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Original article

High-quality endoscope reprocessing decreases endoscope contamination

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ABSTRACT

Objectives: Several outbreaks of severe infections due to contamination of gastrointestinal (GI) endoscopes, mainly duodenoscopes, have been described. The rate of microbial endoscope contamination varies dramatically in literature. The aim of this multicentre prospective study was to evaluate the hygiene quality of endoscopes and automated endoscope reprocessors (AERs) in Tyrol/Austria.
Methods: In 2015 and 2016, a total of 463 GI endoscopes and 105 AERs from 29 endoscopy centres were analysed by a routine (R) and a combined routine and advanced (CRA) sampling procedure and investigated for microbial contamination by culture-based and molecular-based analyses.
Results: The contamination rate of GI endoscopes was 1.3%–4.6% according to the national guideline, suggesting that 1.3–4.6 patients out of 100 could have had contacts with hygiene-relevant microorganisms through an endoscopic intervention. Comparison of R and CRA sampling showed 1.8% of R versus 4.6% of CRA failing the acceptance criteria in phase I and 1.3% of R versus 3.0% of CRA samples failing in phase II. The most commonly identified indicator organism was *Pseudomonas* spp., mainly *Pseudomonas oleovorans*. None of the tested viruses were detected in 40 samples. While AERs in phase I failed ($n = 9$, 17.6%) mainly due to technical faults, phase II revealed lapses ($n = 6$, 11.5%) only on account of microbial contamination of the last rinsing water, mainly with *Pseudomonas* spp.
Conclusions: In the present study the contamination rate of endoscopes was low compared with results from other European countries, possibly due to the high quality of endoscope reprocessing, drying and storage. **P. Decristoforo, Clin Microbiol Infect 2018;▪:1**

Introduction

Several outbreaks of endoscopy-related infections have been reported in literature in recent years mainly associated with duodenoscopic interventions [1–3]. The US Food and Drug Administration received notification of 142 cases of patient infection or exposure from reprocessed duodenoscopes since 2010 [4]. In 2015 the US Food and Drug Administration issued a safety alert and ascertained concerns of an association between multidrug-resistant bacterial infections in patients who had undergone a duodenoscopic investigation [5].

Leffler et al. evaluated 6383 oesophagogastroduodenoscopies and 11 632 colonoscopies (including 7392 for screening) for the occurrence of procedure-related hospital visits with an electronic medical record-based system within 14 days after endoscopy. Hospital visits were recorded in 1.07%, 0.84% and 0.95% of all oesophagogastroduodenoscopies, colonoscopies and screening colonoscopies, respectively and in 0.4% if only signs of infection are considered [6].

Reprocessing of flexible endoscopes by sterilization is difficult due to heat-labile components, and duodenoscopes are probably

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most challenging due to their complex design [7]. Cleaning and disinfection regimens are complicated by narrow lumina and multiple internal channels [8]. Therefore, multiple steps of pre-cleaning, cleaning, high-level disinfection with automated endoscope reprocessors (AERs), rinsing, drying and storage are required within the reprocessing chain to avoid transmission of microorganisms from one patient to another [9]. However, the existing guidelines are inconsistent concerning the frequency and method of the microbiological monitoring [10–12]. The aim of the present study was to evaluate the hygiene level of ready-to-use gastrointestinal (GI) endoscopes and reprocessing quality of AERs in a multicentre prospective study. A further aim of this study was to assess whether a combined routine and advanced (CRA) sampling procedure has an impact on microbial detection, compared with the recommended endoscope routine (R) sampling.

Material and methods

Study participants and design

As this study did not have any influence on the treatment of patients, an institutional review board approval was not required at the Medical University Innsbruck.

Tyrol is one of nine Austrian federal districts with 728 000 inhabitants and approximately 90 000 endoscopic procedures per year. The hygiene status of all available reprocessed endoscopes and AERs was evaluated in two consecutive years (phase I June–December 2015 and phase II June–December 2016). For sampling, an appointment with the study members was fixed in advance. At this time-point all available reprocessed endoscopes and AERs of the respective centre were checked except those out of service or just in use (each endoscope and each AER was checked only once per phase). In phase II the procedure was repeated with the same centres and so, on the whole, the same endoscopes and AERs as in phase I were checked again. The routine (R) sampling procedure was compared with the combined routine and advanced (CRA) sampling procedure, which consisted of the routine (R) and an advanced (A) sample (see Supplementary material, Fig. S1). Samples were analysed in a central microbiological laboratory, which is accredited according to DIN EN ISO 17025 by using culture-based and molecular-based methods including detection of GI viruses. Samples, that were identified as being part of an outbreak (due to the occurrence of the same pathogen in more endoscopes and AERs than expected), were excluded from further data analyses to minimize the influence of extreme results on the overall data set.

