

Investigation of the efficacy of an innovative endoscope drying and storage method in a simulated ERCP setting



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submitted 2.12.2022

accepted after revision 30.12.2022

Bibliography

Endosc Int Open 2023; 11: E419–E425

DOI 10.1055/a-2017-3872

ISSN 2364-3722

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Georg Thieme Verlag KG, Rüdigerstraße 14,
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ABSTRACT

Background and study aims Drying after cleaning and disinfection is essential in the reprocessing of endoscopes

since microorganisms can grow and form biofilms on wet surfaces. In this experimental non-clinical study, we investigated the efficacy of a novel, fast-drying method when reprocessing duodenoscopes.

Methods During a series of 40 tests, three duodenoscopes were exposed to an artificial test soil containing supraphysiological loads of four types of gut microorganisms in a non-clinical ERCP simulation, followed by reprocessing and drying with the PlasmaTYPHOON. Cultures of the distal tip and working channel were acquired immediately after automated decontamination and after drying with the PlasmaTYPHOON. Cobalt chloride paper tests and borescope inspections were used to evaluate drying efficacy.

Results Contamination of the working channels dropped from 86.4% post-decontamination to 33.6% post-drying, with 94% of the positive post-drying samples belonging to one duodenoscope. This duodenoscope showed persistent contamination with *P. aeruginosa* in the working channel. The other two duodenoscopes only showed low levels of *P. aeruginosa* in post-decontamination channel samples, but not after drying. Cobalt chloride paper tests and borescope inspections revealed good drying efficacy.

Conclusions Positive cultures for gut microorganisms were often found in wet endoscopes post-decontamination. The PlasmaTYPHOON is an effective fast-drying method capable of abolishing nearly all remaining microorganisms after decontamination provided no biofilm has developed, even when using a supraphysiological concentration of bacterial load. The clinical use of the PlasmaTYPHOON has the potential to reduce endoscope contamination, the use of wet contaminated endoscopes and therefore the risk of patient infection.

Introduction

Duodenoscope contamination remains an issue that has not yet been overcome by modifications of the design of reusable en-

doscopies or reprocessing methods [1]. While the effect of modifications to endoscope design and reprocessing methods are still not clinically proven, outbreaks, i. e. transmission from traditional duodenoscopes into patients, during the procedure, with both susceptible and drug-resistant microorganisms remain an issue worldwide. The more recent outbreaks often occurred despite strict adherence to reprocessing protocols, indi-

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cating that the concerning microorganisms can survive the comprehensive process of cleaning, automated decontamination and drying that duodenoscopes are currently subjected to [2, 3].

One of the underlying problems of endoscope contamination is the formation of biofilm [1, 4], which especially develops on wet surfaces [5]. Therefore, to prevent biofilm formation in endoscopes, effective drying is crucial [6]. A few different drying methods can be used, such as manual drying or automated drying in an automated endoscope reprocessor (AER) or drying cabinet. To date, limited studies have investigated the drying efficacy of these methods. Until recently, only drying in a drying cabinet seems to be effective in the removal of residual fluid and to result in lower microbial levels both after short- and long-term storage [7]. Some studies show additional decontamination effects of alcohol flushing of the channels prior to drying, but this is not advised by European guidelines due to potentially fixative effects [8, 9].

In a previous study of our group, we found that despite strict adherence to the manufacturer's instructions for reprocessing, persistent contamination can quickly develop in a duodenoscope channel in a non-clinical simulated ERCP setting [10]. In that study, a drying cabinet was used with a drying cycle of approximately ninety minutes. We hypothesize that a shorter, but effective drying procedure would create less opportunity for microorganisms to form a biofilm after decontamination. Recently, the PlasmaTYPHOON (PENTAX Medical/PlasmaBiotics) was developed which is able to dry duodenoscopes within two and half minutes. This drying technique is innovative in that it uses a combination of laminar and turbulent airflows and varied temperatures, to completely dry endoscope channels. It is developed to optimize the drying process and maintain the disinfected state upon storage in a PlasmBAG, but not necessarily designed to reach a zero contamination rate post-decontamination or to remove biofilms. The current study is the first to investigate the drying efficacy and the effect of this novel fast automated drying method on contamination of duodenoscopes after regular cleaning and automated decontamination and whether it can help to remove a persistent contamination. For this, we exposed the endoscope to supraphysiological loads of gut microorganisms in a standardized non-clinical ERCP simulation setting and applied the PlasmaTYPHOON after regular manual cleaning and automated decontamination.

