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# Microbiological surveillance of endoscopes and implications for current reprocessing procedures adopted by an Italian teaching hospital

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*Key words:* Semi-critical devices, endoscopy related infections, endoscope reprocessing, microbiological monitoring, quality assurance in endoscopy

*Parole chiave:* Dispositivi semi-critici, infezioni correlate alle pratiche endoscopiche, reprocessing degli endoscopi, monitoraggio microbiologico, qualità degli endoscopi

## Abstract

**Background.** Hospital acquired infections have been associated with the contamination of flexible endoscopes caused by a failure of the reprocessing procedure. Microbiological surveillance of endoscope reprocessing is valuable for assessing contamination by pathogens. The aim of this study is to evaluate microbiological contamination of endoscopes after reprocessing, and the involvement of reprocessing procedures adopted in endoscopy units of an Italian teaching-hospital.

**Methods.** The study was carried out, on several dates in 2014, in 11 endoscopic operation units equipped with 100 endoscopes (18 bronchoscopes, 41 gastroduodenoscopes, 29 colonoscopes, 12 laryngoscopes) and 9 Automated Endoscope Reprocessors. Presence/absence of common pathogens and indicator micro-organisms (including multi-drug resistant bacteria) and Total Microbiological Count (TMC) were obtained from the biopsy channels of endoscopes after reprocessing, from final rinse water of automated endoscope reprocessors and from tap water applying standard microbiological culture methods. Following the European Guidelines for quality assurance in reprocessing, the post-reprocessing criteria were “absence of indicator micro-organisms and absence of TMC in samples obtained from endoscopes’ channels”.

**Results.** A total of 180 samples were collected (143 endoscopes, 25 Automated Endoscope Reprocessors and 12 water supply). Compliance to the European Guidelines was achieved for 112 out of the 180 (62.2%) samples analyzed. Presence of indicator micro-organisms (mainly *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and other Gram-negative non-fermenting bacteria) was found in 51 out of 143 endoscopes (35.7%). Multi-drug resistant bacteria were also found. Presence of pathogen micro-organisms was statistically associated with the increase of TMC level, but not with time after reprocessing.

**Conclusions.** The study provides information about the microbiological quality of endoscope reprocessing procedures adopted by different endoscopic operation units. The high prevalence of contaminated endoscopes provides evidence of the need to improve the quality of reprocessing.

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## Introduction

Endoscopes are considered semi-critical items from the *Spaulding* Classification (1) for risk of infection, because they come in contact with mucous membranes or non-intact skin (2). Therefore, these devices must be kept free of pathogens. However, endoscopy practice can produce heavy contamination. Some studies have shown that the internal channel of gastrointestinal (GI) endoscopes after use may contain from  $10^7$  to  $10^{10}$  Colony Forming Unit (CFU)/mL and bronchoscopes may contain about  $6.4 \times 10^4$  CFU/mL (3-5). In order to decontaminate these devices and to make their use safe for patients and staff, the endoscopes must be submitted to reprocessing (6).

The reprocessing practices consist of high-level disinfection (HLD) of semi-critical devices, a multi-step process that is expected to inactivate most pathogenic bacteria, viruses, and fungi but probably not certain types of micro-organisms including bacterial spores (7). However, the complex design of endoscopes, with narrow lumens and internal and interconnecting channels, can favor the formation of biofilm and cause failures in reprocessing, contributing to endoscope-related cases and even outbreaks (8-9).

Failure of the decontamination process, at any stage, may cause healthcare-acquired infections (HCAIs) (10-16) caused by all types of endoscopes and, although complications are rare, more HCAIs and pseudo-infection outbreaks have been linked to contaminated endoscopes than to any other re-usable medical device (11). Particularly problematic are outbreaks caused by a Multi-Drug Resistant Organisms (MDRO) linked to endoscopy and recently documented in the literature (8, 10, 17).

However, the number of endoscopy related infections and cross-contaminations, to date, remain underestimated and underdiagnosed, because of inadequate or missing post-

intervention surveillance, or absence of clinical symptoms (18).