All participating centres reprocessed the endoscopes adhering to the complete reprocessing chain (pre-cleaning, manual cleaning, AER, storing) recommended by the Austrian Society for Sterile Supply (ÖGSV) guidelines [10]. Reprocessing of endoscopes was done directly after the GI procedure, enzymatic agents were used for pre-cleaning in 83% of study centres. In six of 52 AERs (11.5%), no regular thermal self-disinfection was performed. The disinfectant used in AERs of all study members was exclusively based on glutaraldehyde.

Samples

All samples were obtained by two hygiene experts and processed under highly aseptic conditions. All specimens were stored on ice and immediately transferred for further analyses. Maximum time from sampling to analyses of the samples was 5 h according to the quality standards in microbiology/infection diagnostics by the German Society for Hygiene and Microbiology [13]. For routine investigation (R sample) of ready-to-use endoscopes, 20 mL of sterile 0.9% NaCl solution was flushed through the biopsy/suction channel from the proximal inlet to the distal end and collected in a 50-mL aseptic microbiological container without any adjuvant. In the case of a duodenoscope, the Albarran lever was moved into the central position and the recess behind and before was investigated with a sterile cotton swab after the flushing of R sampling. For A samples, the same ready-to-use endoscopes were immediately thereafter investigated by steering a sterilized 2.8–5.0 mm id synthetic disc brush PULL THRU™ (Galantai Manufacturing Co. Ltd, Auckland City, New Zealand) in one direction (proximal to distal end) through the biopsy/suction channel with the leading end in first to abrade the inner lumen, including possible biofilms. Once the leading end of the brush appeared at the distal end of the scope, the brush was pulled completely through the endoscope, removed and finally the disc component was cut off and placed into a 50-mL aseptic microbiological container. This procedure was followed by flushing the biopsy/suction channel with 20 mL of 0.9% NaCl and collection of the liquid sample in the same 50-mL aseptic microbiological container (A sample) (see Supplementary material, Fig. S1).

To check reprocessing quality of AERs after a completed cycle of cleaning and high level disinfection, 500 mL of final rinsing water was collected. Technical AER check consisted of check of cleanliness and disinfection performance and examination of temperature and retention time with six temperature data loggers according to the ÖGSV guidelines [10] (Table 1).

Laboratory analyses

R and A samples were vortexed and the disc-brush from A samples was removed under sterile conditions. The samples (R and A samples) were centrifuged at 4600 *g* for 10 min. The virtual pellets were resuspended to a volume of 10 mL with 0.9% NaCl each and used for culture-based and molecular-based analyses. The supernatant was used for molecular-based analyses only.

For molecular-based diagnostics, 5 mL of the resuspended pellet of the A sample was centrifuged again (4600 *g* for 10 min). In addition, the supernatants of R and A samples were pooled and decanted to a 38.5-mL thin wall, Ultra-Clear™ ultra-centrifugation tube (Beckman Coulter, Brea, CA, USA) and ultra-centrifuged at 84 600 *g* at 4°C for 90 min. Both pellets were resuspended in 200 µL of 0.9% NaCl and pooled for further molecular analyses. Fig. 1 shows the flowchart of sample preparation.

For culture-based analyses, the samples were inoculated on blood agar and liquid trypticase soy broth. The final rinsing water of the AER was analysed according to the microbiological requirements of the Austrian drinking water regulations [14]. Bacterial identification was done by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker, Vienna, Austria), antimicrobial resistance testing was not performed. Culture results of R samples were compared with those of CRA samples consisting of R and A samples (A samples were not evaluated separately as the latter are dependent on the R samples due to the sampling procedure) and for interpretation, the guidelines of the ÖGSV, were followed as detailed in Table 1 [10].

