reprocessing have changed from very basic decontamination to more rigorous treatments with better post-disinfection storage options. In this study, we aimed to map scientific knowledge and evaluate the evidence surrounding effectiveness of endoscope reprocessing by a review of the scientific literature through bibliometric analysis using the Web of Science database. This will support evaluation of trends over time and decision-making into the future.

Materials and Methods

Definitions

Web of Science (WoS) is a database set referenced as Science Citation Indexes (SCI) organized by the Institute for Scientific Information (ISI), owned by Thomson Reuters, that provides not only access to approximately 12,000 journals but also supports bibliometric analysis such as citations, references, H index (author-level metric).

HistCite™ is a software that analyzes direct citation interactions of bibliographic records from Thomson Scientific´s Web of Knowledge (WoK) and other sources. The analysis allows the evolution of the field by generating tables and histographs[130].

For didactic purpose, in this paper, a publication citation score is referred as impact and publication records states for the publications that composed the bibliometric analysis dataset. Therefore, the impact indicator used was the Local Citation Score (LCS) which represents the number of times each publication is cited by other publications within the research records used on this study.

Data Collection

A literature review was performed on Web of Science database for assessing endoscope reprocessing publication records. The search was performed in sets: first using the descriptors "endoscopes" AND "reprocessing"; then with "endoscopes" AND "disinfection"; and finally both sets with Boolean operator OR, as following representation in figure 4:

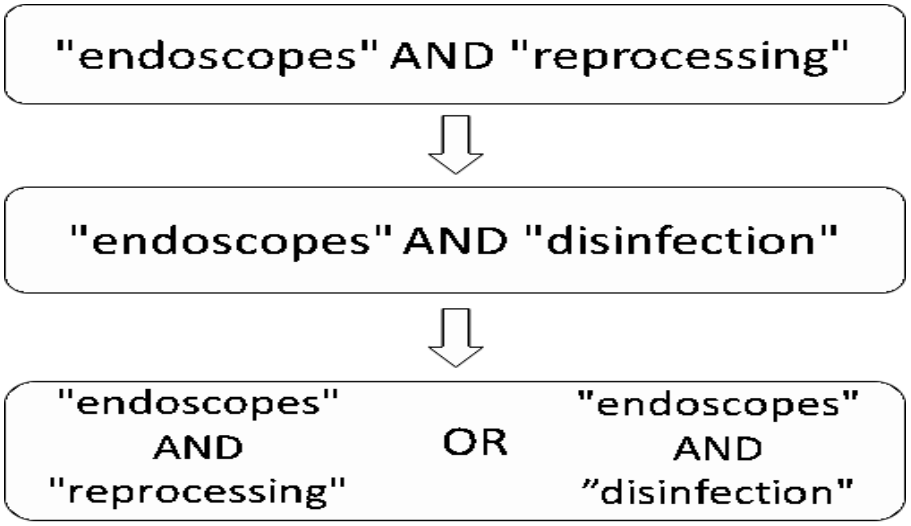

Figure 4. Descriptors.

Data Analysis

Data analysis were divided in two phases: bibliometric analysis of publication records and descriptive analysis of endoscope reprocessing evidence from publications with a higher impact in the area.

Initially all publication records obtained from the search were exported from ISI Web of Science to HistCite™ 12.03.17 software for bibliometric analysis, without any refinement filter for language, year of publication or area of knowledge. The publication records were assessed by results of number of citation score on the following aspects: authorship, country, cited references, words and year of publication; and by specific analysis of histogram from citation´s relationship.

Sequentially, a descriptive analysis was made centered on higher impact publications in the area (LCS) along with the high impact records from the years with more publications numbers at the area (2016 and 2017), and included data of authorship, title, impact score, type of research (review, guideline, in vitro, in vivo) and main result.

Results

A total of 595 publication records on endoscope reprocessing from 1974 to 2017 were identified in the Web of Science database. The overall bibliometric data of endoscope reprocessing shows that the records were published in 168 indexed journals by 2004 authors, in 43 countries (Table 2).

Table 2. Bibliometric analysis of endoscope reprocessing records from Web of Science publications (1974 - 2017).

Bibliometric Data	Total
Publications (records)	595
Indexed journals	168
Authors	2004
Countries	43

Source: Elaborated from Web of Science data.

The description of top ten high impact publications at the area is presented at Table 3 with their respective score. From the most cited endoscope reprocessing publication records in LCS dataset (n=10), 4 are literature review, 2 are guidelines, 4 are articles (experimental/observational studies).

Table 3. Distribution of top ten publications records on endoscope reprocessing by Local Citation Score (LCS).

Local Citation Score *		
Ref	**Publication**	**Impact**
1	SPACH DH, 1993, ANN INTERN MED, V118, P117	142
2	KACZMAREK RG, 1992, ANN INTERN MED, V92, P257	74
3	ALVARADO CJ, 2000, AM J INFECT CONTROL, V28, 138	71
4	BEILENHOFF U, 2007, ENDOSCOPY, V39, P175	46
5	KOVALEVA J, 2013, CLIN MICROBIOL REV, V26, P231	45
6	GORSE GJ, 1991, INFECT CONT HOSP EP, V12, P289	44
7	RUTALA WA, 1991, INFECT CONT HOSP EP, V12, P282	43
8	RUTALA WA, 1995, INFECT CONT HOSP EP, V16, P231	42
9	PAJKOS A, 2004, J HOSP INFECT, V58, P224	41
10	RUTALA WA, 1999, INFECT CONT HOSP EP, V20, P69	40

*LCS refers to citation score among the records included in the research.
Source: Elaborated from Web of Science data.

The description of journals citation was presented by the respective local citation score. Therefore, the values presented in Table 4 represents top ten journals of publications at the endoscope reprocessing area with higher citation local score and their corresponding publications. The journals Journal of Hospital Infection and Infection Control and Hospital Epidemiology presented higher local scores with 15.5% and 14.1% of citations, respectively, from a total of 168 journals. This information can assist researchers and healthcare professionals to easily find the publications at the area with higher impact.

Table 4. Description of top ten journals with higher impact publications on endoscope reprocessing. n=168.

Journal	Impact*	Percentage (%)	Publication records
Journal of Hospital Infection	528	15.5	72
Infection Control and Hospital Epidemiology	480	14.1	43
American Journal of Infection Control	445	13.0	54
Gastrointestinal Endoscopy	431	12.6	73
Endoscopy	275	8.1	31
Annals of Internal Medicine	142	4.2	1
American Journal of Medicine	137	4.0	3
American Journal of Gastroenterology	88	2.6	10
Gastroenterology Nursing	57	1.7	22
Journal of Gastroenterology and Hepatology	57	1,7	12

*Citation score within 595 endoscope reprocessing publication records exported from Web of Science search.
Source: Elaborated from Web of Science data.

The ten authors of publications with the highest citation scores among the 595 records (LCS) are described in Table 5, by citation score, percentage, quantity of publication and country.

Table 5. The top ten authors with most citations received in publications related to endoscope reprocessing. n=2004.

Author	Impact*	Percentage (%)	Publication Records	Country
Rutala WA	333	9.7	24	United States of America
Weber DJ	261	7.6	21	United States of America
Alvarado CJ	150	4.4	7	United States of America
Silverstein FE	142	4.2	1	United States of America
Spach DH	142	4.2	1	United States of America
Stamm WE	142	4.2	1	United States of America
Muscarella LF	141	4.1	14	United States of America
Nelson DB	138	4.0	9	United States of America
Alfa MJ	132	9.3	13	Canada
Vickery K	124	3.6	5	Australia

*Citation score within 595 endoscope reprocessing publication records exported from Web of Science search.
Source: Elaborated from Web of Science data.

The number of publication records included in the analysis over the time period from 1974 to 2017 is illustrated in Figure 5. The first publication dates 1974, however from 1991 the publication rate increases considerably with 17 records (2,9%) and reaches its highest peak in 2016 with 52 (8.7%) publications. From all 44 years of publications in the endoscope reprocessing area, the thirty three publications in 1999 had made this the year with higher citation score (286) (not shown data).

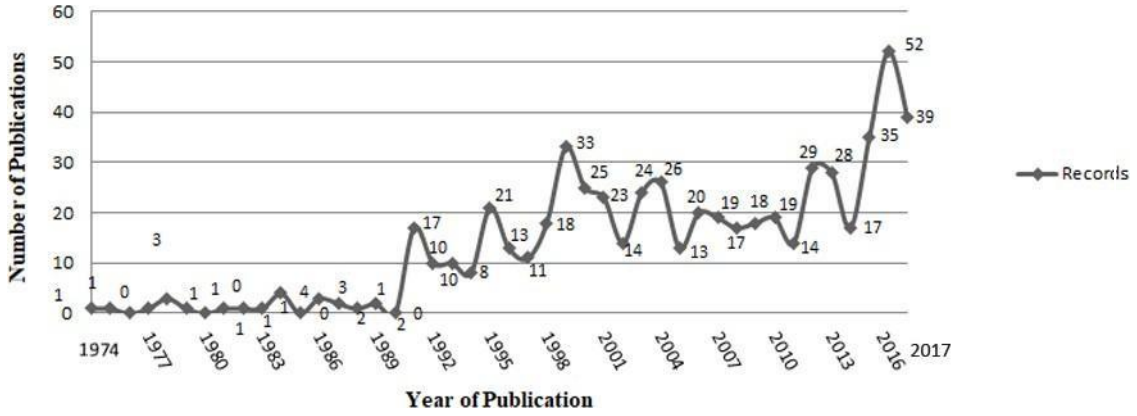

Figure 5. Number of endoscope reprocessing records by year of publication. n=595. (1974-2017).
Source: Elaborated from Web of Science data

Citation linkage of top ten publication records are described by histogram in Figure 6, with each circle representing a publication, the size of the circle indicating the impact of the publication, the arrow demonstrating the citation direction and the number representing the publication identification. Data involves publication from the year of 1991 to 2013.

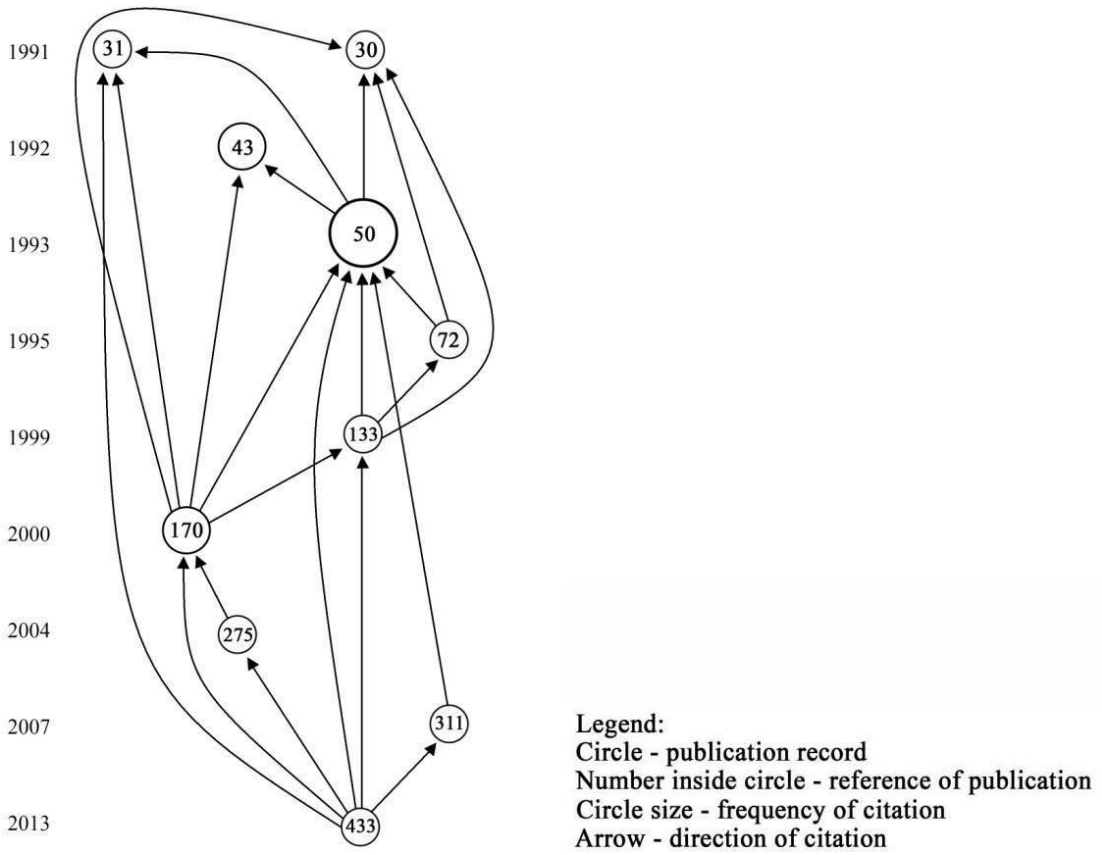

Figure 6. Histogram of citation interactions of top ten endoscope reprocessing publication records.
30 - Rutala WA, et al., (1991); 43 - Kaczmarek RG, et al., (1992); 50 - Spach DH, et al., (1993); 72 - Rutala WA, Weber DJ, (1995); 133 - Rutala WA, Weber DJ, (1995); 170 - Alvarado CJ, Reichelderfer M, (2000); 275 - Pajkos A, Vickery K, Cossart Y, (2004); 311 - Beilenhoff U, et al., (2007); 433 - Kovaleva J, et l., (2013); 31 - Gorse GJ, Messner RL, (1991). *List with full reference details in Appendix A. Source: Elaborated from Web of Science data.

For descriptive analysis it was added the higher impact publications from 2016 and 2017. A brief description of the selected high impact publications included at the descriptive analysis is detailed in Table 6. The analysis of the main results addressed by the authors were gathered on the following issues: association of failures of endoscope reprocessing with endoscope contamination and/or patient infection; importance of the cleaning procedure for achieving adequate reprocessing; the use of glutaraldehyde as a disinfectant agent in most endoscopy units; the importance of staff compliance to endoscope reprocessing recommendations and contamination of endoscope by biofilm presence.

Table 6. Descriptive analysis of selected high impact publications.

Reference	Impact	Processing failure		Importance of cleaning	Use of glutaraldehyde	Staff compliance	Biofilm
		Endoscope contamination	Patient infection				
SPACH DH, 1993	142	+	+	+		+	
KACZMAREK RG, 1992	74	+		+		+	
ALVARADO CJ, 2000	71	+		+	+	+	+
BEILENHOFF U, 2007	46	+	+	+	+	+	+
KOVALEVA J, 2013	45	+	+	+	+		+
GORSE GJ, 1991	44		+	+	+		
RUTALA WA, 1991	43	+		+	+	+	
RUTALA WA, 1995	42	+		+			
PAJKOS A, 2004	41	+		+			+
RUTALA WA, 1999	40	+		+			
NEVES MS, 2016	7			+			+
NARYZHNY I, 2016	7	+	+				
RUTALA WA, 2016	5	+	+			+	
SALIOU P, 2016	5	+					
HERVE RC, 2016	4	+					+
CHAPMAN, 2017	2	+					
OFSTEAD, 2017	2	+		+			

Discussion

The advantages of using flexible endoscopy procedures are well established, and include the management of gastrointestinal pathologies whilst avoiding more heroic surgical procedures[52].

Through mapping of endoscope reprocessing scientific knowledge, in high impact publications demonstrates that the investigation of infection transmission by flexible endoscopy has been related to failure of endoscope reprocessing for decades. In fact, the literature supports the finding that failure of endoscope reprocessing is common in healthcare services[67] and this failure is often associated with patient infection and outbreaks[24,56].

The problems related to endoscope reprocessing failure can be grouped into three main areas: the importance of cleaning to ensure adequate endoscope reprocessing; the importance of reprocessing recommendations compliance; and the presence of contamination remaining on endoscopes even when they are reprocessed using recommended procedures. The last two could appear to be contradictory, however, a high impact publication[18] in the area demonstrated that the presence of biofilm on endoscope channels is a possible explanation for endoscope contamination even when endoscopes are processed according to recommendations. Additionally, high impact publications including guidelines[67] and review articles[29] suggest that the complex design of endoscope influences reprocessing efficacy and may help explain residual endoscope contamination despite following guidelines.

These three main points are crucial and appear to interact. According to the records on this bibliometric search on Web of Science database endoscope cleaning procedures have been addressed since the 1990´s and have been a major concern of the scientific community[29]. It is now well established that the quality of the cleaning

procedure is crucial for adequate endoscope reprocessing[59,67,89] so much so that both the FDA and CDC have introduced new guidelines for cleaning and evaluating cleaning efficacy[56].

Difficulties in performing endoscope reprocessing can be explained by the design of the endoscope. It has long and narrow internal channels which can't be visualized to determine if all soil has been removed, the outer casing is black which increases the difficulty in visualizing soil, and many endoscope models have areas that are particularly difficult to access and clean e.g. the elevator wire of duodenoscopes. All these features mean that endoscope decontamination requires a careful and multistep process to achieve reprocessing[57]. In fact, failure to reprocess all channels have been reported as a fundamental error on endoscope reprocessing in both the early years[29,35] and later years[70,113]. Therefore, adherence to recommended guidelines can be challenging, and practices inconsistent with guidelines in healthcare facilities has been reported throughout the years[82,83].

The importance of following guideline recommendations is undeniable. However, the presence of endoscope contamination[13,15] and patient infection related to endoscopy procedures[12,112] have been reported in literature, despite compliance with guideline recommendations and the absence of reprocessing failure. The detection of biofilm in processed endoscope channels[18] brought a new perspective to endoscope reprocessing, given that biofilms are both tolerant to killing by biocides and removal by detergents[44,68,114,111]. This reality has been well cited in current endoscope reprocessing guidelines[20,54,71,86,106,] , however, recommendations to address these specific issues have not been forthcoming.

Descriptive analysis of publications from 2016-2017, showed that the main issues addressed during this time are investigation of outbreaks of infections related to contaminated endoscopes; the contamination of endoscope by multidrug-resistant

microorganisms (MRO) and methods of monitoring endoscope reprocessing. This means that even though endoscope reprocessing has been studied for at least 44 years, the problems addressed in the early days still exist today.

In the first years of endoscope reprocessing publications the microorganisms reported to be isolated from patient-ready endoscopes were *Pseudomonas*, *Salmonella*, *Mycobacterium* species and Enterobacteriaceae[29,35]. Currently, the most frequently cited microorganisms found associated with outbreaks and contaminated processed flexible endoscopes are multidrug-resistant, particularly Carbapenem-resistant Enterobacteriaceae[104,57]. In addition to professional society and statutory body recommendations[56,61,71,] the importance of monitoring endoscope reprocessing has been frequently addressed in recent publication[115,116]. It is important to note that many recent publications on endoscope reprocessing failure, particularly those from North America, relate to contamination and patient infection associated with duodenoscopes[57,104,117]. In fact, the elevator-wire area of flexible duodenoscopes is a major concern in recent publications[63,86]. However, contamination of all types of endoscopes, including gastroscopes and bronchoscopes, with antibiotic resistant organisms including carbapenemase-producing Enterobacteriaceae have been cited in the literature[101, 118,112]. Contamination of all types of endoscopes, confirms earlier publications that refers to endoscope complex design as one of the key factors for failure of endoscope reprocessing.

In summary, despite extensive investigation and technologic progress, the problem of endoscope reprocessing has endured over the decades. The low number of publications over the first seventeen years is likely due to lack of identification of transmission events and hence little investigation into endoscope processing. The exponential growth of publications over the recent years is likely attributable to transmission events associated with antibiotic resistant organisms and the realization

and consequence of biofilm contamination of endoscopes. The goal of achieving microbiological safe endoscope reprocessing is unlikely to be realized until endoscope design is modified to ensure that instruments can be cleaned and biofilm formation can be prevented.

Appendix A

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CHAPTER 3. MATERIAL AND METHODS

Part I – STUDY OUTLINE

3.1 Design

Observational cross-sectional study structured in two main parts: clinical and laboratorial (Figure 7). The clinical part refers to analysis of clinical data performed for assessing soil level, bacterial contamination level and/or biofilm formation in four different settings: before and after manual cleaning, after endoscope reprocessing (12 to 48 hours after disinfection), following biopsy internal channels extraction for repair (clinically used endoscope from Australia and Brazil) and environmental sampling of endoscope unit surfaces. The laboratorial part refers to analysis of endoscope internal channel surface for damage and its relation to frequency of use.