Reprocessing Guidelines for flexible endoscopes have been produced by various international and national organizations, but this study gives priority to the official European and Italian Guidelines (19, 20) and, in particular, to the following steps: first, *pre-cleaning* immediately after use; second, *manual cleaning* that includes brushing of inner channels; third, the *HLD process*. This step can be carried out either manually, or automatically by an Automated Endoscope Reprocessor (AER); fourth, *rinsing*, preferably using sterile water for the final rinse and *drying*, better with alcohol (21). The final step is *storage* under sterile conditions, preferably in dedicated cabinets, in accordance with the UNI EN 16442:2015 Guideline (22). All these steps are crucial for quality assurance in reprocessing and in order to prevent the formation of biofilm and the survival of pathogens (13).

To assess the effectiveness of reprocessing procedures in endoscopy services, microbiological surveillance by culture-based methods has been proposed as an easy to use approach (9). However, this approach is a controversial issue of many Guidelines due to the long response, time and cost. For example, the Guideline of the American Society for Gastrointestinal Endoscopes (ASGE) (23) does not endorse microbiological routine tests, whereas the Guideline of the European Society of Gastrointestinal Endoscopy (ESGE), together with the European Society of Gastroenterology and Endoscopy Nurses and Associates (ESGENA), in 2007, strongly recommend microbiological surveillance for evaluating the quality of manual or automated reprocessing procedures used in endoscopy (24).

These Guidelines (24) clearly recommend the frequency (every 3 months) and the method for microbiological testing (culture-based methods). The Guidelines

also indicate, as criteria for acceptability to assess the quality of reprocessing, the absence of growth of indicator micro-organisms (Enterobacteriaceae, *Pseudomonas aeruginosa* and other Gram-negative non fermenting bacteria, *Staphylococcus aureus*, atypical mycobacteria and *Legionella* species), and Total Microbiological Count (TMC) and the corrective measures to prevent cross-infections. In Italy, the National Association of Endoscopy Techniques Operators and the National Association of Gastroenterology and Associated Nurses (ANOTE-ANIGEA) have implemented the European Authorities Guidelines and, in 2011, has published the National Guidelines (20), reinforcing the need to perform microbiological surveillance for quality control in endoscopy.

Recently, a rapid and economic method, Adenosine triphosphate (ATP) measurement, has been proposed by the Centers for Disease Control and Prevention (CDC) in the Interim Protocol (25); also a DNA-based method (26) has been suggested as a routine test to evaluate the quality of endoscope reprocessing.

However, a recent Italian survey shows that only 18.2% of endoscopy units had a routine microbiological test within a quality control strategy (27). This highlights the difficulty in implementing a monitoring plan in these operational units, and the need to improve microbiological information about the quality of reprocessing procedures in Italian endoscopy services for the risk assessment of endoscope practices.

The present study aims to explore the microbiological quality of reprocessing procedures through the evaluation of contamination levels of endoscopes post-reprocessing, compared to ESGE-ESGENA microbiological criteria (24). The study was performed in the endoscopy operation units of an Italian teaching-hospital where

a complete microbiological surveillance has not yet been implemented. A secondary objective of the study was to identify an indicator of reprocessing quality, including TMC Levels at 30°C and Time of storage, as easy, economic and rapid tools in surveillance programs.

## Materials and Methods

The study was entrusted to the Hygiene Unit by the Infection Control Committee of the teaching hospital after a pseudo-outbreak of *Mycobacterium gordonae* in a bronchoscopy unit in 2013 (16); a one year survey with microbiological tests was planned to evaluate the quality of reprocessing procedures used in the Endoscopy Services of the hospital.

At the time of survey, the hospital had 11 Endoscopy Operating Units (EOUs), where approximately 1,100 endoscopy procedures/month were performed employing a total of 100 endoscopes (the majority, 87, were produced by Olympus Optical Co. Ltd, followed by 5 Asahi Optical Co Ltd, Medical I and other brands), divided in 70 GI endoscopes - of which 41 gastroscopes and 29 colonoscopes - 18 bronchoscopes, 12 faringo-laryngoscopes and 9 AER (Olympus ETD 3 and MINI ETD 2 - Olympus Optical Co. Ltd) (Table 1).

### Reprocessing Procedure

At the time of survey, between January 2014 and January 2015, the hospital had not established standard procedures for reprocessing, and each EOU had adopted its own reference protocol. A simple questionnaire was developed to collect information about the number of endoscopes, the number of monthly endoscopy practices, and information concerning the protocols for endoscope reprocessing. The data were collected by interviewing the staff of the EOUs and the results are summarized in Table 1.

Table 1 - Number and types of endoscopes used and process features followed by Endoscopy Operation Units. Details are available in the text.