Molecular-based analyses were performed in a total of 40 randomly selected (by random number function of Microsoft Excel) samples, including 20 culture-positive and 20 culture-negative samples and analysed in duplicates. Detection of rotavirus, adenovirus, astrovirus, sapovirus, norovirus (genotype I and II), poliovirus, echovirus, coxsackievirus and human enterovirus 70/71, *Helicobacter pylori* and *Clostridium difficile* was performed using different Rida®Gene RT-PCR assays (R-Biopharm AG, Darmstadt, Germany). More detailed information on the laboratory analyses are given in the Supplementary material (Appendix S1).

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Table 1
Overview of the ÖGSV guideline acceptance criteria for endoscopes and automated endoscope reprocessors

Routine inspections	ÖGSV [10]
Flushing samples from endoscope channels	IO[a] n.d. or total colony count ≤10 CFU/mL
Swabs (Albarran lever)	IO[a] n.d.
Final rinsing water of AER	IO[a] n.d. or total colony count ≤10 CFU/mL
AER performance qualification:	
Check of cleaning efficacy (chamber walls and load carrier with test soil)	No visual residuals of test soil
Check of disinfection efficacy (bioindicator: *Enterococcus faecium*, ATCC 6057, CFU >10^9)	No growth of microorganism
Check of temperature/retention time (temperature data logger)	According to manufacturer and product requirements

Abbreviations: AER, automated endoscope reprocessor; IO, indicator microorganism (Enterococci, Enterobacteriaceae including *Escherichia coli* and others, *Pseudomonas aeruginosa*, gram-negative nonfermenters, *Staphylococcus aureus*, alpha-hemolytic streptococci); n.d., not detectable; ÖGSV, Austrian Society for Sterile Supply.
[a] IO and hygiene relevant microorganism (not specified in ÖGSV guideline).