Methods

Study design

In this experimental study, three duodenoscopes of the same model (DEC ED34-i10T2, PENTAX Medical) were subjected to non-clinical simulated ERCP procedures using an artificial test soil. The ERCP simulations and test soil have been previously described in our previous study [10], and will therefore only be described in short here. Two of the three duodenoscopes (A and B) were used previously in another experimental study, duodenoscope C was a brand new duodenoscope. All three duodenoscopes were confirmed to be clean of any microorganisms of interest by three sets of negative control cultures prior

to the start of the study. All three duodenoscopes were subjected to 40 test cycles including soiling, bedside pre-cleaning, manual cleaning, automated decontamination (cleaning, disinfection and rinsing) and drying with the PlasmaTYPHOON. After these 40 tests, another 20 tests were performed with duodenoscopes that showed persistent contamination, now without additional soiling and with only manual cleaning, decontamination and drying by using the PlasmaTYPHOON. This part of the study was used to examine whether the persistent contamination (suggestive for biofilm) after repeated reprocessing could be cleared. Borescope inspections and cobalt chloride paper tests were used to assess the drying efficacy.

Test soil

The artificial test soil (ATS2015, Healthmark Industries Company Inc. Fraser, Michigan, United States) was inoculated with *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Enterococcus faecium* (ATCC 35667) and *Escherichia coli* (ATCC 25922) in a concentration of 10^8 colony-forming units (CFUs)/mL of each strain. This bacterial load was higher than the 10^{3-5} CFUs/mL usually found in duodenal fluid (supraphysiological load). Positive control cultures collected after soiling and prior to manual cleaning revealed growth of >100 CFUs/sample of all four applied microorganisms.

Soiling procedure

The duodenoscopes were soiled in the same manner as previously described [10]. In short, 50 mL of the inoculated ATS2015 was suctioned through the duodenoscopes while a sterilized reusable biopsy forceps was moved in and out of the working channel ten times.

Reprocessing

Reprocessing of the duodenoscopes was performed according to the manufacturer's instructions for use (IFU). Immediately after soiling, bedside pre-cleaning was performed. Fifteen minutes later (clocked using digital timers), the duodenoscopes were subjected to manual cleaning followed by automated decontamination, including cleaning, disinfection and rinsing in an AER (WASSENBURG WD440 PT, Wassenburg medical, Dodewaard, The Netherlands). Mediclean Forte was used as a detergent during manual and automated cleaning and Neodisher Septo PAC as a disinfectant during automated disinfection (both Dr. Weigert, Hamburg, Germany).

Drying

Within 10 minutes after decontamination (which was timed), the duodenoscopes were dried using the PlasmaTYPHOON according to the manufacturer's IFU. First, the outer surface of the duodenoscope including the channel ports were dried using an air gun and a lint free cloth. Second, the channels of the duodenoscope were connected to the PlasmaTYPHOON. Third, the internal drying process was activated, resulting in a 2.5-minute drying cycle in which all channels are dried simultaneously. Forth, after completion of the drying cycle, the duodenoscope was placed in a sealed plastic bag (PlasmaBAG). Fifth, plasma containing ozone molecules was injected into the Plas-

maBAG for five seconds. Sixth, the sealed bag containing the duodenoscope was stored in a container until the next morning.

Sampling

The sampling method was based on the FDA/CDC/ASM [11] and Dutch guidelines [12] and described in our previous study [10]. Samples of the distal tip and working/suction channel were acquired immediately after decontamination and after overnight storage in the PlasmaBAG. Two samples were collected at each sampling moment: 1) a swab sample (eSwab, COPAN, Brescia, Italy) from the distal tip; and 2) a flush-brush-flush (FBF) sample from the suction and working channel. The FBF sample consisted of two flushes with 20 mL of sterile water and a brush of the channels (CS5522A, Pentax Medical, Dodewaard, The Netherlands). A neutralizing solution (Dey-Engley broth, NutriSelect Plus, Merck KGaA, Darmstadt, Germany) was added in a 1:1 ratio to both samples. The swab solution was poured onto a tryptic soy agar plate and the FBF sample was filtered and placed on an R2A agar plate. After 3 days of incubation at 35°C, colony-forming units (CFUs) were counted per sample with a maximum of 100 CFUs/sample.

Drying efficacy

The drying efficacy was investigated five times per duodenoscope. After overnight storage, air was flushed through all channels while a piece of cobalt chloride paper (Bartovation, White Plains, New York, United States) was placed at the end of the channels to visualize any droplets coming from the channel. In line with the PlasmaTYPHOON manufacturer's instructions, <0.5 cm² droplets on the paper was accepted as effective drying. A borescope (Flexible Inspection Scope FIS-005, Healthmark Industries Company Inc. Fraser, Michigan, United States) was used to inspect the working channels of all duodenoscopes once immediately after the drying procedure to check for residual fluid. Borescope inspections were also performed at the start of the study and after every 10 tests to detect any deviations of the working channel.