3.2 General sample collection and microbiological analysis

The study involved samples collected from endoscopy services in Australia and Brazil. Phases I, II and IV samples were collected by the responsible researcher from an Australian endoscopy unit. Phase III samples were received from endoscopy service repair companies from Brazil and Australia for analysis.

All microbiological processing and analysis of the samples were carried out at the PC2 safety laboratory located within the Biomedical Department of the Faculty of Medicine and Medical Sciences, Macquarie University; Microscopy Unit, Faculty of Science and Engineering, Macquarie University; Clean Room, Department of Engineering, Macquarie University; Biomedical Imaging Facility, University of New South Wales.

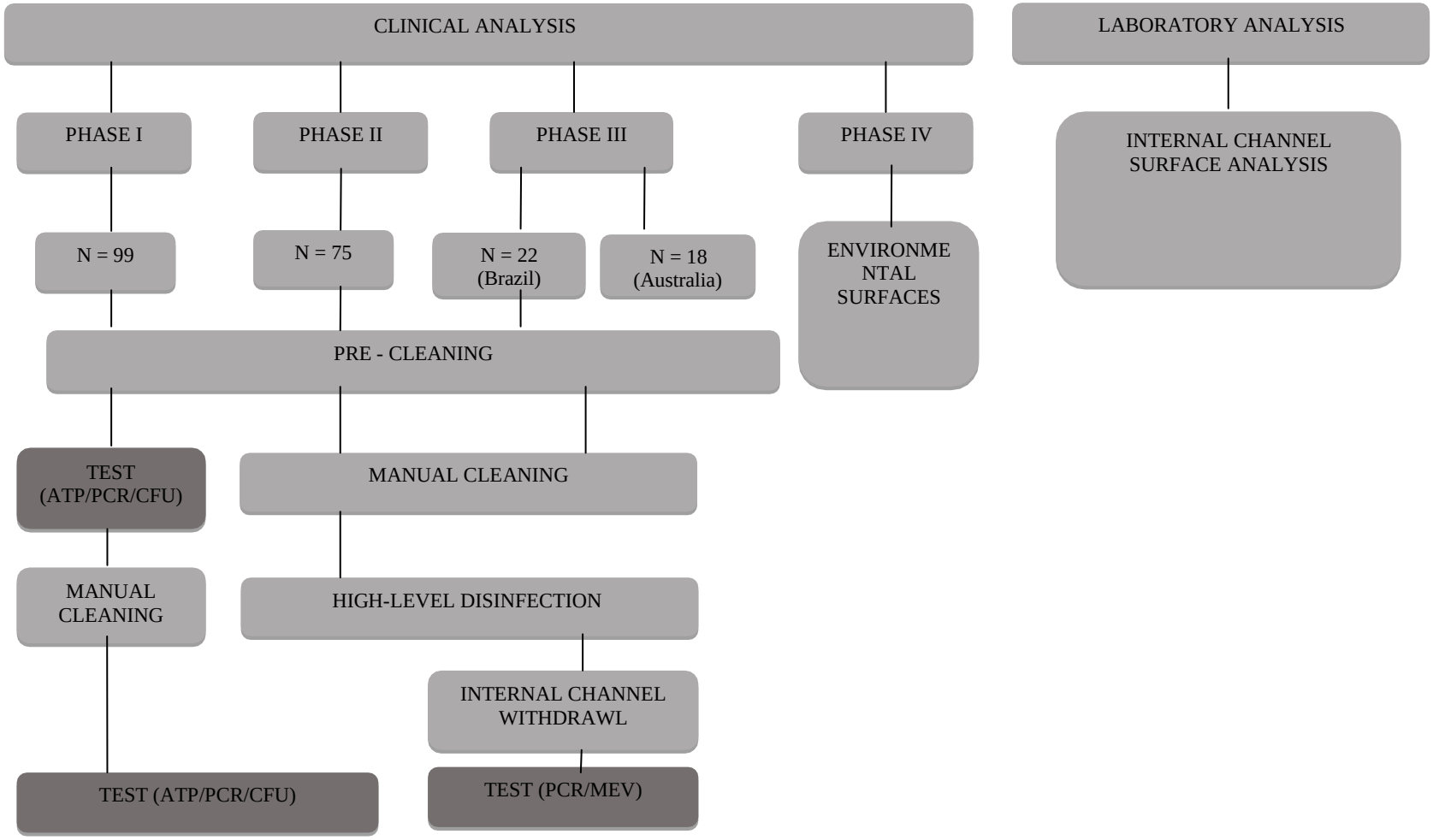

Figure 7. Schematic summary of data collection and processing steps in the clinic and laboratory parts of the research.

3.3 Statistical analysis of data

The results of processed samples were coded and double typed in Excel spreadsheet and transferred to IBM SPSS Statistics version 23.0 software for descriptive and statistical analysis of the data. Mann-Whitney test was used in the comparisons of medians in independent samples and Wilcoxon test for related samples. In addition, a Spearman test was used for correlation of different variables, and chi-square test with a significance level of 5% ($P < 0.05$).

3.4 Ethical aspects

The research involved experiments with endoscopes and surfaces, therefore no data or procedure involving humans was performed.

3.5 Financial support

PhD candidate Lissandra Chaves de Sousa Santos received the scholarships: Overseas Sandwich Doctorate Program (PSDE) (process number - 99999.006642/2015-02) provided by Coordination for the Improvement of Higher Educational Personnel (CAPES) and Macquarie University Research Excellence Scholarship (MQRES) Scheme provided by Macquarie University.

Part II – GENERAL MATERIALS AND TECHNIQUES

3.6 Colony Forming Unit

3.6.1 Liquid Samples

3.6.1.1 Directly on agar plate

For liquid samples, endoscope channel flushes were cultured by pipetting 100 µL of either neat sample or 100 µL of 10-fold serially diluted sample (figure 8) was onto horse agar plates (HBA) (Micromedia Laboratories, Victoria, Australia), applying the spread-plate technique and incubating at 37ºC for 24 hours. After the incubation period, the plates containing from 30 to 300 colonies were read and the number of colony forming units per milliliter (CFU/mL) was calculated.

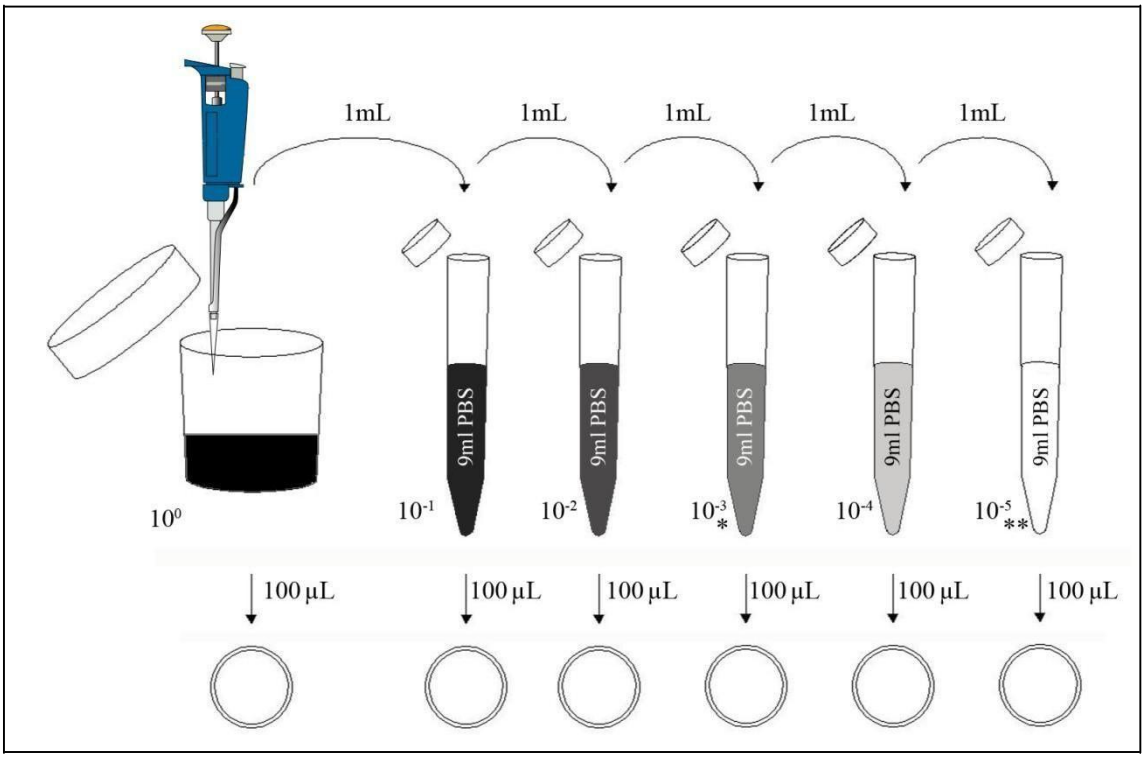

Figure 8. Representation of ten-fold dilution series for determining colony forming unit.

3.6.1.2 Concentration of samples through filter membranes

Microbes present in liquid samples obtained by flushing endoscope channels after reprocessing, were concentrated by filtering the sample through 0.22µm sterile filter membranes MCE membrane filter (Membrane Solutions LLC, WA, USA) (Figure 9). The membrane was then removed from the cartridge and placed onto HBA and incubated for 24 hours at 37^{o}C prior to colony forming counts.

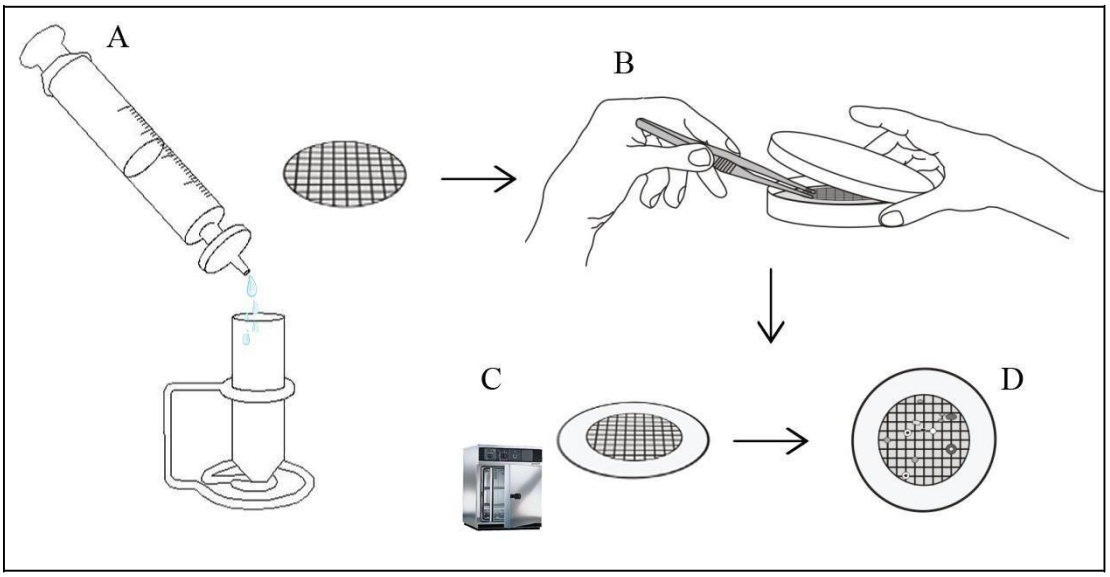

Figure 9. Representation of microbial culture processing by filter membrane. (A) Liquid is passed through filter membrane; (B) Membrane is placed on agar plate with sterile forceps; (C) Incubation of agar plate at 37 ºC for 24 hours; (D) Reading of results by counting colony forming units.

3.6.1.3 Surfaces Samples

Surfaces were sampled by moistening sterile gauze in PBS and rubbing vigorously over the surface. The sterile gauze were then placed in 5 mL of PBS and sonicated at 43 mHz for 20 minutes. A 100 µL aliquot was then spread onto HBA agar plates and incubated in 37°C for 24 hours prior to CFUs counting.

3.7 Bacterial Identification

Culture positive results were plated to obtain single morphology colonies. Isolated single colonies were subcultured onto Chromogenic UTI agar plates (Oxoid) using the streaking technique and incubated at 37ºC for a period of 18 to 24 hours prior to reading, followed by bacterial identification and subculture onto selective culture media including identification of multidrug resistant organisms (MDRO) (Figure 10). The selective media for MDRO identification included BrillanceTM MRSA agar plates (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with 99.7% specificity and 95.4% sensitivity for Methicillin-resistant *Staphylococcus aureus* (MRSA) detection; Brilliance ESBL agar (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with 95% sensitivity and 94% selectivity for detection of extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria; and BrillianceTM VRE agar (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with a sensitivity of 94.7% at 24 hrs, and 100% sensitivity with 100% specificity at 48 hrs for Vancomycin-resistant *Enterococcus* (VRE) detection. For Brilliance ESBL agar plates readings the following sequence was used: coloured colonies were confirmed to be ESBL-positive, *E. coli* colonies were pink or blue in colour; while *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* colonies were coloured green.

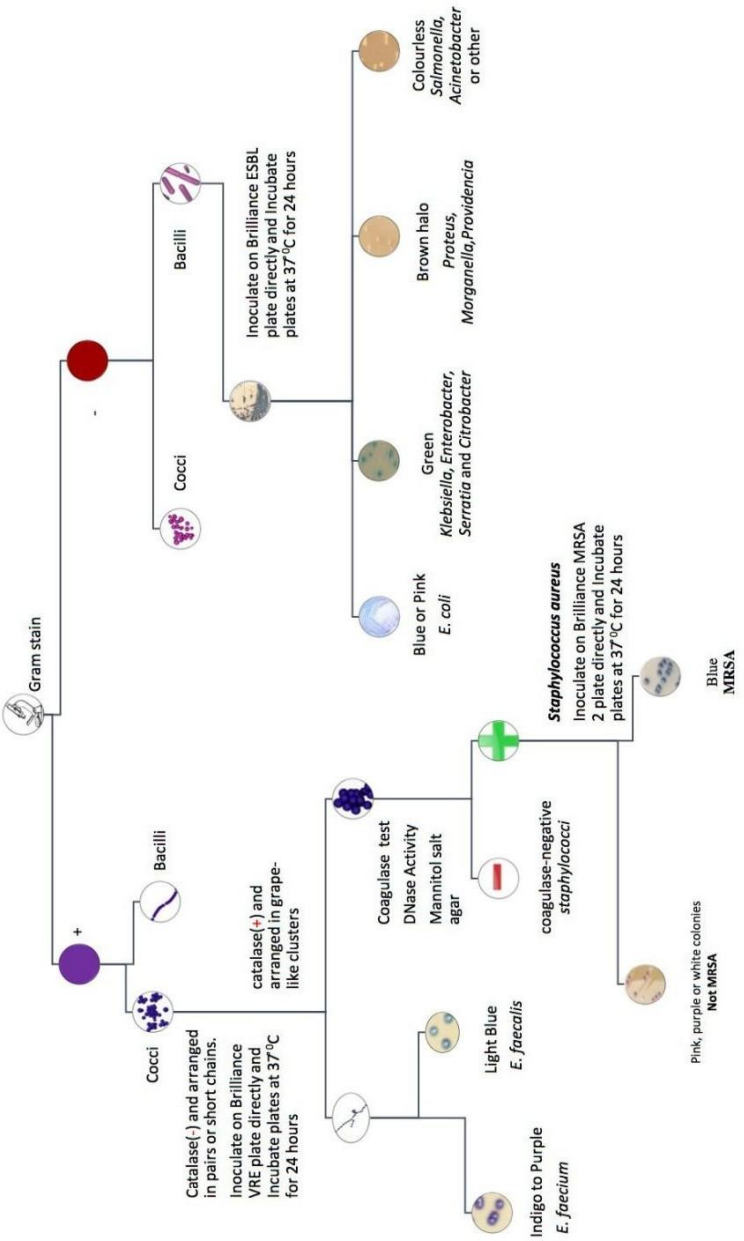

Figure 10. Representation of bacterial identification of culture positive results. Figure drawn by Khalid Johani, 2017.

3.8 Adenosine Triphosphate (ATP) Testing

3.8.1 Aquasnap Total (Hygiena, USA)

Aquasnap Total is a device used to verify the presence of ATP, of organic or microbial origin, in flush water samples obtained from endoscope channels. For sample collection, the device was initially held for 20 minutes at room temperature, then after the device was opened, it was immersed in the sample for 5 seconds and re-coupled into the original tube (withdrawn from 100 μL sample). The upper end of the device was then flexed to opposite sides breaking the seal and allowing the reagents to mix, the device was vertically held while being agitated in a circular motion.

3.8.2 Ultrasnap (Hygiena, USA)

Ultrasnap is a swab device used for testing ATP on surfaces. After collection of the sample on the external part of the endoscopes, the upper end of the device was then flexed to opposite sides to break the seal, the device being stirred in a circular motion, holding the device vertically throughout the process.

3.8.3 Endoswab (Hygiena, USA)

Endoswab is a brush device used for sample collection for ATP quantification of endoscopes internal biopsy channel. To collect the sample, the device was inserted through the channel until it appeared at the distal end of the endoscope and 2 cm was cut using sterile scissors into a collection tube.

3.8.4 System Sure Plus (Hygiena, USA)

All ATP quantification devices, Ultrasnap, Aquasnap and Endoswab, were read in the System Sure Plus, Hygiena bioluminescence monitoring system with results presented at Relative Light Unit (RLU).

3.9 DNA extraction

For extracting DNA from samples the High Pure PCR Template Preparation Kit (Roche, USA) was used as described in following protocol:

- centrifuge sample at 482 *g* for 30 minutes;
- remove supernatant with sterile pipette until final volume of 200 μL;
- transfer volume to 2 mL DNA free Ependorf;
- add 5 μL of 10 mg/mL lysozyme in 10 mM Tris HCl with pH 8.0;
- incubate at 37°C for 15 minutes;
- add 200 μL of Binding Buffer (High Pure PCR Template Preparation Kit - Roche, USA) and 40 μL of reconstituted proteinase K;
- homogenize and incubate at 70ºC for 10 minutes;
- add 100 μL of isopropanol, shake and transfer content to filter coupled to the collection tube (High Pure PCR Template Preparation Kit - Roche, USA);
- centrifuge at 8000 *g* for 1 minute;
- discard flowthrough and collection tube;
- add 500 μL of inhibitor removal buffer (High Pure PCR Template Preparation Kit - Roche, USA);
- centrifuge at 8000 *g* for 1 minute;
- discard flowthrough and collection tube;

- add 500 μL of wash buffer (High Pure PCR Template Preparation Kit - Roche, USA);

- centrifuge at 8000 *g* for 1 minute;

- discard flowthrough and collection tube; (repeat final three steps discharging only flowthrough);

- centrifuge at 13000 *g* for 10 seconds;

- discard collection tube;

- add new collection tube and 200 μL of elution buffer (High Pure PCR Template Preparation Kit - Roche, USA);

- centrifuge at 8000 *g* for 1 minute;

- store DNA at -20ºC freezer.

3.10 Polymerase Chain Reaction (PCR)

Total quantification of bacterial DNA was performed with real-time qPCR analysis for amplification of the 16S rRNA genes of the microorganisms present in the sample using the 16S rRNA_341F universal primers 5'-CCTACGGGAGGCAGCAG-3 'and 16S rRNA_534R 5'-ATTACCGCGGCTGCTGG-3'.

- Quantitative PCR Protocol

- prepare qPCR reaction mix by adding 25 μL of a mixture of 1X Brilliant II Sybr Green qPCR Master mix (Stratagene), 400 nM of forward primer, 400 nM of reverse primer and 2 μL of previously extracted DNA;

- set the reaction for polymerase activation at 95°C for 10 min, 40 cycles of denaturation at 95 ° C for 15 s, annealing at 56°C for 30 s and extension at 72°C for 30 s;

- set a positive control samples for determination of standard reaction curve using pre-established DNA concentrations, determined with serial dilutions of 10^6 to 10^3 copies/μL.

Part III - EXPERIMENTAL SPECIFICATIONS

3.11 Phase One - Microbiological and physicochemical indicators of endoscope reprocessing: manual cleaning procedure.

3.11.1 Data collection

Samples of clinically used flexible gastrointestinal endoscopes were collected before and after manual cleaning procedure, as previously mentioned in Figure 1. Immediately upon arrival of the endoscope in the dirty area, after clinical use and pre-cleaning, the endoscope was sampled in the following sequence:

1) the final 30 cm of the external distal part of the endoscope was sampled and tested for ATP using the Ultrasnap device, with up and down movements and rotations to increase the area of contact.
2) Then, 30 mL of sterile distilled water was flushed into endoscope internal channels (air/water and suction/biopsy channels) (10 mL in each channel). The contents of the flush were collected in a sterile 50 mL falcon tube.