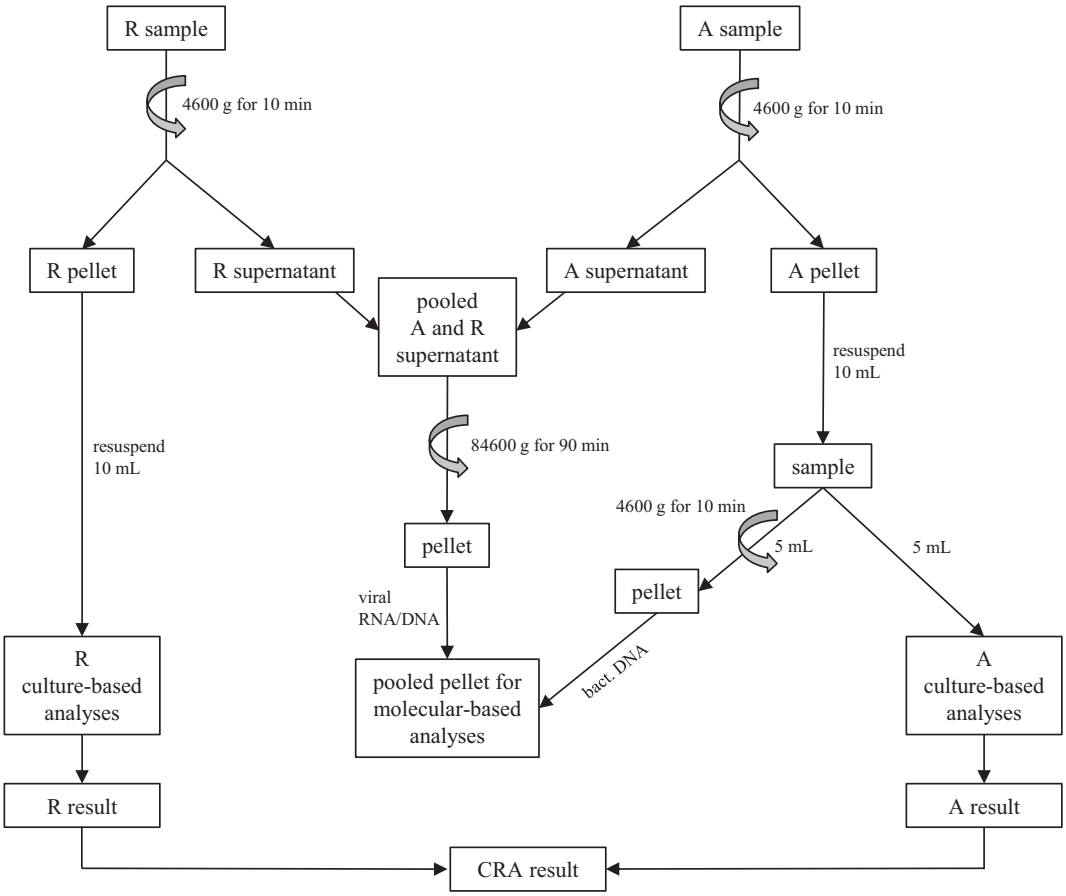

Fig. 1. Flow chart of sample preparation. Routine (R) and advanced (A) samples were centrifuged, the pooled supernatant (R and A), were ultracentrifuged and the pellet was used for viral RNA/DNA analyses. The R pellet was resuspended for culture based analyses. The A pellet was resuspended. One portion (5 mL) was used for the A culture based analyses and the other portion (5 mL) was centrifuged again for bacterial DNA analyses. Both pellets for molecular analyses were resuspended and pooled for RNA/DNA extraction, followed by PCRs for bacterial and viral pathogens.

Statistical analysis

Data entry and descriptive statistics were performed in SPSS version 22. Proportions of failed samples as measured by R versus CRA in each phase were compared using computer software Confidence Interval Analysis (CIA) version 2.2.0, based on Newcombe and Altman [15].

Results

Twenty-nine of 36 (81%) endoscopy centres took part in the anonymous Tyrolean Endoscope Hygiene Surveillance study. In phase I, 218 endoscopes (107 gastroscopes, 95 colonoscopes, 16 duodenoscopes) and 51 AERs were investigated, and in phase II, 245 endoscopes (122 gastroscopes, 105 colonoscopes, 18 duodenoscopes) and 54 AERs were investigated. In phase II, one endoscopic unit showed an outbreak with *Pseudomonas oleovorans* in all 11 scopes and in rinsing water samples of two AERs, indicating a reprocessing problem. These data were excluded for further analyses as specified in the study protocol.

In phase I, the evaluation of 218 GI endoscope rinsing samples yielded microbial growth in 7.3% ($n = 16$) of R and in 27.5% ($n = 60$) of CRA samples. Based on the acceptance criteria of the ÖGSV guidelines, 1.8% ($n = 4$) of R and 4.6% ($n = 10$) of CRA samples taken

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Table 2
Number of failed endoscopes according to ÖGSV guidelines for routine (R) and combined routine and advanced (CRA) samples of phase I (2015) and phase II (2016)

	Phase I ($n = 218$) n (%)			Phase II ($n = 234$) n (%)		
	R	CRA	ΔP (95% CI)[a]	R	CRA	ΔP (95% CI)[a]
Endoscopes (Gastroscopes colonoscopes and duodenoscopes)	4 (1.8%)	10 (4.6%)	2.8% (0.3 to 5.9)	3 (1.3%)	7 (3.0%)	1.7% (−0.3 to 4.4)

Abbreviations: CRA, combined routine and advanced sample from biopsy/suction channel; ÖGSV, Austrian Society for Sterile Supply; R, routine sample from biopsy/suction channel.
[a] Difference in endoscope failure proportion (ΔP) CRA versus R and corresponding confidence interval (95% CI).

from ready-to-use colonoscopes, gastroscopes and duodenoscopes failed the assessment, all due to the growth of indicator organisms or hygiene-relevant microorganisms (none due to total colony count of >10 CFU/mL) (Table 2). Among the 16 Albarran lever swabs from duodenoscopes, one yielded microbial growth with the presence of an indicator microorganism (*Staphylococcus aureus*) and so the device failed the assessment. In phase II, 9.4% ($n = 22$) of R and 28.6% ($n = 67$) of CRA samples showed microbial growth. According to the ÖGSV evaluation guidelines, 1.3% ($n = 3$) of R and 3.0% ($n = 7$) of CRA samples revealed growth of hygiene-relevant microorganisms and failed the acceptance criteria (Table 2). Albarran levers of all 18 duodenoscopes were negative in culture in phase II.

In phase I, colonoscopes yielded markedly more microbial growth than gastroscopes; however, in phase II, the contamination rates for colonoscopes and gastroscopes were comparable (Table 3).