Endoscopy Operation Units	GI endoscopes No.	BFL endoscopes No.	AER No.	Type of reprocessing	Practice/month (mean value)	Affixed reprocessing protocol	Product for pre-cleaning step	Rinsing step	Drying step
EOU_1	26	-	3	Automatic	450	Yes	Tap Water	Demineralized water	Automatic
EOU_2	16	-	2	Automatic	380	No	Tap Water	Demineralized water	Automatic
EOU_3	7	6	1	Automatic	24	Yes	Water and disinfectant	Demineralized water	Automatic
EOU_4	12	7	1	Automatic	120	No	Tap Water	Demineralized water	Automatic
EOU_5	-	5	1	Automatic	80	No	Proteolytic solution	Sterile water	Automatic
EOU_6	5	-	-	Manual	8	Yes	Tap Water	Sterile water	with alcohol
EOU_7	-	6	1	Automatic	24	No	Proteolytic solution	Demineralized water	Automatic
EOU_8	1	-	-	Manual	NA	No	NA	NA	NA
EOU_9	3	-	-	Manual	NA	No	NA	NA	NA
EOU_10	-	2	-	Manual	NA	No	NA	NA	NA
EOU_11	-	4	-	Manual	NA	Yes	Proteolytic solution	Sterile water	Sheet
TOT.	70	30	9	-	1086	-	-	-	-

Legend: GI: Gastro-Intestinal endoscope; BFL: Broncho-Faryngo-laryngoscope; AER: Automated Endoscope Repressor; NA: Not Available.

### Sample collection

Microbiological data were collected during a one-year monitoring plan that involved all the endoscopes in use, all the AER, cleaning and rinsing water; every item has been sampled at least once.

Samples were collected under aseptic conditions according to the European Guideline (24) as follows: the suction/biopsy channel of each endoscope was sampled by flushing 20 mL of sterile saline (0.9%) with a sterile syringe connected to the entry port of the channel. Then, the flux liquid was collected from the distal end of endoscopes in a sterile container. Faringo-laryngoscopes were not tested by flushing and only outer surfaces were sampled using sterile swabs moistened with sterile saline.

Final rinse waters from the AER (100 mL), using a sterile syringe, were collected in a sterile bottle with screw cap.

Tap water (500 mL) used for *pre-cleaning* and for the water supply of the washer disinfectant, were also collected and analyzed according to the Italian Decree for drinking water (28) and for the detection of pathogens as reported in the Guideline (24).

To evaluate whether the time of storage is important for microbiological quality, endoscopes were sampled after different times of storage, immediately after reprocessing inside AER (<1 h), in storage within 72 h from reprocessing and in storage for more than 72 h.

Once the samples had been collected and labeled, they were immediately taken to the bacteriology laboratory and processed with culture-based methods under a laminar flow hood.

### Samples culture

All samples were tested for the detection of indicator micro-organisms as recommended by the Guideline (24) as follows: 5 mL of the sample were inoculated in the same volume of double-concentrated Tryptic Soy Broth (Oxoid, Cambridge, UK) and incubated at

36±1°C for 48 h and the bacterial growth, if any, streaked out on selective agar plates: Violet Red Bile Glucose agar (VRBGA) for Enterobacteriaceae (Oxoid, Cambridge, UK); Brilliance Salmonella Agar Base with supplement (SR0194E) for *Salmonella* spp (Oxoid, Cambridge, UK); Pseudomonas Cetrimide agar (PSELL) for *P. aeruginosa* (Oxoid, Cambridge, UK); Mannitol Salt agar (MSA) for *S. aureus* (Oxoid, Cambridge, UK); and incubated at 37°C for 48 h.

All the colonies grown on the selective media were identified using the VITEK 2 Compact System (BioMérieux, France), according to the manufacturer's instructions. Anti-microbial susceptibility assay was performed using the VITEK 2 and the results were interpreted according to the *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) 2013-2014.

For the detection of *Legionella* spp, the samples were plated on Buffered Charcoal Yeast Extract Agar (BCYE) supplemented with L-cysteine (SR0110) and MWY selective supplement (SR0118) (Oxoid, Cambridge, UK) and incubated at 36±1°C in a humidified environment at 2.5% CO<sub>2</sub> for 10 days.

For the detection of mycobacteria, a decontamination phase used equal volume 4% of NaOH with 0.5% N-acetyl-L-cysteine and subsequent inoculation into mycobacteria growth indicator tube (MGIT) liquid medium vials (MGIT 960, Becton and Dickinson, Maryland, USA) and Löwestain-Jensen slants (Becton and Dickinson, Maryland, USA) and incubated at 36° ±1°C for mycobacteria growth: the observation time was six weeks.