3) Finally, the biopsy channel was brushed with the Endoswab device, cut with sterile scissors and approximately 2 cm collected into the collection tube.
4) The endoscope was then delivered to the professionals responsible for the endoscopy unit service for standard manual cleaning procedure.

3.11.2 Sample processing

Internal Endoscope Channels:

1) For ATP quantification 20 µL of the collected flush was tested by Aquasnap (Hygiena, USA) device.
2) For bacterial CFU counting, 100 µL was cultured as described in section 2.5.1.1. In case of positive results, cultures were checked for purity by colony morphology and bacterial isolation was carried out by spreading one colony on ICU plates by the exhaustion technique and incubating at 37 ºC for 18 to 24 hours.
3) For bacterial load quantification, DNA was extracted (section 3.9) from remaining sample, approximately 30 mL was used, and the number of bacteria determined using real-time qPCR amplification of 16s rRNA genes as described in section 3.10.

3.12 Phase Two - Evaluation of endoscope reprocessing: high level disinfection.

3.12.1 Sample Collection

At this stage, samples were collected using a full protection barrier and sterile equipment (field, apron, glove, syringe, distilled water, scissors, flush connection for endoscopes, brush for endoscope biopsy channel and 50 mL collection tube). All endoscopes to be evaluated were removed from the airflow and storage cabinet after checking the date and time of high-level disinfection (between 12 to 48 hours). It was chosen to collect samples 12 hours after reprocessing, for possible recovery of contamination from biofilm and before 72 hours, the maximum recommended period for storage of endoscopes before reprocessing.

Initially, the distal 30 cm external part of the endoscopes was sampled and tested using Ultrasnap (Hygiena, USA) device, with up and down movements and rotations to increase the contact area evaluated. Then, the endoscope biopsy and suction channels were brushed using an Single-use combination cleaning brush BW-412T by Olympus and the distal 2 cm of the brush removed and collected into the collection tube. Finally, each channel of the endoscope was flushed with 10 mL of distilled water and the flush collected.

3.12.2 Sample processing

- Aliquots of 20 μL of each sample were used to quantify of ATP in internal channels of the endoscopes using Aquasnap (Hygiena, USA) device;
- Aliquots of 20 mL of each sample were used to perform the microbial culture divided into two 10 mL aliquots for each culture medium (HBA - Micromedia Laboratories, Victoria, Australia and Tryptic Soy Contact Agar + LTHTh - ICR - EMD Millipore, Billerica, USA); The 10 mL aliquots of samples were concentrated using a permeable membrane filter as described in section 3.6.1.2, the membrane removed from the filter with sterile disposable forceps and placed

onto the culture plates. The culture plates were then incubated at 37°C for 48 hours prior to reading. In the case of a positive culture result, bacterial isolation was performed by selective culture media (described in section 3.7).

- Aliquots of 10 mL of each sample were used for PCR quantification (as described in section 3.9 and 3.10, respectively for DNA extraction and qPCR).
- Identification of isolated bacteria from patient-ready endoscopes after reprocessing was performed by genetic sequencing of the 16s rRNA gene. Initially, 200 μL of phosphate buffer solution and 10 μL of lysozyme (final concentration of 0.5 mg/mL) were added to the colonies isolated from the bacteria with subsequent incubation at 37ºC for 15 minutes. DNA extraction using the High Pure PCR Template Preparation Kit (Roche) was then performed as described in section 2.9.

Amplification of the 16S rRNA gene was performed using the 16S rRNA_341F universal primers, described in section 2.8. After PCR, the product (10 μL) was then purified with 2 μL of ExoSAP-IT treatment enzyme; centrifuged rapidly and incubated at 37°C for 30 minutes (later inactivation of the enzyme with incubation at 80°C for 15 min); 1 μL of 341F Primer was added to 10 uM and 1 μL of 10 uM Primer 951R and stored at -20°C until all samples were sent to the Australian Genome Research Facility for DNA sequencing analysis. Sequencing was performed using the BigDye Terminator v3.1 Kit (Applied Biosystems) for sequencing and AB3130xl genetic analyzer (Applied Biosystems) for capillary separation. The result was compared to the genetic sequencing available in the GenBank, EMBL and DDBJ databases using the results ≥ 98% similarity.

3.13 Phase III - Microbiological comparison of endoscope channels contamination: Brazil versus Australia.

3.13.1 Sample collection

Internal channels of endoscopes were received from endoscopy repair services located in Brazil and Australia, inside plastic bags. No information regarding equipment information, endoscopy unit and/or patients was provided.

3.13.2 Sample processing

Initially, all endoscope channels had their external surfaces decontaminated by wiping two times sequentially with Matrix (Whiteley Corporation, North Sydney, Australia) marketed as a biofilm remover, 70% ethanol and sterile water.

A. Bacterial Load quantification

For assessing bacterial load, the total amount of DNA present on samples was extracted and quantified. The DNA extraction was performed only on the internal part of the channels, therefore, after aseptic cutting samples into 30 cm sections, a 20 cm piece was marked for DNA extraction (described in Figure 11).

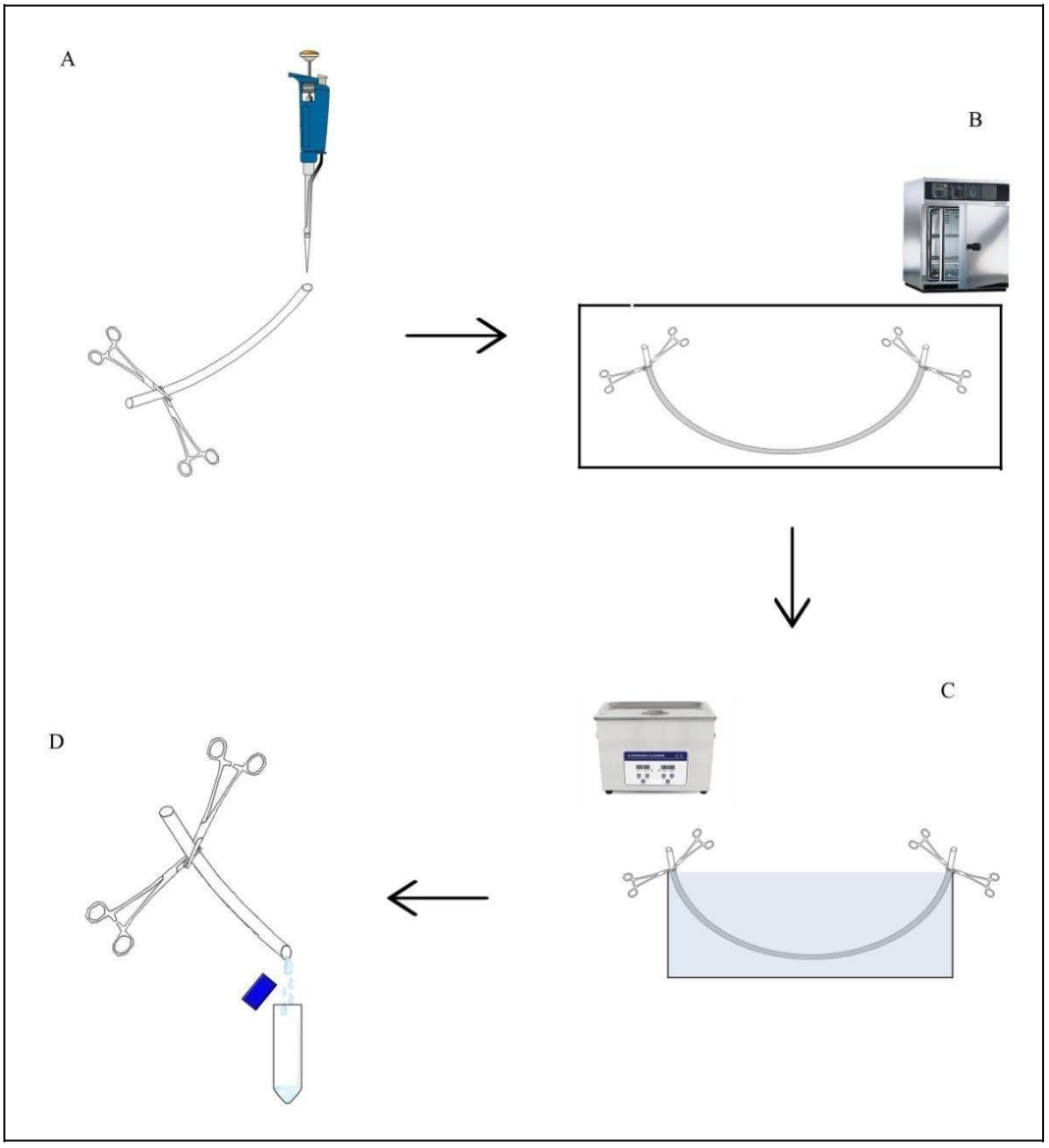

Figure 11. Representation of DNA extraction from endoscope channel internal surface by: (A) clamping one end of the channel for insertion of digestion mix into a 20 cm section; (B) closing both ends of endoscope channel for incubation at 56 ºC overnight; (C) sonication in ultrasonic bath at 43 mHz for 20 minutes; (D) collection of the content into a sterile tube.

Detailed DNA extraction and quantitative PCR used protocols:

- DNA extraction

  - prepare 50 mL of digestion mix (50 mMTris/HCl pH7.5, 150 mM NaCl, 2 mM EDTA, 1% SDS) and add 2.5 mL of 20 mg/mL proteinase kinase;

  - place 2 mL of mixture in each channel, close ends with artery forceps.

  Incubate overnight at 56°C;

- sonicate at 43 mHz in ultrasonic bath (Soniclean, JMR Australia) for 20 minutes;

- drain liquid in 15 mL falcon tube;

- inactivate proteinase kinase by incubating sample at 95°C for 10 minutes;

- add 200 µL of 10 mg/mL lysozyme (Sigma-Aldrich, Castle Hill, Australia) and incubate at 56°C for 2 hours;

- inactivate lysozyme by incubating sample at 95°C for 10 minutes;

- add 640 µL 5 M NaCl to 2000 µL solution to give a final concentration of 1.2 M NaCl;

- mix vigorously and leave mixture in ice for 10 minutes;

- centrifuge at 2500 rpm at 4°C for 15 minutes;

- transfer supernatant to a new tube and centrifuge again at 2500 rpm at 4°C for 15 minutes;

- transfer supernatant to a new tube and measure the volume left;

- precipitate DNA by adding double amount of sample volume of absolute ethanol. Mix well gently. Invert tube up and down 10 times;

- leave the sample in the -20 ºC freezer overnight;

- centrifuge 2500 rpm at 4°C for 15 minutes;

- remove the excess of liquid from tube by aspirating with pipette;

- add 5 mL of 70% ethanol, centrifuge at 2500 rpm for 15 minutes at 4°C and aspirate the excess of liquid;

- place falcon tube in a rack and leave it open in biosafety cabinet until dry (maximum of 30 min);

- add 50 µL buffer (10 mM Tris/HCL pH 8.0, 1 mM EDTA).

- Quantitative PCR

  Was conducted using the same method as described in section 2.5.2.2.

B. Microscopy

The internal surface of each endoscope channel was examined for damage and the presence of patient soil and biofilm by scanning electron microscopy. The endoscope channel was cut transversely to obtain a segment approximately 1cm in length, this segment was then cut longitudinally to expose the inner surface of the channel. The segments were fixed in 1 mL of 2.5% glutaraldehyde for 24 hours at room temperature; dehydrated through increasing concentrations of ethanol and 100% hexamethyldisilazane (HMDS, Polysciences Inc, Warrington, PA, USA) and sputter coated with 20 nm of gold film for microscopic examination. Biofilm presence was defined by visualization of microorganism surrounded by extracellular polymeric substance.

3.14 Phase IV - Assessing cleaning and bacterial load on endoscopy unit surfaces

3.14.1 Sample collection

The Endoscopy Unit's procedure room and nurse station were sampled as shown by red crosses in Figure 12.

Figure 12. Marked areas (red crosses) shows location of sampling of endoscopy unit surfaces in the nurse's station (A) and the procedure room (B).

Microbial sample collection was directed by first determining ATP levels (Ultrasnap, Hygiena, USA) in a 2 cm x 5 cm area. The areas with ATP values higher than the benchmark of 100 RLU were then sampled for culture test. Samples for microbial isolation were collected using sterile gloves and a sterile gauze moistened in PBS. The area (2 cm x 5 cm) was rubbed using rolling movements and placed into a sterile container. The area sampled for microbial isolation was adjacent to the area sampled for ATP determination. Samples were placed in an Esky and transported to Biomedical Department of the Faculty of Medicine and Medical Sciences, Macquarie University, for microbial analysis. The samples were ultrasonicated at 43 mHz for 20 minutes and an aliquot of 100 µL was spread onto HBA and incubation at 37°C for 24

h prior to colony counting. The bacteria from positive cultures were isolated and identified as described in section 2.6.

3.15 Phase V - Evaluation of surface damage and biofilm formation in endoscope channels.

Based on results from Phase III experiments where the SEM showed that biofilm formation on endoscope channels was frequently related to damage on channel's surface, a preliminary investigation was made in order to observe endoscope channel's surface under transmission light microscope.

Therefore, clinically used endoscope channels were cleaned by soaking in NaOH overnight, rinsing and drying. Segments of approximately 1 cm length were cut and fixed on microscope slides for analysis. Microscope images in Figure 13 reveals the presence of damaged areas.

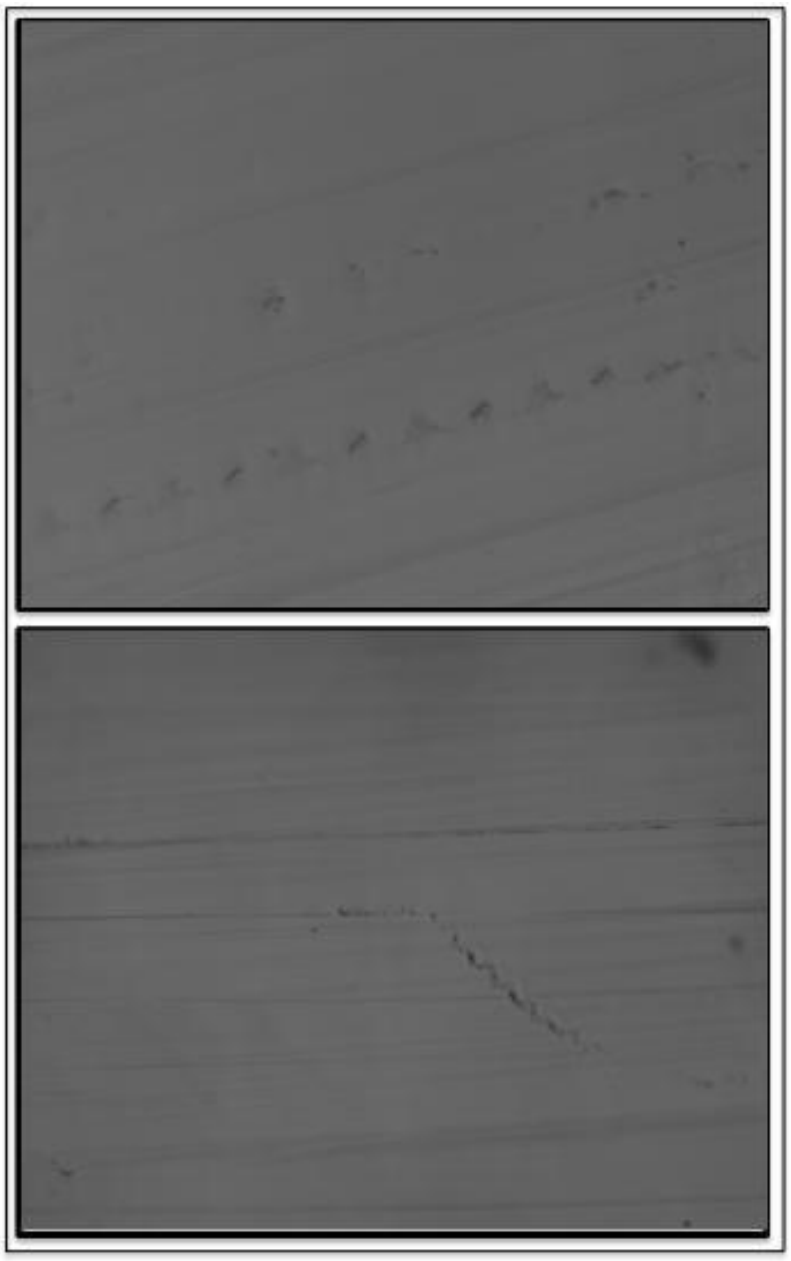

Figure 13. Micrograph image of clinically used endoscope internal channel demonstrating surface damaged area.

Therefore, in order to investigate the relationship between endoscope channels surface damage and the presence of biofilm, three experiments were conducted as detailed in chapter 8:

1) evaluation of relationship between endoscope use and surface damage;

2) determination of how quickly bacteria attach to a new endoscope channel; and
3) determination of the number of times that a biopsy forceps needs to be passed through an endoscope channel before damage occurs.

CHAPTER 4. ASSESSING MANUAL CLEANING OF FLEXIBLE ENDOSCOPE BY MEASURING SOIL AND MICROBIAL CONTAMINATION LEVELS

4.1 Introduction

In order to assess flexible endoscope cleaning we measured the amount of contaminating soil by ATP and microbial load by a combination of qPCR and microbial culture. Ninety-nine flexible endoscopes were sampled both before and after the manual cleaning process.

4.1 Microbiological and physicochemical indicators of flexible endoscope reprocessing: manual cleaning procedure.

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Candidate Contribution:

- Study design

- Laboratorial experiment

- Data analysis

- Manuscript preparation

Overall contribution: 85%

Introduction

Medical devices reprocessing consists of using a validated process to remove patient, microbial and non-organic contamination[56]. The complexity of endoscopes makes their reprocessing challenging. As the reprocessing steps are susceptible to human error and due to the large bioburden present on endoscopes, the margin of safety in processing is small[118], each reprocessing step needs to be performed in a peerless manner to guarantee the validation of the process[31,59].

Flexible endoscopes are reusable, semi-critical devices according to Spaulding's classification[59] and as such are required to be cleaned and then subjected to either high level disinfection or sterilization to remove residual microbial contamination[20,56,58].

Indeed, gastrointestinal endoscope contamination related to endoscopy procedures has been frequently published[10,12,13,26] , including reports of transmission of multidrug resistant microorganisms[25,1,23,24,27,28].

Endoscope contamination and healthcare associated infection (HAI) outbreaks associated with endoscopy have been due to a failure to adhere to reprocessing recommendations and/or equipment damage[20,24,56,57,83,103,110]. However, infection related to use of contaminated endoscopes has been reported even when endoscopes are reprocessed according to guidelines[13,123].

Flexible endoscope reprocessing involves bedside cleaning, cleaning in a dedicated area, disinfection/sterilization, drying and storage in an approved manner[20, 2,61,74,75]. It is well established that the cleaning step is critical for endoscope reprocessing success, as residual organic and inorganic matter within the internal channels of the endoscopes compromises biocide action[20,58,72]. Although automated endoscope reprocessors (AERs) are cited in guidelines[20,63,65,106] and their efficiency have

been demonstrated when compared to manual cleaning[73-75]; manual cleaning is still recommended after endoscopy by some authors[59,71], ensuring brushing of internal channels.

Given the difficulties involved in achieving successful endoscope reprocessing cited above, endoscope reprocessing evaluation can be helpful in detecting possible failures of the process, and thus decrease the possibility that inadequately processed endoscopes are used for patient procedures. In this study, we aimed to evaluate organic soil and microbial contamination levels of clinically used gastrointestinal endoscopes before and after manual cleaning, utilizing adenosine triphosphate (ATP) assays, quantitative polymerase chain reaction (qPCR) and microbial culture.

Material and Methods

Clinically used gastrointestinal endoscopes (colonoscopes and gastroscopes) were assessed for organic soil by ATP bioluminescence and for microbial contamination by qPCR and microbial culture, before and after manual cleaning. All endoscope reprocessing steps were performed by staff of the endoscopy unit as following described:

1) immediately after clinical use, bedside cleaning was done by wiping the external part of the endoscope with wipes soaked in enzymatic detergent and suctioning internal channels also with enzymatic detergent whilst still in the procedure room;

2) the endoscope was then transported to reprocessing dirty area for cleaning procedures where leak testing and manual cleaning were performed;

3) finally the endoscope was transported for disinfection/sterilization to the room housing the AER.