Outcomes

The drying efficacy was defined as presence of droplets found on cobalt chloride paper or on inner surface during borescope inspection. Contamination was defined as presence and number of CFUs of any of the four applied microorganisms in cultures after drying. The difference in contamination rate between post-decontamination and post-drying cultures was assessed as well.

Analysis

Data was managed in SPSS version 25 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 25.0. Armonk, New York, United States: IBM Corp.). Due to the small sample size of three duodenoscopes with repeated measurements dependent of one another, no statistical tests could be performed. Therefore, we only describe the outcomes and present the positive culture results with their 95% confidence intervals.

Results

Between February 16, 2021 and May 26, 2021, a series of 40 tests were conducted with duodenoscopes A and B and sixty with duodenoscope C. The extra twenty tests with duodenoscope C were performed without soiling and consisted only of reprocessing and sampling to see if at some point the contamination would be removed by repeated reprocessing and drying with the PlasmaTYPHOON. This means that 560 samples were collected, 280 of the distal tip (140 post-decontamination and 140 post-drying) and 280 of the channel (140 post-decontamination and 140 post-drying).

Contamination of the distal tip was only seen in duodenoscope C, which was positive for growth of any of the four applied microorganisms in five samples collected after decontamination and one sample post-drying (► **Table 1**). All distal tip samples of duodenoscopes A and B remained negative. Of the channel samples collected post-decontamination, 121 (86.4%, 95% CI: 81%-92%) showed growth of any of the applied microorganisms. In the post-drying samples this was the case in 47 (33.6%, 95% CI: 26%-41%) channel samples, with 44 (94%) of them belonging to duodenoscope C. ► **Table 2** shows the amount of CFUs found per sample.

Duodenoscope A

None of the samples collected of the distal tip of duodenoscope A showed growth of any of the applied microorganisms. Of the channel samples collected post-decontamination, 36 (90%) was positive. 32 (80%) post-decontamination channel samples grew *P. aeruginosa* (► **Fig. 1**), 11 (27.5%) grew *K. pneumoniae*, 4 (10%) grew *E. coli* and 1 (2.5%) grew *E. faecium*. Only two (5%) of the post-drying channel samples were positive, both with *E. faecium*.

Duodenoscope B

The distal tip samples of duodenoscope B were all negative, both after decontamination and after drying in the PlasmaTYPHOON. The channel was contaminated after decontamination in 28 tests (70%). Twenty-two (55%) post-decontamination channel samples grew *P. aeruginosa* (► **Fig. 2**), 10 (25%) grew *K. pneumoniae*, nine (22.5%) grew *E. coli* and three (7.5%) grew *E. faecium*. Only one sample (2.5%) after drying remained positive, in this culture *P. aeruginosa*, *K. pneumoniae* and *E. faecium* were found.

Duodenoscope C

The distal tip of duodenoscope C was contaminated in the first part of the study, with five (8.3%) positive samples post-decontamination and one post-drying (1.7%). Three samples (5%) post-decontamination showed presence of *P. aeruginosa*, one (1.7%) of *K. pneumoniae*, two (3.3%) of *E. coli* and one (1.7%) of *E. faecium*. The positive post-drying sample only contained *E. faecium*. The channel samples were positive in 57 (95%) of the post-decontamination tests, all of them contained *P. aeruginosa* (► **Fig. 3**). Six (10%) of those samples also contained *K. pneumoniae*, two (3.3%) contained *E. coli* and one (1.7%) contained *E. faecium*. Of the channel samples collected after drying

► Table 1 Growth of the four applied microorganisms in the three duodenoscopes in samples collected from the distal tip and suction/working channel after decontamination and after drying with the PlasmaTYPHOON.

	<i>P. aeruginosa</i> , n (%)	<i>K. pneumoniae</i> , n (%)	<i>E. coli</i> , n (%)	<i>E. faecium</i> , n (%)	Any indicator microorganism, N (%)
Duodenoscope A (N = 40)					
Tip after decontamination	0	0	0	0	0
Tip after drying with PlasmaTYPHOON	0	0	0	0	0
Channel after decontamination	32 (80.0%)	11 (27.5%)	4 (10.0%)	1 (2.5%)	36 (90.0%)
Channel after drying with PlasmaTYPHOON	0	0	0	2 (5.0%)	2 (5.0%)
Duodenoscope B (N = 40)					
Tip after decontamination	0	0	0	0	0
Tip after drying with PlasmaTYPHOON	0	0	0	0	0
Channel after decontamination	22 (55.0%)	10 (25.0%)	9 (22.5%)	3 (7.5%)	28 (70.0%)
Channel after drying with PlasmaTYPHOON	1 (2.5%)	1 (2.5%)	0	1 (2.5%)	1 (2.5%)
Duodenoscope C (N = 60)					
Tip after decontamination	3 (5.0%)	1 (1.7%)	2 (3.3%)	1 (1.7%)	5 (8.3%)
Tip after drying with PlasmaTYPHOON	0	0	0	1 (1.7%)	1 (1.7%)
Channel after decontamination	57 (95.0%)	6 (10.0%)	2 (3.3%)	1 (1.7%)	57 (95.0%)
Channel after drying with PlasmaTYPHOON	44 (73.3%)	0	0	0	44 (73.3%)