The liquid samples, collected by flushing endoscope channels and water samples from tap and final rinse water, were also tested for TMC as recommended by the Guideline (25), as follows: 1 mL of the sample was inoculated onto Tryptic Soy Agar (TSA) (Oxoid, Cambridge, UK) in duplicate. 10 mL of the remaining liquid sample was filtered

through a 0.45 µm pore size cellulose esters membrane (Millipore, Billerica, MA) using a KNF Laborport pump (KNF Neuberger GmbH) and the membrane placed on TSA medium and incubated in aerobiosis at 30°C for 48 h.

The results were interpreted according to the Guideline (24) which indicates the absence of growth of the indicator micro-organisms from all the samples and for the TMC levels, the maximum total count from endoscopes channels, and <100 CFU/mL for water samples.

Moreover, to evaluate if TMC is inexpensive for verifying the presence of pathogens that represent a risk of endoscopy-related infection, TMC levels were divided in three categories: absence (<1 CFU/mL) compliant to the Guideline; between 1-20 CFU/mL and >20 CFU/mL.

#### Statistical analysis

Multivariate logistic regression was used to model the effect of TMC and time of storage on the risk of detection of an indicator micro-organism.

## Results

Eleven EOUs located in the multi-building teaching-hospital have been investigated to test the quality of reprocessing procedures. In all the EOUs, reprocessing of endoscopes was preceded by pre-cleaning, using tap water in 4 (36%) cases, proteolytic solution in 3 (27%) cases, water and disinfectant in 1 (9%) case and in 3 (27%) cases this information was not available.

Automated reprocessing with AER consists of: a leak test, a pre-cleaning and cleaning phase with unsterile water at 40°C, a disinfection phase with peracetic acid 0.1% at 35°C (contact time, 10 min), a terminal *rinsing* with unsterile water, and a *drying* through air flow, was performed in 6 (54%) EOUs. Five (45%) EOUs

performed manual reprocessing and only in 2 of these (40%) EOUs, performing manual reprocessing, information about reprocessing was registered and available (Table 1). After reprocessing, all EOUs stored endoscopes in a closed dedicated cabinet with a traceability ticket reporting the reprocessing date.

A total of 180 samples, of which 143 endoscopes, 25 internal surfaces AER and 12 water supplies, were collected (Table 2).

Compliance to ESGE-ESGENA criteria (24) for the absence of indicator micro-organisms was achieved in 112 out of 180 (62.2%) samples analyzed, of which 92 were from endoscopes.

Culture-positive samples for indicator micro-organisms were found in 68 (37.8%) cases, the most frequent were Enterobacteriaceae in 39 out of 180 (21.7%) cases, of which 14 *K. pneumoniae* (1 MDR), 18 *E. coli* (2 MDR), 3 *Enterobacter cloacae* and 4 *Citrobacter freundii*, followed by non-fermenting Gram-negative bacteria in 27 (15.0 %) cases (14 *P. aeruginosa* MDR and 1 *A. baumannii* MDR), and *S. aureus* detected in 2 cases (1.1%). No samples were positive for *Salmonella* spp, *Legionella* spp and atypical mycobacteria.

The post-reprocessed endoscopes had indicator micro-organisms present in 51 out of 143 (35.7%) samples analyzed, with differences according to the type of endoscope. The most frequently contaminated were GI endoscopes in 47 out of 102 (46.1%) samples (gastrosopes 28 out of 57, 49.1%, followed by colonoscopes 19 out of 45, 42.2%) and to a lesser extent broncho – laryngoscopes in 4 out of 41 (9.8%) cases (Table 2). The most frequent indicator micro-organisms detected in endoscopes post-reprocessing were Enterobacteriaceae, in 34 out of 143 (23.8%), followed by non-fermenting Gram-negative bacteria (including MDR *P. aeruginosa*) in 16 (11.2%), and only one reprocessed broncho-scope was positive for *S. aureus*.

Table 2 - Prevalence of indicator micro-organisms isolated from endoscopes post-reprocessing, from AER and tap water in endoscopy services and Non-compliant rates according to the ESGE-ESGENA Guideline (2007) criteria.