The external surface of the endoscope was sampled for ATP quantification by swabbing its distal end; and the internal surface was sampled by a combination of brushing the biopsy channel and flushing each channel (air/water, suction and biopsy) with 10 mL of sterile water, followed by flush of air to collect sample, as described in section 3.11. The flush from each channel and brush end, (removed using sterile scissors, were pooled. From the pooled sample a 20 µL aliquot was used for measurement of ATP levels, 100 µL was used for microbial culture and the remaining flush (approximately 30 mL) was used for DNA extraction and qPCR determination.

To test for differences in soil and microbial load contaminating cleaned and uncleaned endoscopes the Mann-Whitney, Wilcoxon and Spearman tests were conducted using IBM SPSS Statistics software version 23.0.

Adenosine Triphosphate (ATP) Testing

ATP was measured using the commercially available Hygiena ATP devices for endoscopes. The amount of ATP contaminating the external surface of the endoscopes was measured by swabbing the distal 30 cm external surface of endoscopes using Ultrasnap (Hygiena, Camarillo, Calif, USA); the ATP contaminating the internal channels was assessed using Aquasnap (Hygiena, Camarillo, Calif, USA and using Endoswab (Hygiena, Camarillo, Calif, USA) to brush the distal section of the endoscope biopsy channel as described in section 3.6.

All ATP assay devices were placed into the ATP bioluminescence device System Sure Plus (Hygiena, Camarillo, Calif, USA) according to manufacturer's instructions and the amount of contaminating ATP was measured as relative light units (RLU).

Microbial culture

The number of culturable bacteria was determined by spreading 100 µL of flush sample onto Horse Blood Agar and incubation at 37 ºC for 48 hours as described in section 3.6. Positive cultures were subcultured and individual bacterial species identified using selective culture media (as described in section 3.7).

DNA extraction and quantitative PCR

Sample DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, USA), as described in section 3.9.

Total bacterial load was determined by real time quantitative PCR of the 16S rRNA gene as described in section 3.10.

Results

A total of 99 clinically used endoscopes (63 colonoscopes and 36 gastroscopes) were tested both before and after manual cleaning. For determining amount of residual soil or dirtiness the amount of ATP contaminating both the external and internal part of the endoscopes were measured separately.

Gastroscopes had significantly higher ATP values than colonoscopes both internally ($p<0.001$) and externally ($p<0.001$) after manual cleaning (Figure 14).

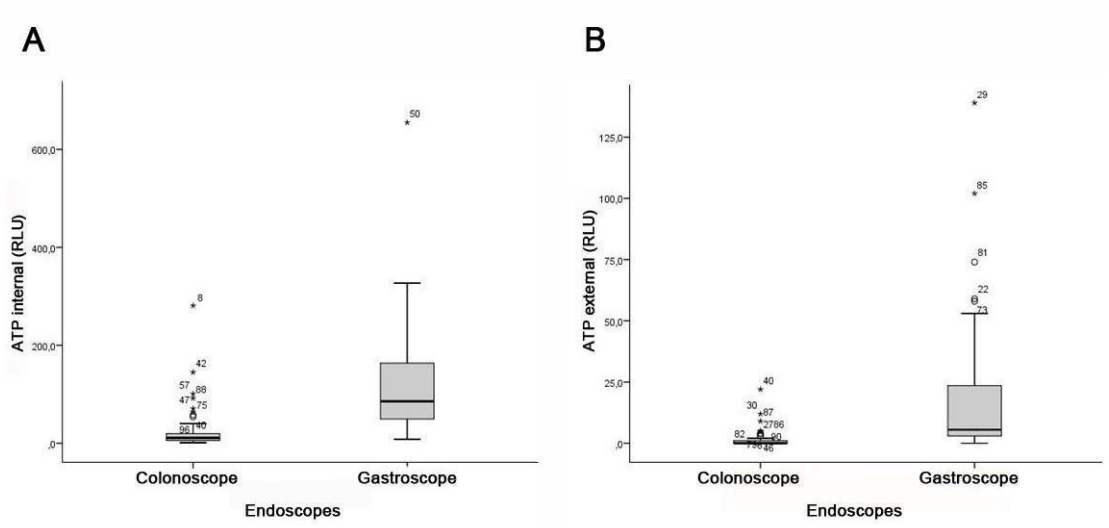

Figure 14. ATP values distribution by endoscope type after manual cleaning tested internally (A) and externally (B).

Manual cleaning of gastroscopes resulted in a significantly greater reduction of ATP contamination than that seen with colonoscopes both internally ($p<0.001$) and externally ($p<0.001$) (Figure 15).

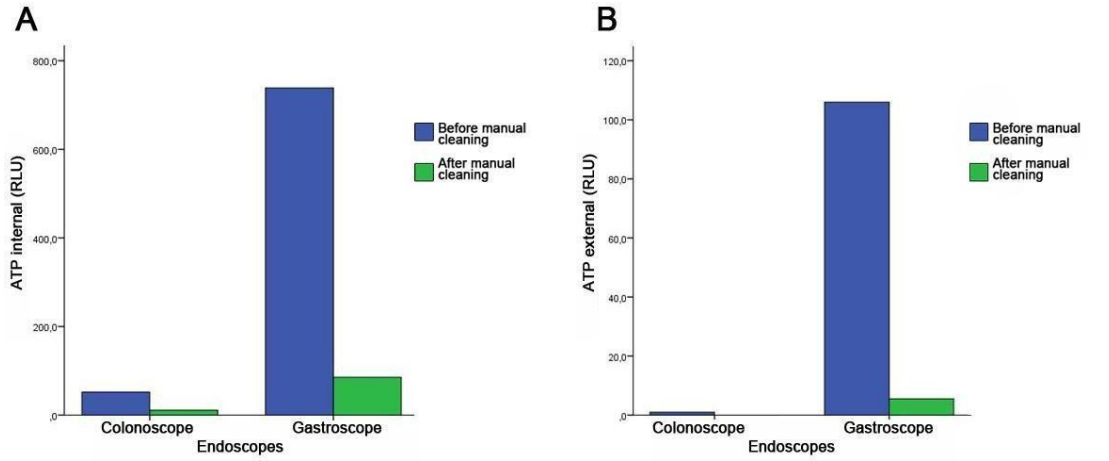

Figure 15. Comparison of ATP results of gastroscopes and colonoscopes tested before and after manual cleaning internally (A) and externally (B).

After manual cleaning, the internal part of the both endoscopes types were more contaminated with biological soil than the external part ($p<0.001$). Overall, 56.5% of endoscopes had ATP levels greater than 200 RLU before manual cleaning and 8% after cleaning.

For determining bacterial contamination levels, endoscopes flushes were tested by a combination of microbial culture and qPCR for total bacterial load. Microbial culture analysis demonstrated that 33% of the endoscopes tested grew bacteria before manual cleaning and 11% grew bacteria after manual cleaning. The maximum number of culturable bacteria prior to manual cleaning was 4 $\log_{10}$ and 2 $\log_{10}$ /mL after manual cleaning. Microbial culture results are presented in Figure 16.

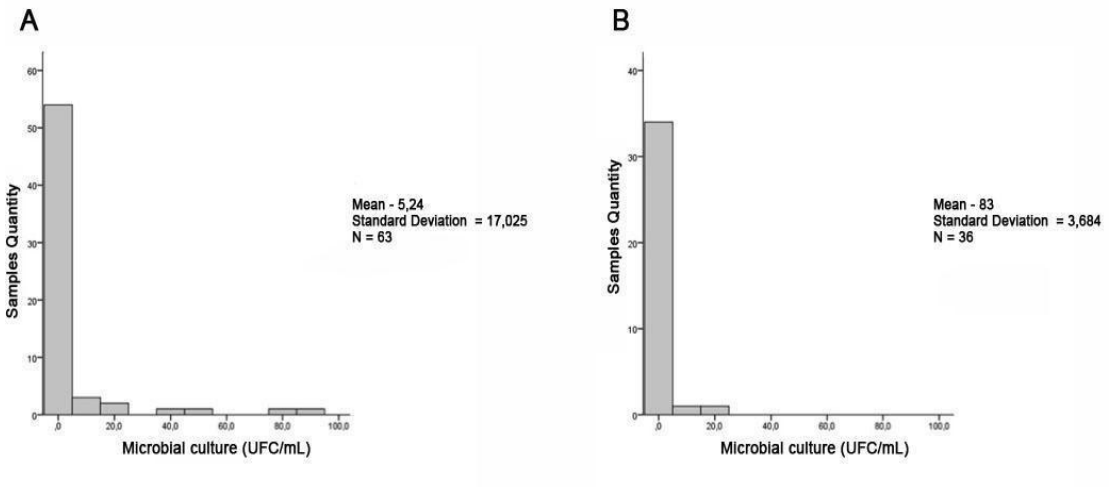

Figure 16. Distribution of microbial culture results on colonoscopes (A) and gastroscopes (B) after manual cleaning.

Isolated microorganisms were principally *Escherichia coli* (39%), *Staphylococcus* coagulase negative (19%) and *Klebsiella spp*. (17%) (Figure 17).

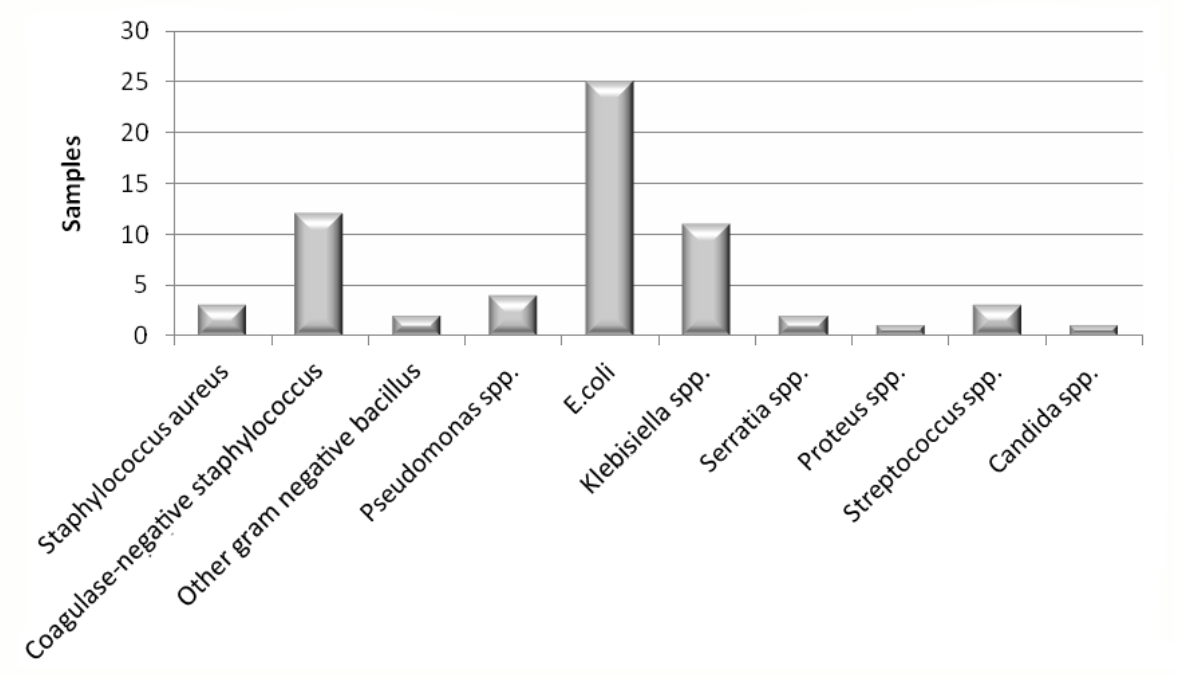

Figure 17. Microorganisms isolated from clinically used endoscopes sampled before and after manual cleaning.

Working endoscope channel bacterial load analysis is presented in Table 1, the median values from before and after manual cleaning is 6 $\log_{10}$ bacteria/mL (range 3 $\log_{10}$ to 7 $\log_{10}$ bacteria/mL). There was a significant reduction in total bacterial load as measured by qPCR after manual cleaning ($p=0.03$) (Figure 18). The total bacterial load of colonoscopes following manual cleaning was significantly higher than the total bacterial load on gastroscopes ($p<0.001$).

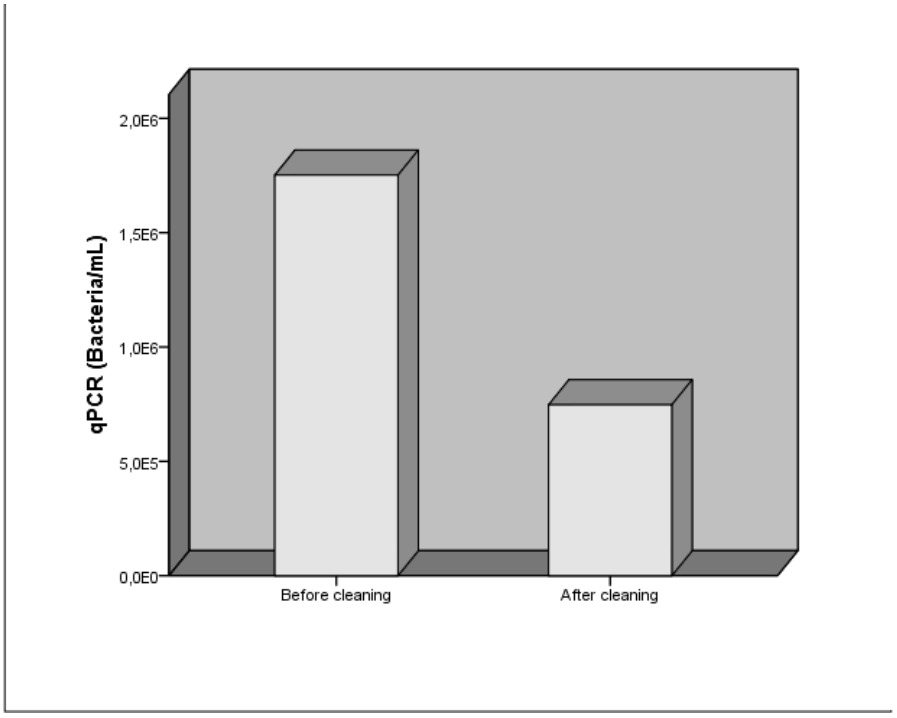

Figure 18. Comparison of qPCR results (Bacteria/mL) tested on clinically used gastrointestinal endoscopes before and after manual cleaning.

The correlation analysis among the variables cited above showed that ATP and qPCR values presented a positive and significant correlation ($p<0.001$ for colonoscopes and $p=0.035$ for gastroscopes).

Discussion

The efficacy of the reprocessing process of flexible gastrointestinal endoscopes was evaluated by measuring biological soil utilizing various ATP test kits and comparing ATP results to microbial load. There is frequent warning about the peculiarities of the physical structure of endoscopes that threaten reprocessing quality and safety. Therefore, a suitable bedside test to determine if an endoscope is clean prior to disinfection is a necessary quality control tool.

Accomplishing endoscope reprocessing recommendations involves a careful practice based on the range of procedures, equipment and potential risks involved in the process. Failures of endoscope reprocessing can result in contaminated endoscopes being used on patients and result in infection and outbreaks of HAI[56,71]. This reality justifies the arsenal produced at the area, such as, disinfection agents, automated equipment, guidelines and reprocessing quality control aimed at improving patient safety[15,26,54,61,101]. Endoscope reprocessing failures can be related to human performance, inadequate materials and equipment with structural and maintenance problems; and also organizational and environmental problems[81-83].

Even though endoscope reprocessing evaluation may detect possible failures and to avoid patient contamination, some recommendations are controversial among international guidelines. Microbial culture is being used for detecting failures of endoscope reprocessing and endoscope damage, during endoscope contamination investigation[14,85,101], but is also used in clinical practice for quality control[20,119]. Nevertheless, some guidelines do not include surveillance cultures as an endoscope reprocessing quality control given the time necessary for results, cost and difficulties in interpreting results, especially regarding environmental microorganism isolation[106,71].

Considering that endoscope reprocessing is a multistep process and that cleaning is considered crucial for disinfection success[119, 89]; monitoring cleaning procedures can be effective on endoscope reprocessing quality control[122]. Currently, visual inspection is recommended as gastrointestinal endoscope manual cleaning evaluation procedure[53,1].

However, visual inspection recommendation has limitations, such as the subjectivity inherent to the qualitative evaluation process without pre-established criteria; the black color of the outside of the endoscopes that makes detecting soil difficult; and the presence of narrow internal channels in the endoscopes that make it

impossible to visualize the endoscope internal surface[87,88]. We have found that gastrointestinal endoscope internal surfaces were contaminated with higher amounts of biological soil than the external part of the endoscope. Visual inspection obviously is inadequate for detecting soil contaminating internal channels.

We measured endoscope contamination utilising ATP, qPCR and culture before and after manual cleaning. ATP test has been refereed as method to evaluate endoscope manual cleaning efficiency[89-91,121-123]. ATP is present in all living organisms as an energy source, and in commercial test kits its presence is detected by the reaction of ATP with luciferase which converts ATP into visible light[94-95]. The use of ATP as an indicator of biological soil on test surfaces, including gastrointestinal endoscopes, has the advantage of being rapid with testing time taking less than 5 minutes[96,97,121].

However, some possible limitations on the use of ATP as a cleaning test monitoring are presented in the literature, such as, low accuracy and reproducibility among commercially available brands[99] different brands of ATP luminometer read on different RLU scales so comparison between brands is difficult and limited sensitivity in detecting low levels of ATP[99,100]. Thus, it is essential to use in clinical practice an institutional protocol with recommendations for interpretation of the results obtained with each type/brand of ATP test adopted.

The mean ATP values assessed on internal channels of gastrointestinal endoscopes tested prior to manual cleaning were 738 RLU in gastroscopes and 52 RLU in colonoscopes. After cleaning, these values decreased significantly to 85 and 11 RLU, respectively.

Similar study[122], using different brand, demonstrated a reduction from 1315 RLU in the biopsy channels and 39.3 RLU in the colonoscopes air and water channels before cleaning to 20 RLU and 15.2 RLU, respectively, after cleaning.

Clinical studies evaluating gastrointestinal endoscopes cleaning procedure with the same ATP devices used in this study have not been found in the literature. However, comparison of three ATP test brands with in vitro contamination on metal surfaces demonstrated statistically significant difference before and after cleaning using Hygiena Ultrasnap device[124].

Although we haven't found significant correlation between the results of ATP tests and microbial culture, the correlation between biological soil (ATP) and total bacterial load, as determined by qPCR, was statistically significant. It should be noted that the majority of samples post cleaning had no microbial growth, this suggests that the pre-cleaning and manual cleaning of the endoscopes were effective in reducing their levels of contamination.

Endoscope manual cleaning analysis demonstrated the efficiency in reducing biological soil ($p < 0.001$) and microbial contamination ($p = 0.03$), with a low percentage of the sample with viable microorganisms.

Financial Support

As described in section 3.5.

CHAPTER 5. EVALUATION OF ENDOSCOPE REPROCESSING: HIGH LEVEL DISINFECTION.

5.1 Introduction

Reprocessed endoscopes were evaluated for soil and contamination level by ATP, qPCR and microbial culture. Additionally, culture samples were tested by both blood agar and neutralizer plates for assessing possible influence of the endoscope´s residual disinfectant on microbial culture results.

5.2 Evaluation of soil and contamination level of reprocessed gastrointestinal endoscopes.

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Candidate Contribution:

- Study design

- Laboratorial experiment

- Data analysis

- Manuscript preparation

Overall contribution: 70%

Introduction

Gastrointestinal endoscopy consists on an important diagnostic and therapeutic procedure that allows visualization and access to gastrointestinal injuries, without surgical interventions[52]. In the United States, approximately 18 million gastrointestinal endoscopy procedures are performed annually with an estimated cost of US $ 32.4 billion[51]. Recently, the evidences that associate gastrointestinal endoscopes with infection outbreaks are significant[24,11], especially the ones caused by multidrug-resistant organism [12,22,23,27,28]. Periodic surveillance of endoscope reprocessing can improve failures detection and prevent infections related to endoscopy procedures.