The most commonly identified hygiene-relevant microorganisms detected in GI endoscopes were *Pseudomonas* spp. ($n = 3$ for R samples and $n = 6$ for CRA samples) and *Streptococcus* spp. ($n = 2$ for R samples and $n = 5$ for CRA samples) (Table 3). In five cases, hygiene relevant microorganisms were only detected in R samples, not in A.

From 51 monitored AERs in phase I, nine (17.6%) failed the acceptance criteria recommended by the ÖGSV (Table 4). One AER failed due to microbiologically contaminated last rinsing water (2.0%) and in this case also, the respective endoscope was contaminated (with *Pseudomonas oleovorans*). Eight AERs failed (15.7%) because of ineffective cleaning, disinfection or inadequate temperature/retention time (AER check). In these eight AERs exhibiting technical problems, the last rinsing water samples were not contaminated and also the respective endoscopes did not yield microbial growth. In phase II, 52 AERs were checked and six AERs (11.5%) failed the assessment criteria all due to growth of hygiene-relevant microorganisms in the last rinsing water (all of these six AERs lacked a regular thermal self-disinfection). In three of the six contaminated AERs, the respective endoscope also showed microbial growth. In 2016, none of the 52 AERs showed any failures in AER checks (Table 4).

All 40 samples, including 20 culture-positive and 20 culture-negative rinsing samples, from GI endoscopes were negative for rotavirus, adenovirus, astrovirus, sapovirus, norovirus (genotype I and II), poliovirus, echovirus, coxsackievirus and human enterovirus 70/71, *H. pylori* and *C. difficile* (including *C. difficile* toxin A and toxin B), respectively, by RT-PCR.

Table 3
Distribution of indicator and hygiene-relevant microorganisms in different scope types and in last rinsing water of automated endoscope reprocessors

Failed device	R (CFU/mL)	CRA (CFU/mL)	Indicator and hygiene-relevant microorganism
Phase I			
Gastroscope	2	>100	*Sphingomonas parasanguinis*
Gastroscope		1	*Streptococcus viridans*
Gastroscope		1	*Moraxella osloensis*
Colonoscope	1	1	*Streptococcus viridans*
Colonoscope	>100	>100	*Pseudomonas pseudoalcaligenes*
Colonoscope		>100	*Pseudomonas oleovorans*
Colonoscope		1	*Pseudomonas luteola*
Colonoscope		2	*Streptococcus mitis*
Colonoscope		1	*Moraxella osloensis*
Duodenoscope	1	1	*Staphylococcus aureus*
Failed last rinsing water	Water quality (CFU/mL)		
AER	3		*Pseudomonas oleovorans*
Phase II			
Gastroscope	2	2	*Pseudomonas oleovorans*
Gastroscope		22	*Pseudomonas aeruginosa*
Gastroscope	1	1	*Streptococcus sanguinis*
Gastroscope		1	*Moraxella osloensis*
Colonoscope	1	1	*Pseudomonas oleovorans*
Colonoscope		3	*Streptococcus salivarius*
Colonoscope		1	*Moraxella osloensis*
Failed last rinsing water	Water quality (CFU/mL)		
AER	14		*Pseudomonas aeruginosa*
AER	10		*Pseudomonas oleovorans*
AER	>100		*Pseudomonas oleovorans*
AER	2		*Pseudomonas oleovorans*
AER	14		*Pseudomonas oleovorans*
AER	>100		*Pseudomonas oleovorans*

Abbreviations: AER, automated endoscope reprocessor; CRA, combined routine and advanced sample from biopsy/suction channel; R, routine sample from biopsy/suction channel.

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Table 4
Results of automated endoscope reprocessor surveillance phase I (2015) and phase II (2016); assessment according to ÖGSV guidelines [10]

	Phase I ($n = 51$) n (%)	Phase II ($n = 52$) n (%)
Failed AER check [a]	8 (15.7%)	0 (0.0%)
Cleaning efficacy	1	0
Disinfection	6	0
Temperature/retention time	5	0
AER failed final rinsing water	1 (2.0%)	6 (11.5%)

Abbreviations: AER, automated endoscope reprocessor; ÖGSV, Austrian Society for Sterile Supply.
[a] Some AERs failed because of more than one part of AER check.