Indicators micro-organisms	BFL-endoscopes	Gastro-duodenoscopes	Coloscopes	Final rinse water from AER <sup>(*)</sup>	Tap Water	Total No.
Tot. No.	41	57	45	25	12	180
<i>Klebsiella pneumoniae</i>	0	7	5(1*)	1	1	14(1*)
<i>Escherichia coli</i>	0	10 (1*)	7 (1*)	1	0	18(2*)
<i>Salmonella</i> spp	0	0	0	0	0	0
Other enterobacteriaceae	0	2	3	2	0	7
<i>Pseudomonas aeruginosa</i>	1*	6*	1*	6*	0	14(14*)
Other Gram-negative nonfermentant	2 (1*)	3	3	5	0	13(1*)
<i>Staphylococcus aureus</i>	1	0	0	0	1	2
<i>Legionella</i> spp	0	0	0	0	0	0
Atypical mycobacteria	0	0	0	0	0	0
Total positive culture No.(%)	4 (9.8)	28 (49.1)	19 (38.8)	15(60.0)	2 (16.7)	68 (37,8)

\* Number of MDR micro-organisms; BFL: Broncho-Faryngo-laryngoscope <sup>(\*)</sup>Automated Endoscope Reprocessor

The outcome of AER showed the presence of indicator micro-organisms in 15 out of 25 (60%) samples (Table 2).

All water supply samples satisfied drinking water standards, (except for free chlorine level, because the water is continuously hyperchlorinated between 0.5 and 1 ppm free chlorine). However one sample was non-compliant for ESGE-ESGENA criteria (24), for presence of *K. pneumoniae* and for *S. aureus* (Table 2).

Also TMC was evaluated and compared to the recommended levels reported in the ESGE-ESGENA Guideline.

The TMC level exceeded the limits for the quality assurance of endoscopes post-reprocessing in 85 out of 130 (65.4%) inner channels analyzed (Table 3); the outer surfaces of the 13 faringo-laryngoscopes sampled were only tested for indicator micro-organisms. Of the 130 endoscopes tested only 45 (34.6%) had TMC levels <1 CFU/mL, in 36 (27.7%) the TMC level was 1-20 CFU/mL, and in 49 (37.7%) it was >20 CFU/mL (Table 3).

The time since the endoscopes were reprocessed was specified in 123 cases and ranged from <1 h to 40 days, with 91.1 % of the samples taken within 72 h of storage (Table 3).

Multivariate analysis showed that the odds of the detection of an indicator micro-organism is strongly related to TMC level,

Table 3 - Multivariate odds ratios (OR) and 95% confidence intervals (CI) for the risk of detection of indicator micro-organisms in relation to Total Microbiological Count (TMC) level and time from reprocessing.

Variables	Samples No (%)	OR
TMC <1	45 (34.6)	Ref
TMC 1-20	36 (27.7)	5.38 (1.02-28.32)*
TMC >20	49 (37.7)	23.39 (4.78-114.5)**
Time <1h	47 (38.3)	Ref
Time 1-72h	65 (52.8)	0.47 (0.17-1.29)
Time >72h	11 (8.9)	0.61 (0.01-5.10)

Legend: TMC: Total Microbiological Count; Time: Time of storage; \* p<0.05; \*\* p<0.01

showing a 5.38 and 23.39 times increased risk for TMC levels between 1 and 20 CFU/mL and >20 CFU/mL, respectively, whilst the time of storage does not have a significant effect (Table 3).

## Discussion

Flexible endoscopes are extremely sophisticated medical devices and widely used in numerous diagnostic procedures (29). The complexity of these devices creates considerable challenges for the reprocessing, which takes a lot of time, needs great diligence and qualified staff and any deviation from the reprocessing protocols could lead to failure of decontamination, increasing the risk of cross-infections (8).

This study has provided useful information on the quality of the reprocessing procedures adopted in the endoscopy services of an Italian Teaching Hospital which had not implemented a monitoring plan before.

The non-compliance rate of endoscopes after reprocessing was mainly due to exceeding TMC levels (65.4%), followed by presence of *Enterobacteriaceae* (23.8%) and non-fermenting *Gram*-negative bacteria *P. aeruginosa* (11.2%).

Our results show a relatively low rate of compliant outcomes compared to other studies. For example, Saviuc *et al.* (30) found 86% compliant GI-endoscopes in accordance to French Guideline (30) while our results report 54% of compliant GI-endoscope.