Given endoscope structural particularities, reusable and thermosensitive semicritical devices, high level disinfection is recommended for gastrointestinal endoscope reprocessing[59,64]. High level disinfection process, carried out by means of chemical agents and set temperatures, it is possible to eliminate most of the microorganisms, with the exception of some spores[54,7]. Some factors can affect the success of the disinfection process, among them, the object configuration, temperature, pH, concentration and contact time of the disinfectant agent and the effectiveness of the cleaning of the object, with consequent amount of organic matter present[63]. The removal of disinfectant agents from endoscope surfaces after reprocessing must be performed by effective rinsing for residual toxic biocide removal. However, the possible effect of disinfectant, not fully rinsed from endoscopes, on surveillance culture have been reported in literature[118] but substantial evidences at the area are lacking. In this study we aimed to (1) assess soil and contamination level on reprocessed gastrointestinal endoscopes, and (2) evaluate the effect of residual disinfectant on microbial culture results.

Material and Methods

Preliminary study

In order to assess the effect of possible residual disinfectant on endoscope culture result, a preliminary study was performed for determining the residual disinfectant on flexible endoscopes after high level disinfection process. Initially, a high level disinfection cycle was performed on a gastrointestinal endoscope using automated endoscope reprocessors and peracetic acid disinfectant. The initial and final concentration of the disinfectant in contact with the endoscope during the cycle was calculated using two stage titration using a Mettler Toledo T70 autotitrator fitted with two 20 mL burettes, an auxiliary pump and a DMI-140SC platinum ring redox electrode. Additionally, both the initial and final solution of disinfectant in contact with the scope during the cycle were tested with specific peracetic acid test strip and analyzed by comparing the strip colour with the standard. The concentration of residual liquid in AER at the end of the disinfectant cycle tested was 2 ppm.

Sample Collection

For assessing soil and contamination level, reprocessed endoscopes were sampled. All endoscopes evaluated were removed from the endoscope drying cabinet after checking the date and time of high-level disinfection. We chose to collect samples within 12 hours after reprocessing, for possible recovery of contamination from biofilms and before 72 hours, maximum recommended period for endoscope storage

before new reprocess. After sample selection criteria, the collection was performed using a total protection barrier and sterile equipment.

Initially, the 30 cm of the endoscope external distal part was tested by Ultrasnap device for ATP assay, with up/down and rotations movements to increase the contact area evaluated. Then, biopsy and suction channels were brushed with an Single-use combination cleaning brush BW-412T by Olympus and 2 cm tip of the brush was cut and collected on sterile sample collection tube. Finally, using the flexible endoscope flush connection, as described in section 3.12.1.

ATP assay

Reprocessed endoscopes were assessed for soil level by ATP assay tested both on external part of the endoscope using Ultrasnap (Hygiena, USA) device and internal channels using Aquasnap (Hygiena, USA) device, by taking aliquots of 20 μL of each flush sample from internal endoscope channels. All ATP quantification was performed using the System Sure Plus (Hygiena, USA), bioluminescence monitoring system, and results presented in relative light unit (RLU).

Microbial culture

Isolation of viable bacteria was obtained by culturing 20 mL aliquots of endoscope internal flush samples into two types of agar plates (10 mL each), horse blood agar (Micromedia Laboratories, Victoria, Australia) and neutralizer agar plates, Tryptic Soy Contact Agar + LTHTh - ICR (EMD Millipore, Billerica, USA), commercially available agar plates containing neutralization for peracetic acid.

All samples were cultured by passing flush volume through MCE membrane filter (Membrane Solutions LLC, WA, USA) with 25 mm diameter and 0.22 µm pore size coupled to MS® re-usable syringe filter holder (Membrane Solutions LLC, WA, USA), after steam sterilization. Then the filter was removed from the membrane with sterile disposable forceps and placed on respective culture agar plates for incubating at 37 ° C for 48 hours prior to positive/negative reading. If culture positive, then each bacterial colony was re-cultured on Chromogenic UTI agar plates (Oxoid) by incubation at 37ºC for 18 to 24 hours for initial identification (as described in section 3.8).

Microbial identification

Bacterial species identification of isolates from culture positive samples was performed through genetic sequencing of 16s rRNA. Initially, 200µL of phosphate buffer solution and 10µL of lysozyme (final concentration of 0.5 mg/mL) were added to the colonies isolated from the bacteria with subsequent incubation at 37 ºC for 15 minutes. Then DNA was extracted from samples with High Pure PCR Template Preparation Kit (Roche) for sequential PCR amplification of 16S rRNA gene (16S rRNA_341F universal primers 5'-CCTACGGGAGGCAGCAG-3' and 16S rRNA_534R 5'-ATTACCGCGGCTGCTGG-3'). The product (10 µL) was then purified with 2 µL of ExoSAP-IT treatment enzyme; centrifuged rapidly and incubated at 37°C for 30 minutes (later inactivation of the enzyme with incubation at 80°C for 15 min); 1 µL of 341F Primer was added to 10 uM and 1 µL of 10 uM Primer 951R and sent to Australian Genome Research Facility for DNA sequencing analysis. Sequencing was performed using the BigDye Terminator v3.1 Kit (Applied Biosystems) for sequencing and AB3130xl genetic analyzer (Applied Biosystems) for capillary separation. The result

was compared to the genetic sequencing available in the GenBank, EMBL and DDBJ databases using the results ≥ 98% similarity.

Quantitative polymerase chain reaction

Aliquot of 10 mL of each flush sample was assessed for bacterial load by qPCR. Samples were centrifuged on Heraeus™ Multifuge™ X1 Centrifuge (Thermo Fisher Scientific) at 482 *g* for 30 min, and supernatant was removed until final volume of 200 µL. On final content, 5 µL of lysozyme (Sigma, Sydney, Australia) was added to each sample and incubated at 37 ºC for 15 min. Then, 40 µL of proteinase kinase (Sigma, Sydney, Australia) was added, followed by incubation at 70 ºC for 10 min. Genomic DNA was extracted using High Pure PCR Template Preparation Kit (Roche). DNA sample was subjected to real-time qPCR for amplification of the 16S rRNA genes (16S rRNA_341F universal primers 5'-CCTACGGGAGGCAGCAG-3 'and 16S rRNA_534R 5'-ATTACCGCGGCTGCTGG-3') using as cycle conditions 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 min, annealing at 56°C for 30 s and extension at 72°C for 30 s.

Statistical analysis

Data statistical analysis was performed by IBM SPSS Statistics version 22.0 software. Mann-Whitney test was used in the comparisons of medians in independent samples, and chi-square test with a significance level of 5% ($p < 0.05$).

Results

A total of 88 gastrointestinal endoscopes were tested from 12 to 72 hours after reprocessing, sampled on internal and external part of the endoscope, by ATP, qPCR and microbial culture. All samples presented ATP results under 50 RLU, and qPCR analysis demonstrated a median bacterial load of 3 $\log_{10}$ bacteria/cm (Table 7).

	GASTROINTESTINAL ENDOSCOPES N=88		
	Soil level		**Bacterial level**
	ATP Internal	ATP External	BAC/cm
Minimum	0	0	0
Maximum	12	39	1.1E+04
Mean	1.15	2.46	2.1E+07
Median	0	0	1.8E+03
St Deviation	5.7	2.5	2.0E+03

Table 7. Distribution of descriptive analysis of reprocessed gastrointestinal endoscope by ATP and qPCR.

Nine of the 88 (10.2%) samples presented culture positive result. The comparison of different media used for culturing samples demonstrated that HBA presented higher culture positive samples (6/88) than disinfectant neutralizer media (3/88), however, this difference was not significant (p=0.5).

The bacterial species isolated were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Roseomonas gilardii* and *Micrococcus luteus.*

Discussion

Gastrointestinal endoscope (GI) reprocessing involves a multifactorial and complex process that if not successful can compromise patient safety and cause related infections[24]. The endoscope becomes highly contaminated during endoscopy procedures[62] and the path of infection transmission between patient and contaminated endoscopes has already been reported in outbreaks investigations[28]. Therefore, achieving optimal endoscope decontamination is clearly important for ensuring the microbial safety of endoscopy procedures.

Thus, in order to surpass the difficulties involved in GI endoscope reprocessing, such as endoscope structure characteristics of heat-sensitivity and complex design; an extensive decontamination process involving manual and/or automated procedures; and high demand with rapid turnover being the common endoscope use conditions, the surveillance of endoscope reprocessing can help detect errors and prevent infections.

In this study, microbial culture of reprocessed GI endoscopes resulted in 10.2% of positive results with isolations of viable bacteria, identified as common environmental microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus capitis, Roseomonas gilardii* and *Micrococcus luteus*).

Similar study design for evaluating contamination level on reprocessed gastrointestinal endoscopes detected through microbial culture was found by Ofstead et al. (2015)[108] and Alfa et al. (2012)[125] reporting 9% and 14% of the samples, respectively, with the presence of viable microorganisms. Even though, ideally after endoscope reprocessing it would be no grow of bacteria, the count of 10^2 CFU/mL was recommended as threshold for indicating endoscope reprocessing contamination level, which was pointed as achievable in clinical practice[125]. However, different realities

were also reported in literature, indicating higher endoscope contamination levels. In a study carried out in two Brazilian hospitals, Machado et al. (2006)[126] showed that 48.3% of the gastrointestinal endoscopes evaluated presented a positive microbial culture and levels of contamination ranging from 10^4 to 10^6 CFU/mL after reprocessing. Ofstead et al. (2016)[127] reported 47% of positive microbial culture, with increasing to 60% when the same gastrointestinal endoscopes were tested after two months period.

The use of microbial culture for endoscope reprocessing surveillance is well reported[101,14,20,85,119]. However, some guidelines[1,59,71] don´t include the method as a recommendation for routine clinical practice claiming difficulties for sampling and interpreting results. The Gastroenterological Society of Australia (2003) endoscope reprocessing guideline recognize that appropriate bacteriological surveillance on endoscopes and automated reprocessors is challenging, but, also states that culture is the only method for detecting damage of the endoscope internal channels in clinical practice. In order to overcome the difficulties related to sampling and interpretation of microbial culture results of endoscope reprocessing, the guideline also recommend the frequency that endoscopes must be tested and which microorganisms should be investigated, thus directing the procedure and interpretation of cultures.

The role of microbial culture as a periodic quality control of endoscope reprocessing is still unclear[56] and outbreaks of MDRO related to contaminated endoscopes despite negative culture results have been reported[32,62]. In order to try to improve results of microbial culture, the use neutralizer to flush endoscope channels has been suggested as more sensitive method than using sterile water[116].

On preliminary study (unpublished data), we found that after high-level disinfection cycle on Soluscope AER, residual disinfectant of 2 ppm is present on endoscope internal channels. For assessing possible effect of residual disinfectant on

endoscope culture results in clinical practice, surveillance culture on eighty eight reprocessed endoscopes were performed by using both regular and disinfectant neutralizer plates. The results indicated that there´s no significant difference on using disinfectant neutralizer media plates for performing surveillance culture when compared to regular non-selective culture media, if culture tested on a period over 12 hours after reprocessing.

This study was conducted in a single center endoscopy unit, and therefore we suggest that similar research involving different units be undertaken. We have found that disinfectant neutralizer media plates seem to have no effect on surveillance culture results (done ≥12 hours after processing), however we recommended the use of commercially available disinfectant neutralizer plates if routine surveillance endoscope culture tests is performed immediately after reprocessing for confirming results.

Acknowledgement

We thank Dr. Trevor Glasbey for assisting on preliminary´s study design and execution.

Financial support

As described in section 3.5.

CHAPTER 6. ASSESSING CLEANING AND BACTERIAL LOAD ON ENDOSCOPY UNIT SURFACES

6.1 Introduction

A brief report on soil and bacterial load of endoscopy unit surfaces was performed after surfaces decontamination at the end of the list. Therefore, the surfaces that are most likely to be often touched by healthcare professionals on an endoscopy unit were sampled for identifying possible contamination.

6.2 Identifying contaminated areas on endoscopy unit´s surfaces: a brief report.

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Candidate Contribution:

- Study design

- Laboratorial experiment

- Data analysis

- Manuscript preparation

Overall contribution: 80%

Introduction

Healthcare associated infections represents a major concern and a worldwide challenge, especially for the variability of resources and behaviors of practices related to the control and prevention of infections. In a healthcare setting, pathogens can be transmitted by both human and environmental sources[61]. The contact with surfaces and equipment in healthcare settings provides a common path of microorganism transmission[130] which can directly or indirectly colonize healthcare professionals and/or patients[81].

In the endoscopy unit scenario, the focus of infection control and prevention recommendations has been on endoscope reprocessing. However, with the reports of infection transmission and outbreaks related to endoscopy procedures[118], evaluation of other factors in endoscopy units should be further addressed[132]. In this study, we aimed to identify areas with high bacterial load on endoscopy unit surfaces.

Material and Method

The endoscopy unit samples were collected from nurse's station and two procedure rooms after decontamination at the end of the day´s list. The areas chosen to samples were the ones often touched by healthcare professionals. Initially, areas to be tested were marked with 2cm by 5cm sampling area in duplicate for each surface. Then, ATP test was performed by rubbing the swab (Ultrasnap, Hygiena) with rolling movements across the area and sequential ATP analysis undertaken by reading results in System Sure Plus, Hygiena bioluminescence monitoring system, and comparing results with 100 RLU threshold. For all values above 100 RLU, a surface sample from a second sampling area was collected, using sterile gloves, by rubbing the surface area with

sterile phosphate buffered saline (PBS) wetted sterile gauze with rolling movements. For culture, each sample was placed inside a sterile tube containing 5 mL of PBS that was then placed in an ultrasonic bath (Soniclean, JMR Australia) at 43mHz for 20 minutes sonication. Finally, an aliquot of 100 µL was spread on HBA for incubation at 37 ºC for 24 hours.

Separated bacterial colonies were isolated and initial identification was performed by selective media, MacConkey agar (MAC, Oxoid), Mannitol salt agar (MSA, Oxoid) and Chromogenic UTI agar (Oxoid) (as described in section 3.7); additionally, biochemical characteristics were assessed by Analytical Profile Index system (API-20E) to identify different members of Gram negative bacteria as manufacturer's instructions for use. The results are presented in CFU/cm^2.

Results and Discussion

Between the nurse station and the procedure rooms, a total of 24 areas was initially sampled for soil load by ATP assay. Sequentially, a total of 8 areas presented ATP results higher than 100 RLU and were tested for bacterial load by microbial culture. A description of the areas sampled is illustrated in Table 8 and 9.

Table 8. Description of the endoscope unit sampling areas from nurse station tested by ATP and Culture.

Sampling area	ATP (RLU)	Culture (CFU/cm^2)$^+$
Table	188	5
Chair top	143	20
Chair lift handle	241	165
Telephone	259	40
Keyboard	155	25

($^+$) performed if ATP result higher than 100 RLU; (-) not applicable

Table 9. Description of the endoscope unit sampling areas from procedure room tested by ATP and Culture.

Sampling area	ATP (RLU)	Culture (CFU/cm^2)$^+$
Mouse	251	50
Keyboard	24	-
Table	81	-
Stool	121	0
Door endoscope storage cupboard	25	-
Sink tap	16	-
Sink pipe	4781	Unc.*
Door to cleaning room	26	-
Bench up	35	-
Bench mobile pc	25	-
Keyboard mobile pc	14	-
Infusion pump	38	-
CPR cart	37	-
Medication cart	11	-
Mobile bench	9	-
Endoscope light connection	15	-
Storage cabin	39	-
Door to recovery area	99	-
CPR computer	16	-

(*) uncountable culture result; ($^+$) performed if ATP result higher than 100 RLU; (-) not applicable

The analysis of ATP assay indicates the nurses' station presented higher median values than the procedure room´s surfaces. However, the results of soil level on endoscopy unit procedure room surfaces were heterogeneous with discrepant values in particular areas, such as sink pipe showing ATP value of 4781 RLU (Figure 19).

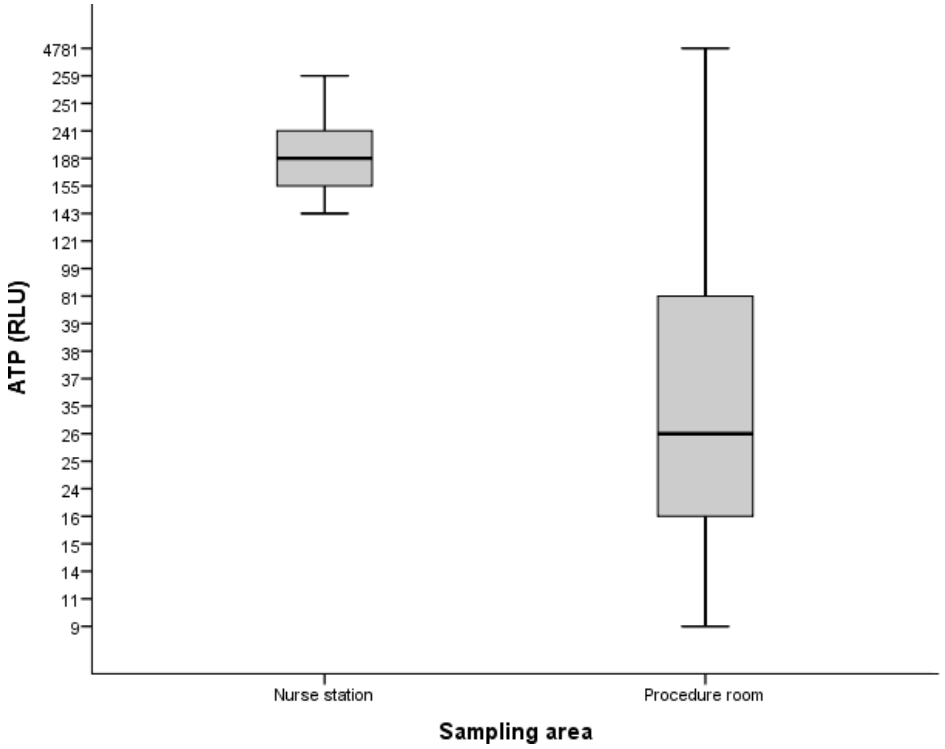

Figure 19. Distribution of ATP values of nurse station and procedure room´s surfaces at a endoscopy unit demonstrating minimum, maximum and median values.

The areas with higher soil load level were the telephone at the nurse station and the sink´s pipe at the procedure room. However, at the nurse station, the area with higher bacterial load was the chair lift handle.

The culture results show that one of the samples presented uncountable bacterial growth (pipe) and one with no growth; from the other six areas sampled the results varies from 5 to 165 CFU/cm^2. All culture positive results had multispecies bacterial growth and the microorganisms isolated from endoscopy unit surfaces include *Staphylococcus*

aureus, *E.coli*, *Pseudomonas* spp., *Shigella* spp., *Pantoea* spp., other *Staphylococcus* and *Enterococci*.

Staphylococcus aureus is associated with more than 10% of healthcare associated infections[130] and infections caused by *Staphylococcus aureus* resistant to methicillin can have severe progression with high mortality rate[134].

The presence of microorganisms in a dry biofilm form and adherent to environmental surfaces in an intensive care unit has already been reported[135] along with the resistance of dry-surface biofilm to biocides[136,137].

Recovering microorganisms from surfaces and environment can be challenging, given some particularities of detection methods. ATP is present in all cells and constitutes their main source of energy. It can be quantified by the detection of its bioluminescence in the reaction with the enzyme luciferase, thus, ATP assay can represent the presence human cells and microorganisms[94,95]. Thus, the use of ATP as an indicator of the level of surface contamination is advantageous by the practicality of the sample collection and fast results (less than 5 minutes), allowing the detection of failure and repetition of the cleaning process[96,97]. Alternatively, microbial culture method requires the growth and detection of viable, culturable, microorganisms, and this can explain the inconsistency of ATP and culture results in this study. In fact, in a investigation of contamination on an intensive care unit surfaces using different detection methods, Hu et al. (2015)[137] reported low culture results or non-culturable samples despite high bacterial load tested by PCR along with detection of live bacteria, confirmed by microscopy and live dead stain.

Overall, the sampling method for assessing microbial hygiene evaluation and pathogen detection are not yet well established in the literature[138], therefore, comparisons involving different methods can be limited.

Conclusion

By sampling different endoscopy unit areas which are often touched by healthcare professionals, we were able to identify that the nurses' station presented higher soil level than the procedure room. In addition, potential pathogenic microorganisms were isolated in different areas of the endoscopy unit´s nurses' station. These findings suggest that not only the procedure room, but the nurses' station area is also subjected to contamination with pathogenic organisms and therefore should be carefully addressed by healthcare professionals for more rigorous cleaning and decontamination. The presence of viable and possibly pathogenic bacteria detected through microbial culture reinforces the risks of environmental contamination in healthcare settings as a possible source of patient infection.