Discussion

In the Tyrolean Endoscope Hygiene Surveillance study 1.3%–1.8% of R samples and 3.0%–4.6% of CRA samples failed the acceptance criteria. Most studies have shown a significantly higher degree of endoscope contamination [16,17]. Saliou et al. [16] have found 264 of 762 (34.6%) GI endoscopes with a total microbial count of >25 CFU/mL. Of these 264 samples, 225 even showed a count of >100 CFU or the presence of indicator organisms. However, they incubated the samples for 8 days [16]. In a study performed by Chiu et al. [17], 57 of 420 (13.6%) samples gained by rinsing the biopsy channel of gastroscopes and colonoscopes yielded microbial growth (>10^3 CFU/mL). Possible reasons for a lower endoscope contamination rate in the present study may be a high standard of cleaning and disinfection of endoscopes with a pre-cleaning and manual cleaning step, use of AERs, and proper drying and storage as recommended by European guidelines [10,11,18]. Another cause may be the use of different disinfectants—glutaraldehyde in the present study versus peracetic acid in the study of Saliou et al. [16]. Chiu et al. [17] did not give any information on endoscope reprocessing.

Furthermore, comparison of different endoscope contamination rates is difficult because different guidelines are applied for interpretation of results and because different sampling and monitoring methods are used.

In the present study, the combined routine and advanced sampling procedure using a brush and collection of rinsing water thereafter led to an increase in pathogen detection, which was statistically significant only in phase I according to the ΔP (95% CI). However, one relevant pathogen, *Pseudomonas aeruginosa*, was detected only when applying the advanced sampling procedure. On the other hand, it should be noted, that in five cases indicator organisms were detected in R only, but not in the respective A samples. Based on these results, we cannot give any recommendation for the use of an advanced sampling procedure for microbial monitoring, especially when considering the increased effort and resource consumption.

In the present study, the microorganism found most often was *Pseudomonas* spp., and here mainly *Pseudomonas oleovorans*. *Pseudomonas* spp. is preferably located in moist environments [19], it is able to form biofilms and so is extremely difficult to remove from plumbing, AERs and endoscope channels [8,20]. Several healthcare-associated post-endoscopic infections due to *Pseudomonas aeruginosa* have been reported, including sepsis, liver abscess and ascending cholangitis. To our knowledge *P. oleovorans* has so far not been described in endoscope contamination and infections mainly occur in immunocompromised individuals [21]. Besides *P. aeruginosa*, which was present in one case, another pathogenic bacterium, *Staphylococcus aureus*, that can cause severe infection, also in immunocompetent hosts, was isolated.

Furthermore, in the current study, molecularly based methods did not reveal any GI viruses, *H. pylori* or *C. difficile*. Both *H. pylori* and *C. difficile* are important pathogens that have been found as contaminants of GI endoscopes in other studies [22,23]. Contamination of endoscopes with GI viruses has not been investigated so far.

Overall, the strengths of the present study are the multicentre character and the large sample size with 81% of all endoscope centres of Tyrol taking part in the study. However, limitations are the voluntary participation including the previous announcement of sampling, which might have created a positive selection bias as well as a Hawthorne effect. Furthermore, no detailed information concerning product types/manufacturers was collected so as to not influence study results and to protect anonymity of study participants.

In conclusion, a number needed to treat of, for example, at least five for colorectal polypectomy performed by an experienced colonoscopist [24] has to be weighed against the risk of 1.3 to 4.6 patients out of 100 who could have had contact with hygiene-relevant microorganisms through endoscopic intervention, as revealed here. To elucidate the real number needed to harm, implementation of a proper and personalized surveillance system should be mandatory for developed health systems. In the present study, contamination rate of GI endoscopes was low compared with results from other European countries, probably due to the excellent quality of endoscope reprocessing, drying and storage, and training of staff.

Tyrolean Endoscope Hygiene Surveillance study group

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Transparency declaration

All authors declare no conflicts of interest

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Author contributions

PD and JK have contributed substantially to the conception and design of the work, collection, analyses and interpretation of data, drafting and revising the article for important intellectual content, and final approval of the submitted version. AF, WP and DW have contributed to the design of the work, development of methodology, interpretation of data, and final approval of the submitted version. ME was involved in data analysis and interpretation, revising the article for important intellectual content, and final approval of the submitted version. CLF conceived the project, designed the study, and critically revised the manuscript. DHO developed methods, revised the article for important content and contributed to final approval of the submitted version.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.01.017.

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