Another study shows 89% compliance rate of duodenoscopes tested after reprocessing according to the Guideline of the Viennese District Health Authorities (31).

One more study reported only 13.6% of positive samples obtained from internal channels of GI endoscopes (32), whilst we have found that the GI endoscopes showed the presence of indicator organisms in 46.1% of the samples. The same study (32) shows

that culture positivity obtained from AER was 1.7%, whilst our data show that 60% of final rinse water from AER was contaminated. However, the different Guidelines adopted in these studies, that differ for sampling method, culture protocols, interpretation thresholds and parameters analyzed, make a real comparison of results impossible. In fact, as shown by Cattoir *et al.* (33), just the sampling methods significantly influence the recovery rate of micro-organisms and thus the interpretation of results.

In any case, our study found a poor quality of reprocessing endoscopes compared with other studies, and this may be due to the confusing situation caused by these being large, small and very small EOUs with different kinds of reprocessing protocols where information may be lacking. Greater difficulties arose in the smaller units, where manual reprocessing is used and treatment registration does not occur. Even in the largest units, the high use of GI endoscopes (on average 415/month) and the limited number of endoscopes, compared to Saviuc *et al.* (30), may affect the high rate of non-compliant outcomes. This may cause insufficient pre-cleaning and/or inappropriate brushing, poor maintenance of equipment, and carelessness in all phases of the reprocessing, especially when the service is overloaded (29).

Carelessness in the first steps of reprocessing is confirmed by the microbiological results, because the high prevalence of *Enterobacteriaceae* from biopsy channels implies insufficient cleaning and/or disinfection procedures such as skipping brushing as indicated by the European (19) and Italian (20) Guideline. Likewise, the high number of samples positive for *P. aeruginosa* and other *Gram*-negative non-fermenting bacteria, in particular from AER, may indicate poor maintenance of washer disinfectors, contamination of final rinse water or insufficient drying of endoscopes before storage. This implies that the water

supply systems used in reprocessing should be investigated.

It is important to consider that MDROs, in particular *Enterobacteriaceae* and *P. aeruginosa*, are responsible for several outbreaks related to endoscopy use in the last few years (10), and our data highlight the need to improve the whole reprocessing cycle to prevent post-endoscopy infections.

These results have led to corrective actions by the health management such as reorganization of EOUs (centralization of the GI endoscopy service) and a renovation of reprocessing procedures based on updated Guidelines. However, to date, no microbiological study has confirmed the effectiveness of this approach. The microbiological protocol to test the quality of reprocessing procedures in endoscopy was submitted by the Hygiene unit to the health management evaluation.

It is important to emphasize that during the survey, no cases of infections or outbreaks related to use of endoscopes were registered.

Our study shows that routine microbiological surveillance of the endoscopy procedures is important for the quality assurance of the reprocessing of endoscopes and provides information about any deviations, errors and failures of the reprocessing cycle. To date, microbiological surveillance is reported in the scientific literature (8, 9, 34) and several Guidelines (7, 19, 20, 35) to be a component of a prevention strategy in endoscopy. U.S. Guidelines do not endorse routine culturing (23), however, after recent outbreaks of MDRO related to endoscopic practices, regular microbiological monitoring to assess contamination of endoscopes after reprocessing has been introduced (25). A systematic review shows that it is possible to prevent more than 91% of endoscopy-related infections if the quality control system is improved and implemented (11).

Despite the evidence-based recommendation to perform microbiological surveillance in endoscopy, in particular for the prevention of MDRO infections (8), there remain impediments (27), such as the cost (36) and the long response time for the detection of some pathogens. Our results may suggest that using only the TMC levels could be an easy, rapid, economic routine quality control test, and this could lead to increased controls after reprocessing of endoscopes, but larger studies are needed for confirmation. Also an alternative method, such as ATP Bioluminescence Assay (ATPmetry) is even faster (<1 minute) and easy to use for monitoring the reprocessing of endoscopes. However, some studies show that ATP measurement cannot be used as an alternative to microbiological tests for monitoring the quality of reprocessing (33, 37). At the same time, DNA-based techniques, in particular Real-Time PCR, are a rapid method (within 3-4-hours, including both DNA extraction and amplification) and some simplified protocols represent a promising approach to reprocessing surveillance (26). However, this approach needs further studies for validation and implementation and, to date, culture based-methods remain the only reference methods reported by the international Guidelines.