This was the result of a single center study and should be repeated in different scenarios for better evidence. Another limitation of the study is that samples were collected at a single time point and decontamination procedure compliance was not tested. Further research is needed at the endoscopy unit area to characterize the microbial conditions of the area and strengthen evidences for specific application of decontamination protocols.

CHAPTER 7. ENDOSCOPE CHANNEL CONTAMINATION EVALUATION

7.1 Introduction

Endoscope channels received by endoscope repair services from Brazil and Australia endoscopy units were analyzed for assessing contamination level.

7.2 Microbial contamination on flexible endoscope channels

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Candidate Contribution:

- Study design

- Laboratorial experiment

- Data analysis

- Manuscript preparation

Overall contribution: 80%

Introduction

Flexible endoscopy is minimally invasive and thus provides diagnosis and treatment without the morbidity associated with more invasive surgical techniques. However, the reprocessing of flexible endoscopes is a challenge, given their complex structure with long and narrow channels. Inadequate cleaning compromises the decontamination process and may lead to endoscope damage and reprocessing failure, resulting in transmission of infectious organisms[20,57].

Microbial contamination of reprocessed endoscopes has been reported in the literature, not only after quality control investigations[13,14,16,18,19], but also following outbreaks of healthcare associated infection (HAI)[12,24,26,11].

The risk associated with endoscope procedures was thought to be very low[29]. However, the recent transmission of multidrug resistant microorganisms via endoscopy resulting in patient colonization, infection and even death has increased health professionals' awareness that endoscopy-related transmission of infection occurs and is a real risk to the patient. This is in part due to the antibiotic resistant nature of the bacteria involved making detection of outbreaks easier[11,13,22,23,24,28].

Endoscope contamination has often been related to failure of reprocessing[56,24,11] but the presence of biofilm on endoscope channels could compromise decontamination even if conducted rigorously under ideal conditions[114]. The presence of biofilm on reprocessed endoscope channels was confirmed in 2004[18] and despite improved awareness by healthcare professionals biofilm contamination of endoscope continued to be reported decade later[19].

In this study, we aimed to evaluate microbial load and the presence of biofilm within processed endoscope channels obtained from a middle income country (Brazil) and those obtained from a high income country (Australia).

Materials and Methods

Forty gastrointestinal flexible endoscope biopsy channels (twenty-two from Australia and eighteen from Brazil) were analyzed for bacterial load by 16s rRNA quantitative polymerase chain reaction and for biofilm presence by scanning electron microscopy (SEM). The samples were received from endoscope servicing departments in Brazil and Australia after endoscope channel replacement. Information about time of use or conditions of endoscope reprocessing was not provided. All endoscope channels had their external surfaces decontaminated by wiping two times sequentially with Matrix (Whiteley Corporation, North Sydney, Australia) marketed as a biofilm remover, 70% ethanol and sterile water.

Quantitative Polymerase chain reaction (qPCR)

For bacterial load determination, DNA was digested from inside the endoscope channels by syringing 2 mL of digestion mix (50nM Tris/HCl pH 7.5; 150nM NaCl; 2 nM EDTA; 1% SDS; proteinase K 20mg/mL) inside a 20 cm section of channel and incubating at 56°C overnight. The channel section was then sonicated at an average of 43 mHz in ultrasonic bath (Soniclean, JMR Australia) for 20 minutes and the sonicate collected into a clean tube. The proteinase K was inactivated by heating to 95°C, 200 μL of 10 mg/mL lysozyme (Sigma-Aldrich, Castle Hill, Australia) added and incubated at 56°C for 2 hours.

DNA was then extracted by salt precipitation. Briefly, 640µl of 5M NaCl was added to the digested sample, mixed vigorously and cooled on dry ice for 10 minutes prior to centrifugation at 2500 rpm at 4ºC for 15 minutes. The supernatant was transferred to a new tube before centrifuging at 2500 rpm on 4ºC for 15 minutes and transfer of supernatant to new tube prior to overnight alcohol precipitation at -20ºC and centrifugation. DNA pellets were resuspended in 50 µl buffer (10mM Tris/HCL pH 8.0, 1mM EDTA).

Contaminating bacterial numbers were determined by real-time quantitative PCR using 16S rRNA Eubacterial universal primers 341F 5´-CCTACGGGAGGCAGCAG-3` and 534R 5`-ATTACCGCGGCTGCTGG-3` as described previously (JACOMBS et al., 2012).

To test for differences in number of bacteria contaminating endoscope channels the Mann-Whitney rank sum test was conducted using IBM SPSS Statistics software.

Scanning electron microscopy

A 1 cm section of the internal surface from twelve biopsy channels was analyzed by SEM. Following qPCR analysis, six samples with high microbial loads and six samples with low microbial loads were fixed in 2.5% glutaraldehyde for 24 hours. Samples were then dehydrated through increasing concentrations of ethanol and 100% hexamethyldisilazane (HMDS, Polysciences Inc, Warrington, PA, USA) and sputter coated with 20 nm of gold as described previously[149]. Biofilm presence was defined by visualization of microorganism surrounded by extracellular polymeric substance.

Results

Bacterial Load

The majority 28/40 (70%) of the samples were negative for bacterial contamination by qPCR. However, 4/40 (10%) endoscope channels had bacterial loads higher than 3 log_{10} bacteria/cm (1000 bacteria/cm), with a maximum of 5 log_{10} bacteria/cm (100000 bacteria/cm) (Figure 20). Endoscope channel samples from Brazil showed significantly higher bacterial load than Australian samples (p=0.02).

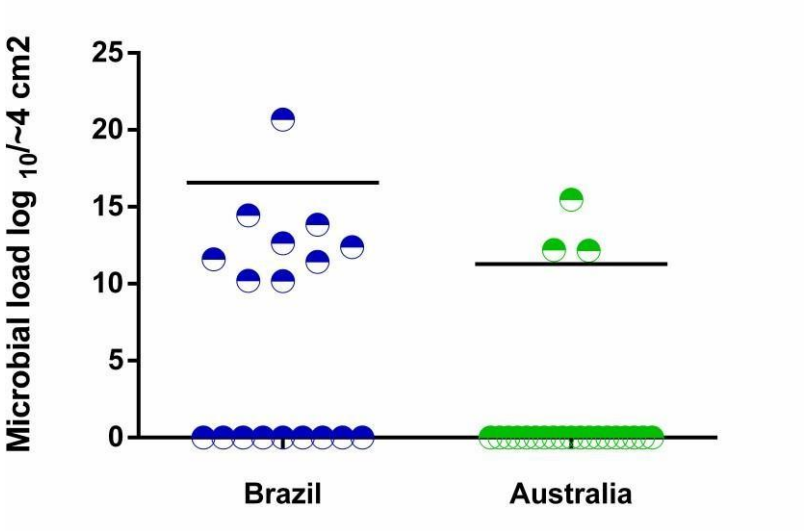

Figure 20. Frequency of bacterial load quantification results on endoscope channels analysis by country.

Microscopy Visualization

For internal surface visualization, 8 samples of endoscope channels from Brazil and Australia were examined by SEM. An overall description of SEM images analysis compared with qPCR results is illustrated in Table 10. The presence of biofilm was detected on samples, despite low values of qPCR, including extensive and multilayer biofilm formation. Additionally, areas of surface damage were detected on samples from both countries. On all samples, including the negative samples for bacterial load

quantification, presence of soil was detected, regardless the country of origin (unshown data).

Table 10. Description of endoscope channel samples analyzed by SEM.

Country origin	Bacterial load (qPCR)	Biofilm	EPS*	Soil	Surface damage
Australia	Med.		+	+	+
Australia	Med.			+	+
Australia	High	+	+	+	
Brazil	Low	+	+	+	
Brazil	Low		+	+	
Brazil	High	+	+	+	+
Brazil	Med.			+	+
Brazil	Med.	+	+	+	

* Extracellular Polymeric Substances (EPS).

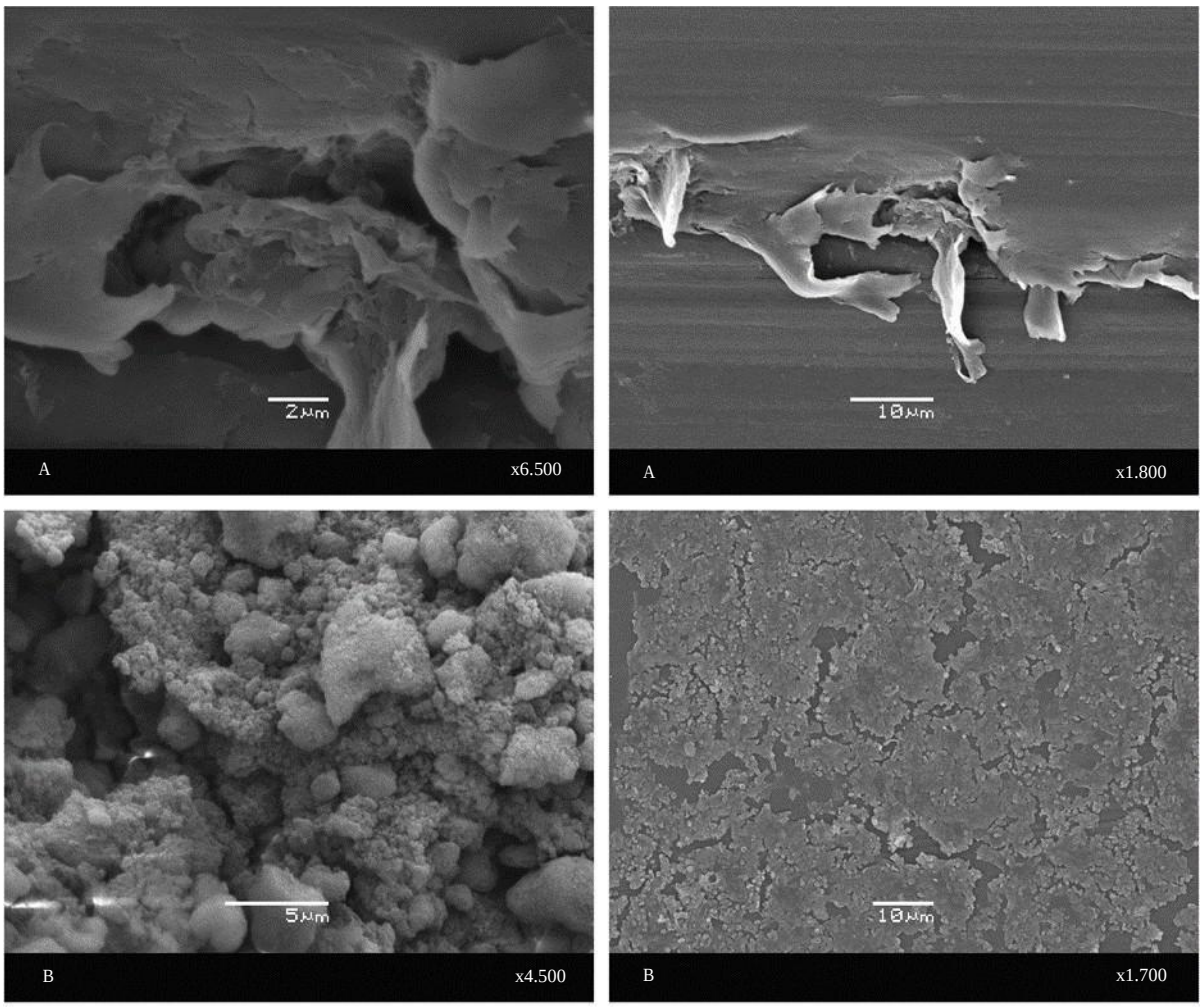

Figure 21. Scanning electron micrograph of endoscope channels internal surface from Australia (A) and Brazil (B) showing extensive EPS.

Even though samples from both countries demonstrated biofilm presence, Brazilian samples also presented blood cells, neutrophils cells and fungal hyphae structures (Figure 22).

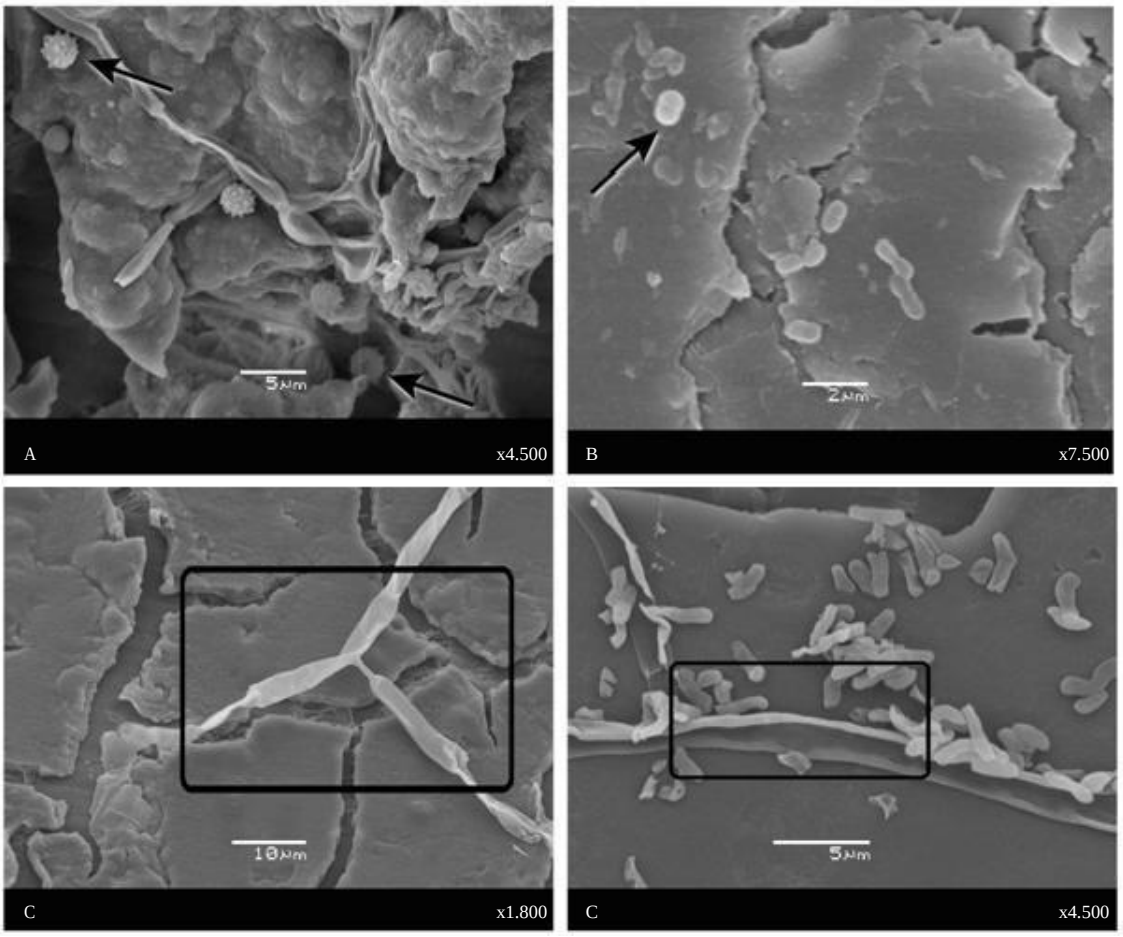

Figure 22. Scanning electron micrograph of Brazilian endoscope channels internal surface demonstrating presence of (A) neutrophils cells (B) blood cells and (C) fungus hyphae on biofilm formation.

On samples from both, Australia and Brazil, it was possible to identify soil presence and/or biofilm formation associated with surface´s damaged area (Figure 23). However, on some Brazilian samples the biofilm was multilayered and with extensive EPS. On some channels it was difficult to visualize the channel surface (Figure 24).

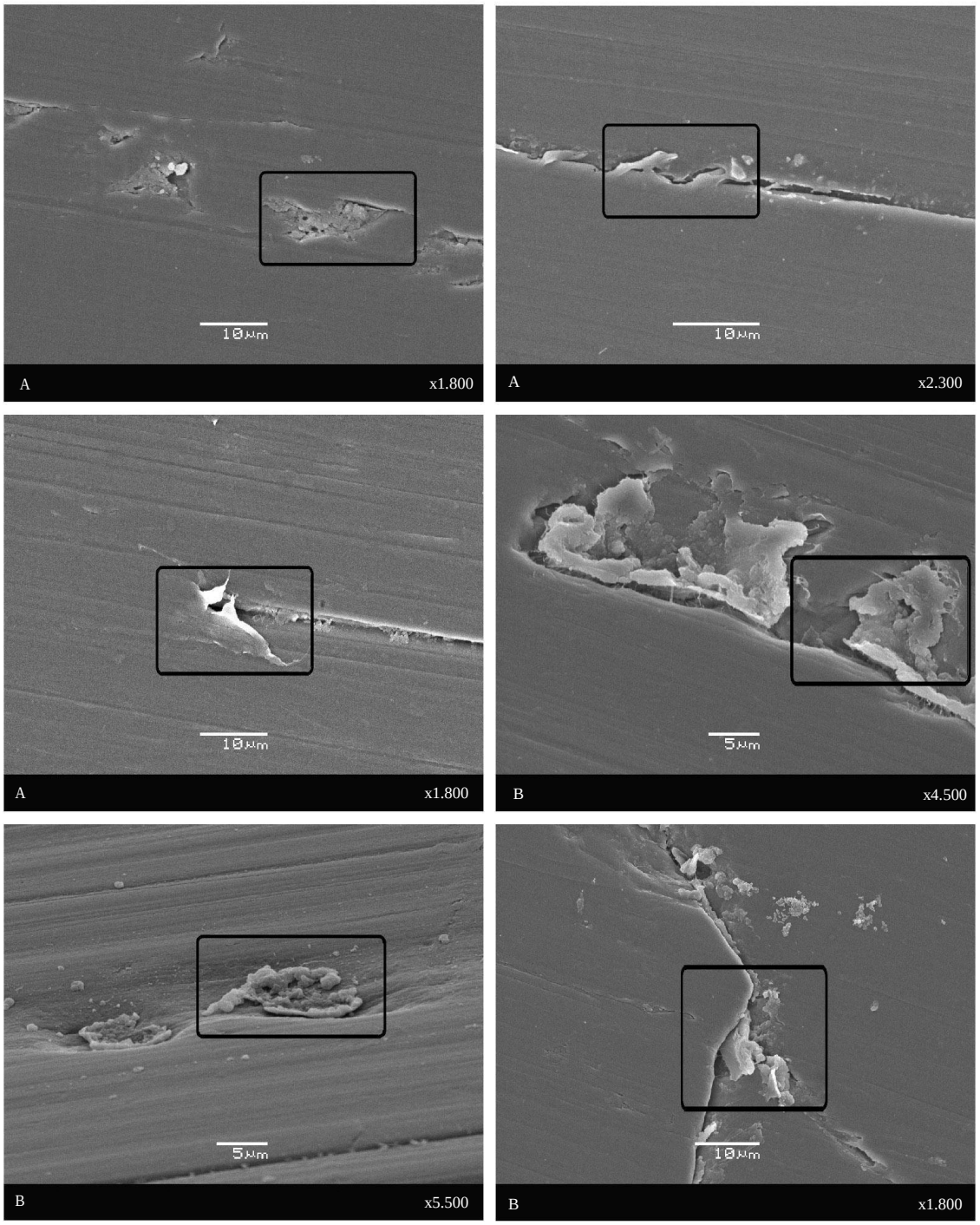

Figure 23. Scanning electron micrograph of endoscope channels internal surface demonstrating biofilm formation on damaged areas of samples from (A) Australia and (B) Brazil.

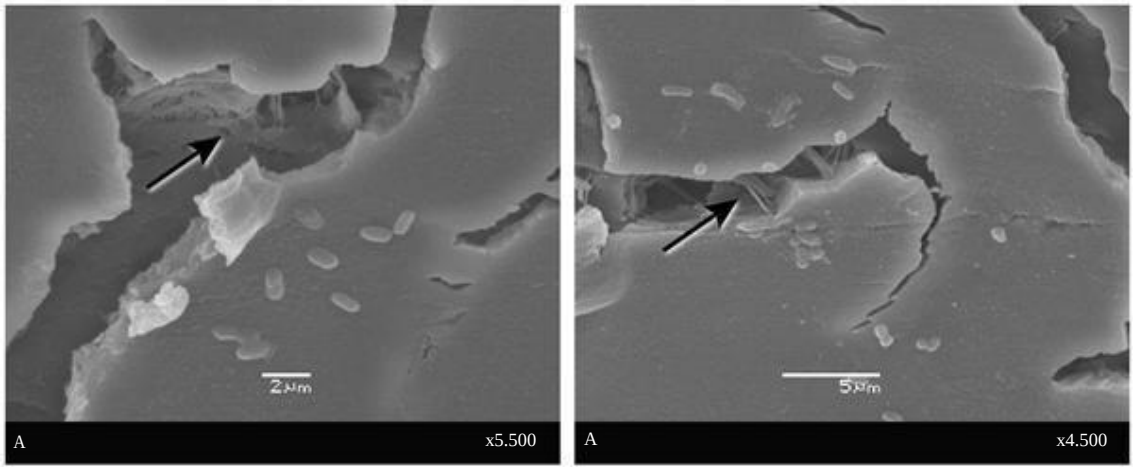

Figure 24. Scanning electron micrograph of Brazilian endoscope channels internal surface showing extensive EPS and a multilayered biofilm.

Discussion

Processing endoscopes to render safe instruments for patient use is challenging given the complexity of endoscopes rendering them difficult to clean[31,68]. In addition it is difficult to access to endoscope channels to assess validation of cleaning.

Although, endoscope reprocessing recommendations have been aimed at improving standardization and prevention of healthcare associated infections (HAI) [1,7,26,54, 64,77] endoscopy-related patient infection and outbreaks continue [11,24,56].

Generally, worldwide recommendations regarding endoscope reprocessing follow the same pattern involving five basic procedures (e.g. cleaning, disinfection, rinsing, drying and storage)[54,59,64]. Accordingly, recommendations from Australian and Brazilian´s official manuals[20,64] states basically the same endoscope reprocessing methods and recommended materials.

Staff performance, equipment and materials conditions, organization problems or high patient demand can cause endoscope reprocessing failure[81]. Studies[82-83] verified that the most frequent causes of endoscope reprocessing failure were automated reprocessors and staff practice different from standard recommendation.

Endoscope channels from Brazil presented significantly more bacterial load when compared to Australian samples and up to 5 $\log_{10}$ bacteria/centimeter. Although, qPCR does not differentiate between viable and dead bacteria, which can be a limitation of the assay, any microbial load persisting through the reprocessing process indicates that current reprocessing recommendations are insufficient for removing all soil from clinically used endoscopes[108].

However specific realities related to healthcare services demands and staff compliance to reprocessing recommendations could be present in one or both countries described in this study. Investigations of endoscopy units from Brazil demonstrates that structural and technical conditions are not always suitable and can compromise proper endoscope reprocessing[139,140].

Further studies involving multicentre observations in both addressed countries would be necessary for further comparison of organizational and personal conditions from respective endoscopy units. Overall, the direct observation of the researcher identified differences in the endoscopy services visited in Australia, in such as the use of automated endoscope reprocessors, the use of peracetic acid as a disinfectant agent and the storage of endoscopes in drying cabinets, which can be difficult to achieve in lower-middle income countries, such as Brazil.

We found that, even though the majority of the endoscope channels analyzed showed low bacterial load on qPCR, endoscope channels were frequently seen to be damaged and soil and/or biofilm was present on all channels examined by SEM.

Residual soil and biofilm on medical devices act as a barrier to disinfectants thus compromising disinfectant efficacy[41,141]. Biofilms are complex three-dimensional structures composed of communities of microorganisms incorporated into a matrix of exopolymeric substances. The biofilm matrix is composed of water, proteins,

extracellular DNA and polysaccharides and diffusion into the biofilm structure is limited. This combined with biofilm bacterial physiology results in a bacterial phenotype that is often more tolerant to disinfectant or antibiotic action when compared to the same organism growing as individual planktonic cells[41,45,48]. Biofilm presence on endoscopes has been shown to both decrease cleaning efficacy of instrument detergents[44] and disinfectant activity in vitro[68,114].

In areas of endoscope channel damage, there was increased amount of biofilm and this has been reported in other studies[20]. It´s worth mentioning that damaged areas were detected on endoscope channels from Brazil and Australia, suggesting that physical damage, like bacterial contamination, can be present even with different uses and reprocessing scenarios, therefore, also needs to be prevented.

In conclusion, persistent contamination of endoscopes even after reprocessing can be related to biofilm presence on endoscope internal channels, which are complex and resistant bacterial communities. With these results, we not only demonstrated bacterial contamination of reprocessed endoscope channels but we also confirmed the presence of biofilm formation, extensive soil presence and surface damage of endoscope channels from a high income and a lower-income country. Needless to say that endoscope reprocessing recommendations should be reviewed based on possible biofilm presence to provide an effective and microbial safe endoscopy procedure.

Acknowledgments

We would like to thank Dr Vanderlei Hass for helping with statistical analysis.

Financial support

As described in section 3.5.

CHAPTER 8. A PRELIMINARY STUDY ON THE CONTRIBUTION OF ENDOSCOPE CHANNEL DAMAGE TO BIOFILM FORMATION

8.1 Introduction

In chapter 5 we visually showed evidence of damage to the working channels of clinically used endoscopes. Often biofilm was present in these damaged areas. Channel damage could be a result of brushing during cleaning or the passage of instruments, such as biopsy forceps, during procedures. The number of times the clinical endoscopes we evaluated had been used was unknown. We therefore, developed an in vitro assay to investigate the relationship between biopsy forceps passage and endoscope channel surface.

8.2 Endoscope channel damage: evaluation of clinically used endoscopes and development of an in vitro model.

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Candidate Contribution:

- Study design
- Laboratorial experiment
- Data analysis
- Manuscript preparation

Overall contribution: 90%

8.2.1 Introduction

The number of endoscopy related infections and outbreaks reported in the literature has increased rapidly over the last few years[24,28,112]. This increase is also reflected in the number of FDA medical device reports related to endoscopy since 2010[56]. The increase in the number of reports and publications, is likely due, at least in part to increased detection of outbreaks as many have been associated with multidrug resistant microorganisms [23,56]. Of major concern is carbapenem-resistant Enterobacteriacae (CRE) due to limited treatment options and high mortality rate[12]. Many of these outbreaks have been associated with inadequate cleaning, particularly of the elevator mechanism of duodenoscopes, and subsequent biofilm formation. Past studies have also shown biofilm formation on endoscope channels[18-19]. In the studies by Pajkos and Rei-Pen, the biofilm was found frequently in damaged areas of the channel such as in pits and scratches. Protection of biofilm within pits and scratches makes it almost impossible to physically remove during cleaning. The passage of instruments and brushes down channels during procedures and cleaning are thought to be the likely cause of channel damage.

The development of biofilm whether by inadequate cleaning or by growth in protected damaged areas of the channel adversely impacts on subsequent cleaning and disinfection. Many instrument grade detergents have been shown to have poor efficacy against removing biofilm [41,44,142-144] . Additionally, the efficacy of disinfectants against biofilm has also been shown to be reduced as reviewed by Bridier[145]. Both *Enterococcus faecalis* and *Pseudomonas aeruginosa* survived disinfection by two commonly used endoscope disinfectants, glutaraldehyde and accelerated hydrogen peroxide, when grown as an aged, mature biofilm[68].

Failure of endoscope decontamination resulting in patient infection has been reported even when professional society guideline recommendations for endoscope reprocessing have been followed[23,112]. These transmission events may relate to biofilm formation in visually non-detectable, damaged areas of the endoscope.

The bioburden within used gastrointestinal endoscopes, as estimated using a brush/flush technique, can be as high as 9.4 Log_{10} organisms per device[9]. However, how many of the bacteria attached to the channel are sampled using this technique? Nor is it known how quickly bacteria can attach to undamaged Teflon tubing. When compared to surgical interventions, endoscopy procedures are less time consuming. Therefore, bacteria remain in contact with host tissues for a shorter time and hence may impact on bacterial attachment. Alfa et al. (1999)[9] found average procedure times of 32 minutes for duodenoscopes and only 22 minutes for colonoscopes.

The objectives of this study were:

(1) to determine how quickly bacteria can attach to new Teflon tubing.

(2) to determine the extent of channel surface damage of clinically used endoscopes obtained from Australia

(3) to develop an in vitro assay to model the effect of repeated clinical use on endoscope surface integrity.

8.2.2 Material and Methods

8.2.2.1 Bacterial attachment to new Teflon tubing

Bacterial attachment to PTFE Teflon tubing was determined utilizing an in vitro flow system developed in the Vickery laboratory as a method to form reproducible biofilm on Teflon tubing for efficacy testing of detergents against biofilm[44].

The bacterial inoculum was prepared by removing a single, fresh colony of *E.coli* (Strain K12 from Reeves laboratory) from a horse blood agar plate and emulsifying it in 100 mL of TBS and incubating at 37 ºC for 7 hours. The absorbance of the resulting culture was diluted to give a reading of between 0.2 to 0.3 at a wavelength ʎ of 620 nm to give approximately 10^8 bacteria/mL. One milliliter of this bacterial culture was added to 99 mL of Tryptic Soy Broth (TSB) and acted as the inoculum for the bioreactor. The Teflon tubing was connected to a peristalic pump and media using sterile gloves and the media circulated at 75 mL/hour. Media and tube were kept in water bath at 37 ºC during experiment (Figure 25).

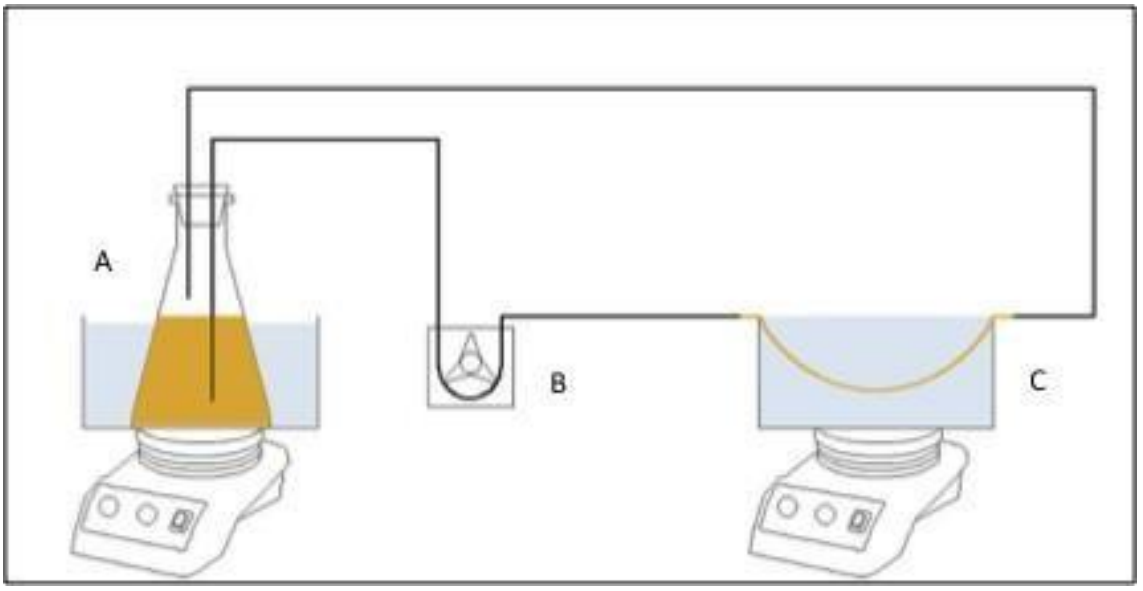

Figure 25. Schematic of apparatus used for bacterial attachment assay to Teflon tubing.

At set time points of 30 minutes, 1, 2, 4 and 6 hours, the pump was stopped and a pre-marked 25cm length of tube removed for analysis before reattaching remaining tubing aseptically and the pump re-started. The external surface of the removed tube was wiped serially with Matrix (Whiteley Corporation, North Sydney, Australia) marketed as a biofilm remover, 70% ethanol and sterile water. The 25m length of tube was then aseptically cut into 5 pieces of 5 cm each, to obtain 5 replicates for each time point.

Non-attached bacteria were removed from the samples by placing each 5 cm piece of tubing into 10 mL of PBS and gently inverting 2 times, the PBS was aspirated and the washing procedure repeated 3 times. Attached bacteria were harvested by aseptically cutting each segment into five one cm pieces and placed them all into same sterile tube with 5 mL of PBS. The samples were then subjected to sonication in an ultrasonic bath (Soniclean, JMR Australia) for ten minutes with a sweeping frequency of 42-47 kH at 20°C, followed by vortex for 1 minute and serial ten-fold dilutions from 10^{-1} to 10^{-3} for 30 min, 1, 2 and 4 hours and 10^{-1} to 10^{-5} for the 6 hour time point

samples. A 100 µL aliquot of every sample and dilution was spread onto HBA and incubated at 37 ºC for 24 hours before counting CFU on all plates containing between 30 and 300 colonies.

8.2.2.2 Surface profiling of channels removed from clinically used colonoscopes

Clinically used endoscope channels from a variety of brands were received from endoscope repair services in Australia and ten clinically used biopsy channels were subjected to surface profile analysis by contact profilometer. For comparing results, seven new endoscope biopsy channels were also assessed.

All biological material was removed from a 2 cm section of the channels by soaking in 5M sodium hydroxide overnight at room temperature, rinsing in distilled water, cut longitudinally, and dried utilising filtered nitrogen gas. The samples were then processed in the Alpha-Step 500 Surface Profiler (Tencor, Mountain View, California) which uses a stylus to scan the surface profile and calculates the arithmetic average deviation of the channel profile from the centre line or average roughness (Ra).

The surface profile was measured in two sequential areas of the channels chosen randomly and scanned by stylus with 200 µm length and 5 seconds speed each. An example of the read out obtained is shown in figure 26. The average of both roughness areas of each sample was calculated. A one-tailed t test was used to test the null hypothesis that surface roughness of biopsy channels is increased following clinical use.

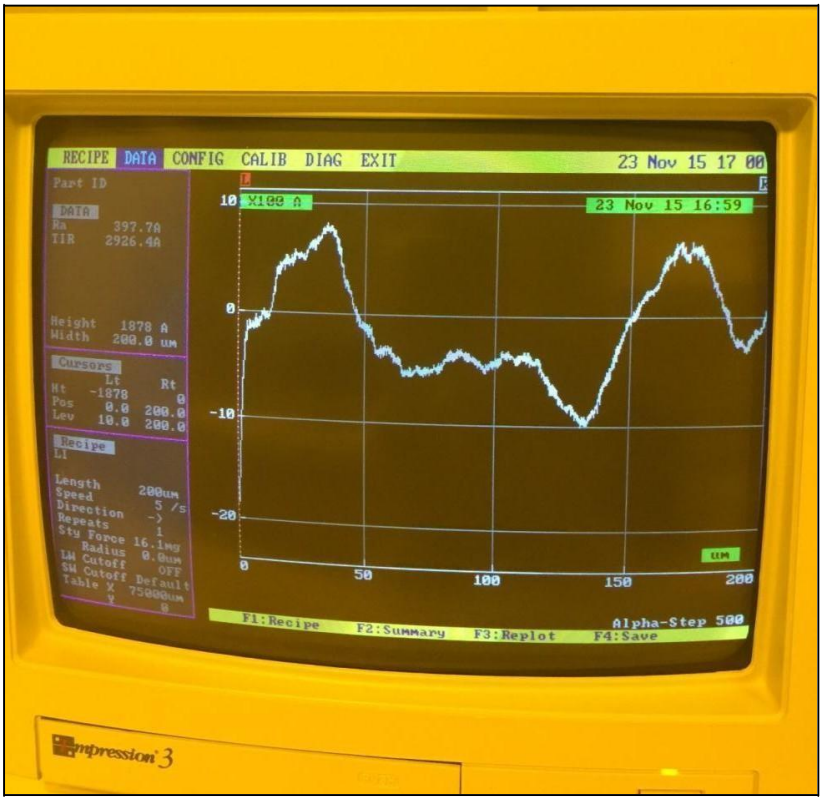

Figure 26. Illustration of endoscope channel surface profile data generated by Alpha-Step 500 Surface Profiler.

8.2.2.3 Development of an in vitro assay to model channel surface damage

We assumed that endoscope channel surface damage can frequently occur due to the passage of instruments, such as biopsy forceps, so an in vitro experiment was performed to simulate clinical use. The central portion of a 60 cm piece of PTFE Teflon tube was bent at an angle of between 90º to 120º and a flexible endoscope biopsy forceps with 2.8 mm of diameter was repeatedly passed through the Teflon tube as shown in figure 27. The number of passages of the biopsy forceps was fixed at 50, 100, 200, 500 and 1000 times.

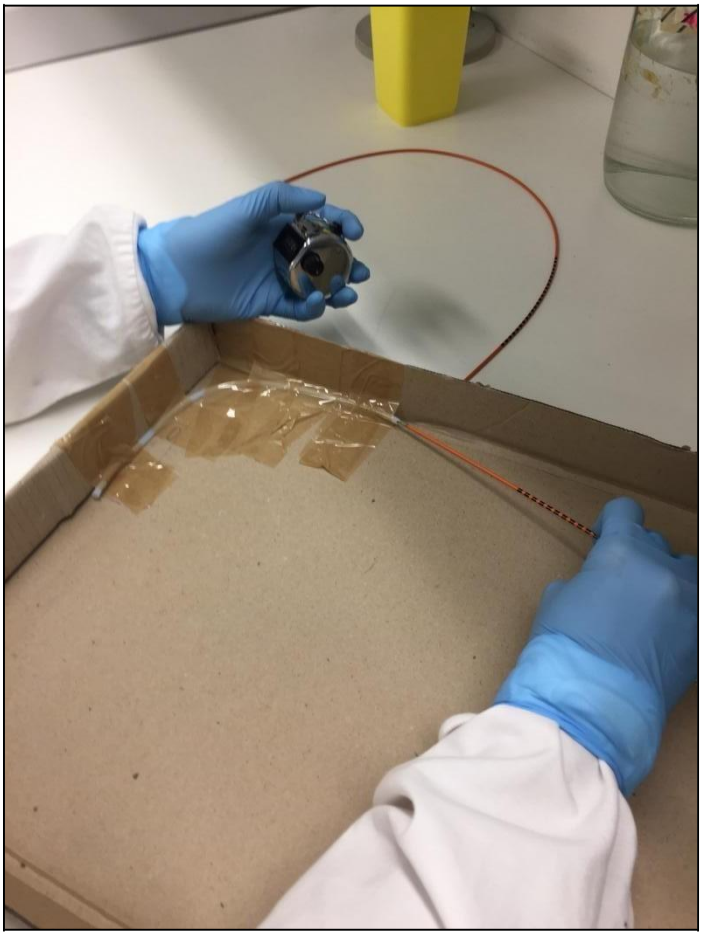

Figure 27. Illustration of flexible endoscope biopsy forceps passage through Teflon tube.

A 2 cm section of the tube was removed from the bent area of tube, (30 cm from the end of the tube) for microscopy analysis. Images of 10x10 µm (512x512 pixels) was acquired by Bruker MultiMode® 8 in scanasyst mode in air with scanasyst air probe (tip radius 2 nm, spring constant of 0.4 N/m). Five random images were taken of each sample for posterior second order plane fit for assessing roughness values of the samples. The average of roughness values of each sample was calculated using the nanoscope analysis software.

8.2.3 Results

Bacterial attachment to new Teflon tubing

The attachment of bacteria to the Teflon tubes was evident by 30 minutes (the earliest time point tested). By the 2 hour time point more than 10-fold bacteria had attached and by 3 hours 100-fold more bacteria had attached to the tubing when compared with the number attached at 30 minutes (Figure 28).

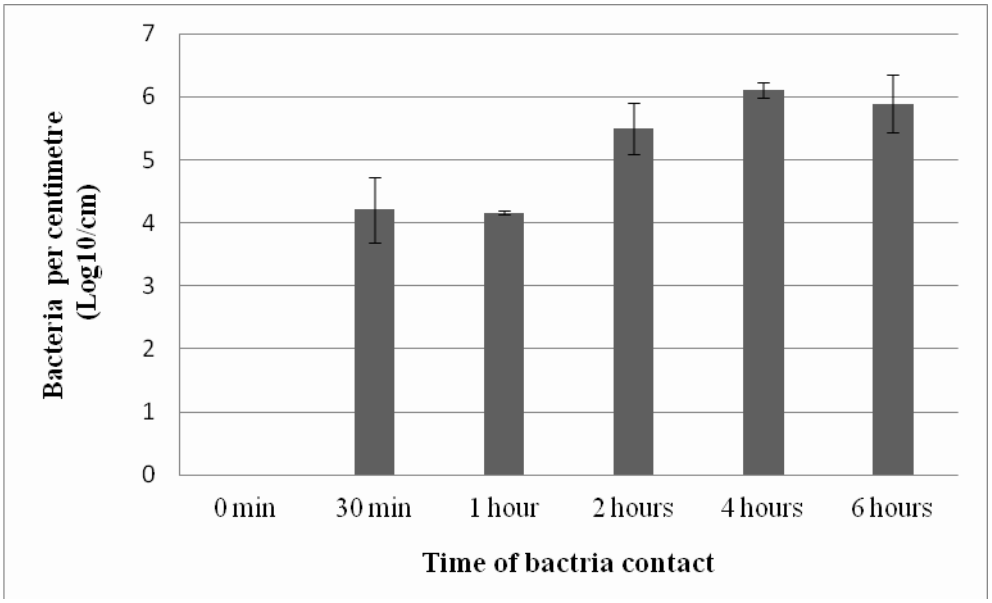

Figure 28. Viable bacteria attachment to Teflon tubes generated by in vitro model of flow contamination.