Our results found no statistically significant association between longer storage times and the risk of detection of pathogens. It should be noted that the number of samples stored >72h was small and the statistical power of our study might be low. However, this result is consistent with other studies on the safe storage time after reprocessing of endoscopes (38, 39). This result can be considered in the ongoing debate about the real need to reprocess endoscopes after 72 h of storage, as shown by the Italian Guideline (20).

Although our study provided strong evidence on the microbiological quality of reprocessing of endoscopes, it has some

limitations. In the study, we did not use any neutralizer in the saline solution in order to neutralize traces of disinfectant, as suggested by the European Guideline (24), and this may have led to the underestimation of the detection of micro-organisms. Moreover, unlike the European Guideline (24), we tested only the suction-biopsy channel. This sampling selection did not permit all the internal endoscope channels to be adequately checked, where any small damage can be a source of bacterial contamination within the endoscope (8). However, because the focus of our study is to explore the effectiveness of the decontamination process of endoscopes through microbiological culture-based methods, limited resources have forced to the selection of the most representative endoscopic channel for this purpose (32).

In conclusion, our study has provided information about the microbiological outcome of the reprocessing of endoscopes and the approach adopted in the different endoscopy operation units of a large Italian Hospital. The data show a high percentage of contaminated endoscopes, and these led the health director to take evidence-based corrective action to improve the quality of reprocessing.

However, a microbiological monitoring plan is essential to assess the hygiene quality of reprocessing in the newly reorganized EOUs, in order to prevent the risk of HCAs related to the uses of endoscopes.

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*Competing Interest:* All Authors declare that they have no competing interests and they developed the methodology, analyzed the data and written the manuscript independently of the financier.

*Authors' contributions:* AC and LM initiated and designed the study, AC, GV, CP and LM, implemented the study, monitored data collection for the whole study, performed laboratory tests, AGS designed data collection tools and wrote the statistical analysis plan, cleaned and analyzed the data, AC wrote the manuscript. MDG,

ADC and JO revised the draft paper. All Authors read and approved the final manuscript.

*Availability of data and materials* All data generated and analyzed during the study are included in this article.

*Ethics approval and consent to participate* Not applicable

*Consent for publication* Not applicable.

#### **Riassunto**

##### ***Il monitoraggio microbiologico degli endoscopi e implicazioni sulle procedure di ricondizionamento adottate da un ospedale universitario italiano***

La contaminazione degli endoscopi a seguito del fallimento delle procedure di decontaminazione è associata ad infezioni correlate all'assistenza. L'obiettivo dello studio è stato valutare il grado di contaminazione degli endoscopi dopo la sanificazione e le implicazioni circa le procedure di sanificazione adottate nei servizi di endoscopia di un ospedale universitario italiano.

**Metodo.** Lo studio, ha interessato 11 unità di endoscopia dislocate all'interno del nosocomio, con una dotazione complessivamente di 100 endoscopi (18 broncoscopi, 41 gastroduodenoscopi, 29 colonscopi, 12 laringoscopi) e 9 apparecchiature per la sanificazione automatica degli endoscopi. Gli esiti microbiologici sono stati interpretati sulla base delle Linee Guida Europee sulla qualità microbiologica della sanificazione post-reprocessing degli endoscopi le quali prevedono "assenza di microrganismi indicatori e assenza di Carica Microbica Totale" in campioni ottenuti dal flussaggio dei canali endoscopici.

**Risultati.** Sono stati raccolti in totale 180 campioni ambientali (143 endoscopi, 25 apparecchiature per la loro sanificazione automatica e 12 campioni di acqua di rete). La conformità ai criteri di qualità della sanificazione riportati dalle Linee Guida Europee è stata raggiunta nel 62,2% dei campioni analizzati. La presenza di microrganismi indicatori (principalmente *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* e altri batteri Gram-negativi non fermentanti) è stata confermata in 51 dei 143 endoscopi (35,7%). Sono stati rinvenuti anche microrganismi multi antibiotico-resistenti (MDRO). La presenza di microrganismi patogeni è risultata statisticamente associata con l'aumento dei livelli di Carica Microbica Totale ma non con il tempo trascorso dopo la sanificazione degli endoscopi.

**Conclusioni.** Lo studio ha fornito informazioni sulla qualità microbiologica della sanificazione degli endoscopi di un grande ospedale italiano. L'alta prevalenza di endoscopi contaminati comprova la necessità di azioni correttive al fine di migliorare la qualità delle procedure di sanificazione.

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