Surface profiling of channels removed from clinically used colonoscopes

Clinically used colonoscope biopsy channels (median=526.82 A) were significantly rougher than new colonoscope biopsy channels (median=357.8 A) (P=0.03) (Figure 29). This result indicates that used endoscopes biopsy channels have more

deviations in their surface profile when compared to a medium line, which demonstrates that the surface profile of endoscopes channels changes with the use of the device.

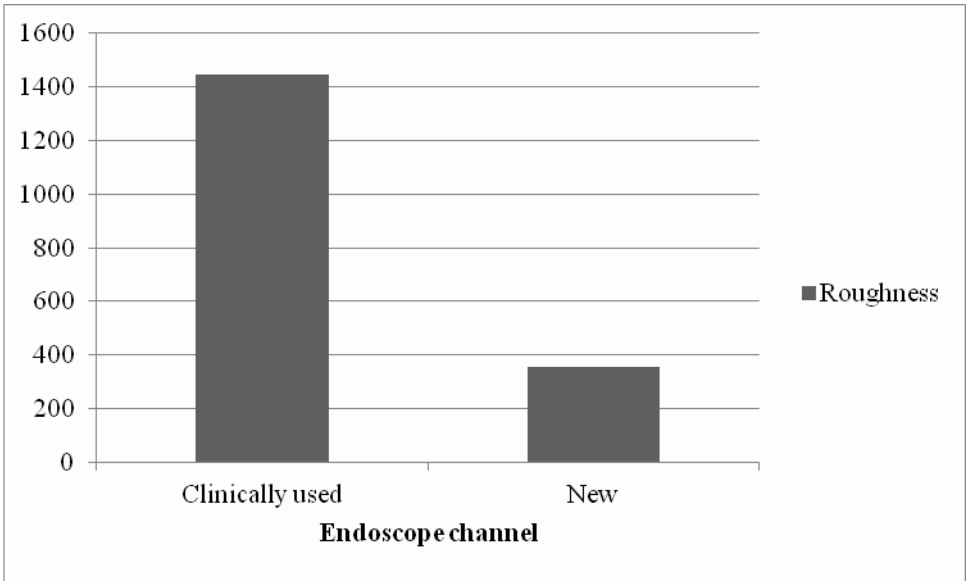

Figure 29. Comparison of roughness values (ångström) of clinically used and new endoscope biopsy channels tested by surface profiling (p=0.03).

Development of an in vitro assay to model channel surface damage

The average roughness values of damaged Teflon tubes as assessed by AFM is illustrated in figure 30. The number of passage of biopsy forceps trough Teflon tube seems to affect surface integrity after 200 times. AFM images of the control channels and channel surfaces following 20 and 100 times passage of biopsy forceps is shown in figure 31.

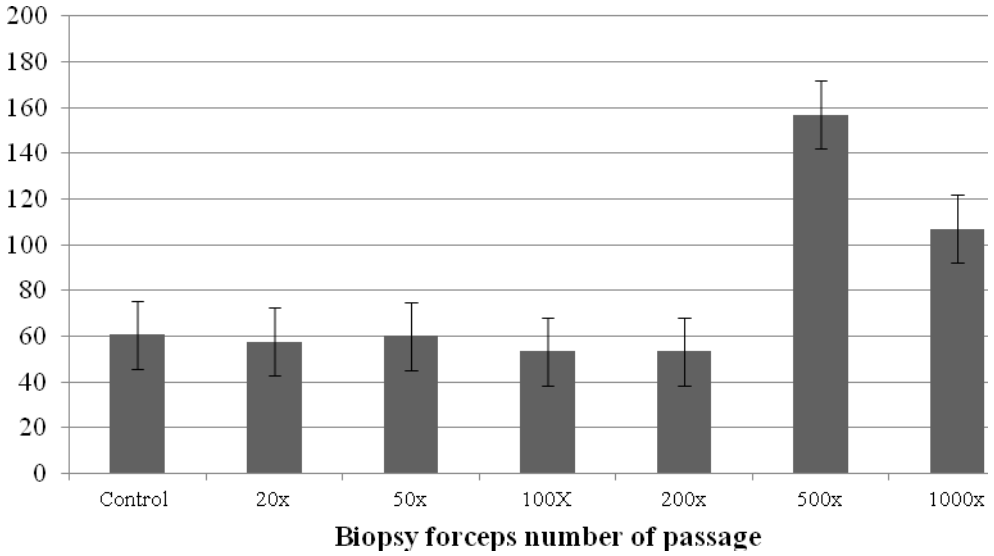

Figure 30. Comparison of Roughness values of Teflon tubes with different number of biopsy forceps passage analyzed by atomic force microscopy.

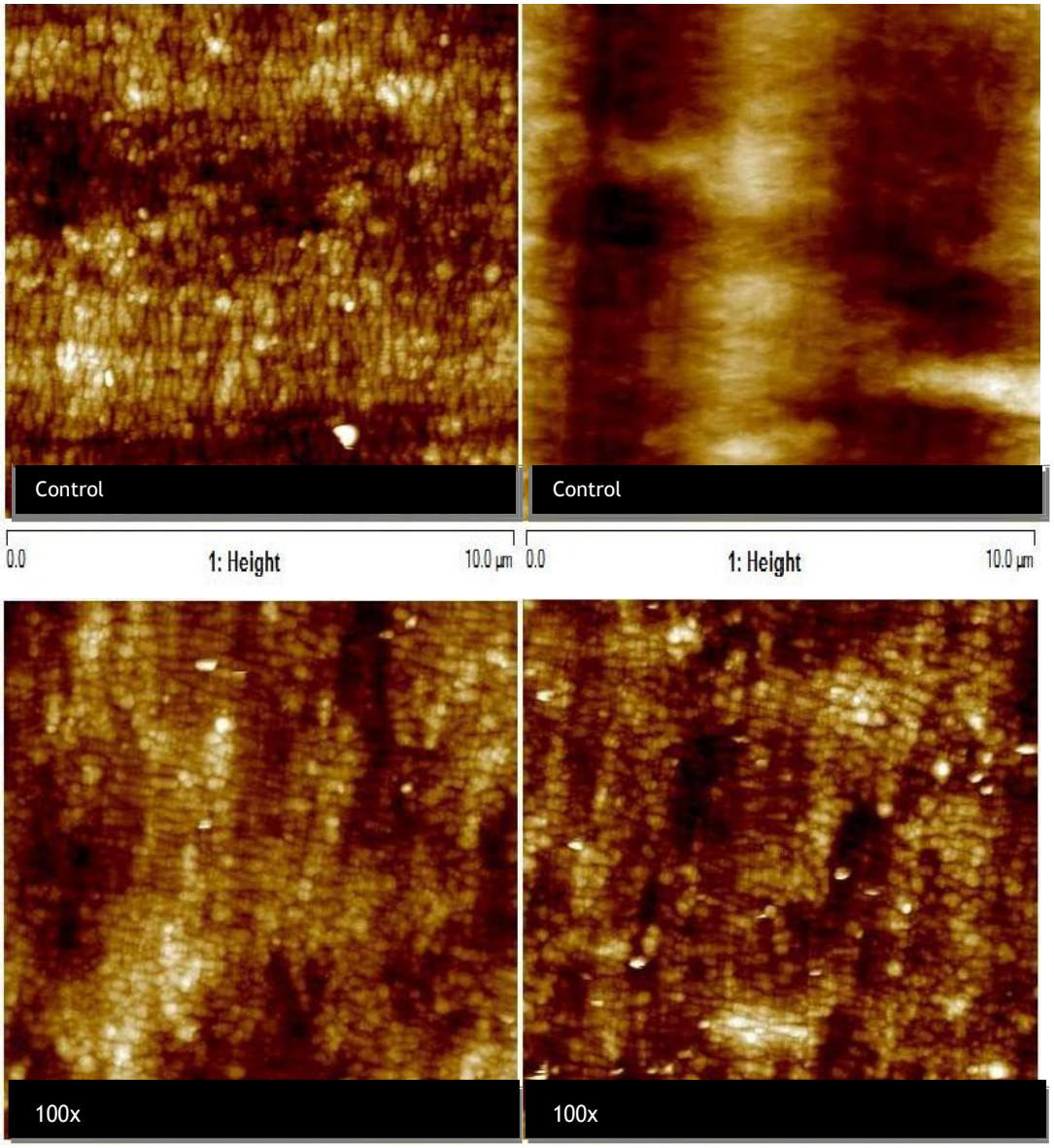

Figure 31. Micrograph of Teflon tube internal surface analyzed by atomic force microscopy.

Discussion

Endoscope reprocessing can be affected by a variety of factors. Problems with automatic endoscope reprocessors (AER) and failure to follow recommendations in guidelines are most frequently cited as the cause of endoscope reprocessing failure[82,83,103]. Additionally, improper maintenance of both endoscopes and AER can

also compromise endoscope reprocessing[81]. Unfortunately, endoscope reprocessing failures can be associated with the occurrence of patient infection and even outbreaks[11,24,56]. However, infection outbreaks linked to endoscopy has even occurred when guidelines are rigorously adhered to and no errors of endoscope reprocessing were found[23]. Continued endoscope contamination has even occurred following repeated decontamination by high-level disinfection[146].

Given the complex design of gastrointestinal endoscopes they can easily be damaged. Scanning electron microscopy has visually confirmed damage to endoscope channels and the presence of biofilm containing bacteria of various morphologies associated with that damage[18,19]. Endoscope damage has resulted in patient infection[10,112,147]. In this study, internal channels of clinically used endoscopes were found to be significantly rougher than new endoscope channels, which demonstrates that the surface profile of endoscopes channels changes with routine use. A recent evaluation of gastrointestinal endoscopes channels utilizing borescopes over a 2 months period, demonstrated that not only was there channel damage but that the channel irregularities changed with time[127].

In addition, increased roughness associated with channel damage provides a good habitat for bacteria and patients' soil to attach. Within dips and crevices the soil and bacteria are partially protected, which increases cleaning difficulty and facilitates the growth of biofilms. Rough surfaces have been shown to retain more bacteria when in the presence of test soil containing blood[148]. In this scenario, the presence of damage on endoscope surface may contribute to bacterial adherence.

In this study, surface roughness was evaluated using two different scales, angstrom and nanometers, and using two different methods. Neither method proved to

be ideal and better methods are needed so that roughness assessment can be conducted over a larger area of the endoscope channel, thus decreasing sampling error. A literature review on the use of surface roughness values for microbial studies found that different scales/methods but that traditional microscopy methods can´t distinguish soil from microbial retention on surface defects. The nanometer scale, such as provided by AFM examination, provides visualization of defects on the microbiological scale, but the area being examined is reduced[149]. In order, to try to overcome this issue, in this present study the surface sample was examined in five different randomly selected areas, however, the extent of the damage caused by biopsy forceps on Teflon tube exceeded the nanometer scale. Despite this we found that passing biopsy forceps through Teflon tubing 500 times increases surface roughness and therefore causes damage.

In clinical practice, monitoring endoscope damage can be a challenge given the complex structure while research methods involve destructive procedures for assessing endoscope internal surface. Currently, visual inspection and conducting a leak test are recommended as being indicative of endoscope defects[56,61,119]. However, the internal surface of channels can't be visually assessed except by using a borescope. Repeated positive microbial cultures from the one endoscope are also suggestive of endoscope damage[54], however, both culture and leak tests are not reliable methods for determining endoscope damage[31,54].

Even though endoscopes are often in contact with patient for a short period during endoscopy procedure, given the high numbers of bacteria resident in the gastrointestinal tract, gastrointestinal endoscopes are routinely contaminated with high microbial loads. In this study we have shown in an in vitro model that large numbers of bacteria attach to Teflon tubing in 30 minutes. This finding suggests that clinically, bacteria not only contaminate the internal channels of endoscopes but also could adhere to endoscopes' surface during endoscopy procedures, therefore, additional

recommendations regarding endoscope surface bacteria attachment prevention should be addressed. As bacterial adherence to surfaces can be facilitated by the deposition of organic material in medical devices, minimizing the time between clinical use of flexible endoscopes and reprocessing is fundamental for reducing contact time of bacteria with the device surface[41,141].

Indeed, the relationship between internal damage and endoscope contamination should be further addressed in order to improve professional society guidelines and contribute to better patient safety. Thus, the search for an appropriate method for assessing endoscope channel damage is still necessary, given that: (1) in this study, viable bacteria attached to Teflon tubing within 30 minutes of surface contact; (2) current endoscope reprocessing recommendations appear to be ineffective in preventing bacterial attachment and biofilm formation on endoscope channels surface; and (3) recommendations on endoscope maintenance and repair are still empirical in guidelines.

A limitation of this study is that only one species of bacteria was analyzed for their attachment to Teflon tubing and that additional species should be assessed. Suggested additional work, would be to analyze the effect of channel damage on bacterial attachment.

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CHAPTER 9. GENERAL DISCUSSION AND CONCLUSION

The continuing outbreaks of healthcare associated infection (HAI) with carbapenem resistant Enterobacteriaceae (CRE) surviving endoscope disinfection/sterilization procedures has focused not only the medical community, but regulators and industry on the difficulty in decontaminating endoscopes. Adequate decontamination of endoscopes plays a major role in preventing transmission of infections. Monitoring endoscope reprocessing allows detection of failures on the endoscope reprocessing stages, thus reinforcement or improved procedure or guidelines can be made to ensure adequate decontamination of endoscopes to prevent infection transmission and promoting patient safety.

In this study, a broad investigation of gastrointestinal endoscope reprocessing and its possible contributing factors for failure was performed in order to facilitate elucidating gaps on infection prevention. The scientific studies conducted in this thesis provide evidence in area of endoscope processing that could contribute to better patient safety. The difficulties on gastrointestinal endoscope reprocessing, contamination risk and outbreaks are a recognized challenge in science. Overall, the issue was assessed by analysis of microbiological, physicochemical and structural conditions of endoscopes and in both clinic and laboratorial settings.

The investigation on efficiency of cleaning procedure of gastrointestinal endoscopes after clinical use was performed by assessing endoscopes before and after endoscopy. The analysis of endoscope flush samples by ATP, culture and qPCR allowed reinforcement of previous findings that endoscope cleaning procedures on endoscope reprocessing guarantees a significant reduction on how dirty or how much protein was left on the scope ($p < 0.001$) as well has a significant reduction in the number of contaminating bacteria ($p = 0.03$). Even though it wasn´t the purpose of the

study to compare different methods, the use of these three different surveillance tests on the same cleaning process on each endoscope sample it was possible to confirm the cited reduction by comparing the results from each method.

The use of ATP for evaluating endoscope cleaning process has been well cited by researchers and guidelines and it´s implementation in clinical practice would provide a fast point of use result on how well the endoscope had been cleaned and how much biological soil remained on the endoscopes. The use of microbial culture for contamination surveillance is well established among endoscopy facilities and guidelines, however, it´s use on a regular basis from every endoscope would be unlikely, given the time required and costs involved with culture. However, the main difficulty is the delay, of up to 48 hr in getting the results, which means that the endoscopes need to be quarantined or that the scope is reused prior to obtaining the results. These same reasons also apply to qPCR test in regular clinical practice, although the delay in obtaining results is shorter. We conducted qPCR in this study as one of our objectives was to include at the analysis of contamination level not only culturable bacteria but also the unculturable and dead bacteria, reducing bias of method analysis results.

It had been suggested in the literature that residual disinfectant remaining in endoscope channels after reprocessing could affect surveillance culture results by killing the bacteria. We therefore, assessed microbial contamination on ready to use endoscopes by culturing by using regular culture media (non selective) without a neutralizer and by using disinfectant neutralizer media plates. Samples were obtained from 12 hrs post processing (the minimum time required by the Australian GESA guidelines). The results revealed that the use of a disinfectant neutralizer had no effect on bacterial counts if the samples were obtained after 12 hours from endoscope reprocessing. Therefore, additional studies on use of neutralizer immediately after

disinfection are necessary in order to confirm if residual disinfection present on endoscope channel would affect culture results by improving culture method sensitivity given that currently its largely used method for endoscope reprocessing surveillance.

Overall, the investigation of ready to use endoscopes demonstrated that the reprocessing wasn´t effective in all instances as bacterial contamination was detected, even though present in low numbers. These findings suggest that current endoscope reprocessing protocols should be improved for reliable results and patient safety.

The success of endoscope decontamination has been related to the possibility of biofilm attachment to endoscope surface. The presence of biofilm on endoscope internal channels has been well established in literature with the proximity of biofilm in area with obvious surface damage, however, the extent of this relationship is not known. Initially, by comparing surface roughness of clinically used and new endoscope channels by using a stylus probe, it was possible to conclude that used endoscopes are significantly rougher than never used ones. This method involves scanning the endoscope surface by the contact of the probe with the surface throughout a determined length, so reporting it´s irregularities. We then investigated the relationship between biopsy forceps passage and endoscope channel surface. We found that passing biopsy forceps through Teflon tubing 500 times, increases surface roughness and therefore causes damage. This finding suggests a benchmark for using the endoscope biopsy channel before it should be replaced, so as to reduce contact of microorganisms with rougher and more damaged surface area.

In order to compare different social and economic realities, this study analyzed endoscope biopsy channels from endoscopy services in Australia and Brazil. The results demonstrated that even though the majority of the endoscope channels analyzed showed low bacterial load by qPCR analysis, endoscope channels were frequently damaged and soil and/or biofilm was visually confirmed on all channels. Biofilm was also associated

with damaged areas but in some endoscopes covered the entire surface. This finding also suggests that qPCR isn't a sensitive method for detecting biofilm on endoscope channels, which can be related to difficulties in collecting the samples, the difficulty in extracting the DNA due to DNA damage by disinfectants and the possible presence of PCR inactivators.

Even though endoscope channels from both countries presented similar amount of contaminating soil and biofilm presence, the samples from Brazil were also contaminated with red blood cells and neutrophils cells. Thus, the detection of extensive soil presence along with biofilm formation in biopsy channels despite decreased bacterial load of endoscopes from sites with different regulatory and structural realities suggests that the presence of biofilms contributes to the failure of reprocessing of endoscopes even under optimal conditions of infrastructure and with sufficient resources to ensure that guideline recommendations are observed.

These findings support previous findings on the necessity that endoscope reprocessing needs to be improved. One important aspect of this reality is understanding the relation between surface damage and biofilm formation and more work needs to be done in this area. Other important aspect of endoscope reprocessing is reducing the time between the endoscopy procedure and processing. This will reduce the contact time between the microorganisms and biologic soil with the endoscope surface and so preventing or, more likely, reducing biofilm formation. However, how fast can bacteria attach to an endoscope channel was uncertain. Thus, this study demonstrated, on an in vitro model of Teflon tube contamination by controlled flow, that bacteria attached to the tubes internal surface within 30 minutes. This result suggests that bacteria can rapidly attach to endoscope channels surface which emphasizes endoscope reprocessing guidelines recommendations of minimizing the time to reprocessing following clinical use.

Overall, the findings of biofilm presence on clinically used endoscope channels in addition to bacterial attachment on Teflon tubing even with a low duration of contact reinforces that the paradigms about endoscope reprocessing must be constantly updated in terms of the paradigm of preventing biofilm formation and/or removal and it is not only the planktonic cells that need to be removed. The results presented indicate that most endoscope channels have bacteria attached to them, which can promote patient infection, despite endoscope reprocessing in conformance with current guidelines.

The recent endoscopy related outbreaks involving antibiotic resistant bacteria has raised the public interest in the safety of endoscopes and their reprocessing worldwide. This study is not limited to producing microbiological results, but rather reflects on reprocessing conditions, as well as "rethinking the reprocessing management processes", in order to standardize behaviors and eliminate risks for patients and healthcare professionals.

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