► Table 2 Range of colony-forming units (CFUs) found of the four applied microorganisms per sample.

	0 CFU	<10 CFU	10–50 CFU	50–100 CFU	≥ 100 CFU
<i>P. aeruginosa</i>	401	79	43	13	24
<i>K. pneumoniae</i>	531	27	2	0	0
<i>E. coli</i>	543	16	0	0	1
<i>E. faecium</i>	550	7	1	1	1

with the PlasmaTYPHOON, 44 (73.3%) showed growth of *P. aeruginosa*, the other three microorganisms were not found in the post-drying samples. In these last 20 tests, in which this duodenoscope was no longer exposed to the soil, all channel samples except three post-decontamination and two post-drying samples were positive for *P. aeruginosa*, including the last three tests.

Drying efficacy

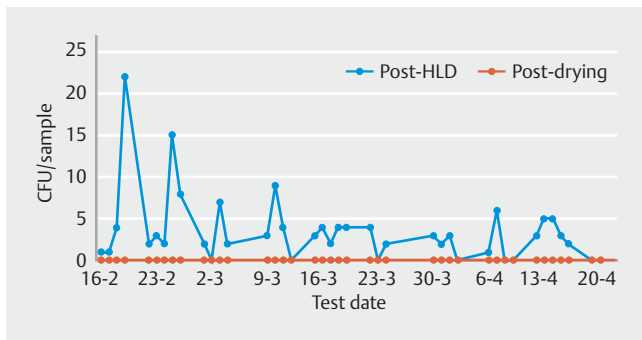
Per duodenoscope, five drying efficacy tests were performed using cobalt chloride paper. In only one test (test number 40) in duodenoscope C, one small droplet (approximately 0.05cm²) was blown out from the suction cylinder after overnight storage. No droplets were blown from the other suction, working or air/water channels. In all other tests of the three duodenoscopes, no droplets were found on the cobalt chloride paper. During borescope inspections, no droplets were seen in the working channel. This inspection was performed once on all duodenoscopes immediately after drying with the PlasmaTYPHOON and also after overnight storage in the PlasmaBAG after

every ten tests (five inspections of duodenoscopes A and B and seven of duodenoscope C). During these inspections, some superficial scratches were seen in all duodenoscopes at the entrance of the working channel, but no damages that can explain the persistent contamination in duodenoscope C.

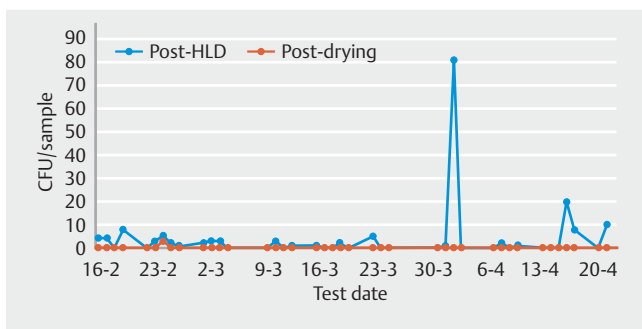
Discussion

In this experimental non-clinical ERCP simulation study, we show that a quick drying procedure with the PlasmaTYPHOON was able to consistently remove nearly all remaining gut microorganisms that were still present after decontamination in two duodenoscopes after exposure to supraphysiological levels of gut microorganisms. In a third endoscope, drying with the PlasmaTYPHOON showed a significant reduction in bacterial load, however the PlasmaTYPHOON was not able to additionally prevent or remove persistent contamination with *P. aeruginosa* from the working channel.

We found that 86.4% of the cultures collected from the channels after decontamination still contained any of the ap-



► **Fig. 1** Presence of *P. aeruginosa* in post-decontamination and post-drying samples after PlasmaTYPHOON of the channel of duodenoscope A. CFU, colony-forming unit.



► **Fig. 2** Presence of *P. aeruginosa* in post-decontamination and post-drying samples after PlasmaTYPHOON of the channel of duodenoscope B. CFU, colony-forming unit.

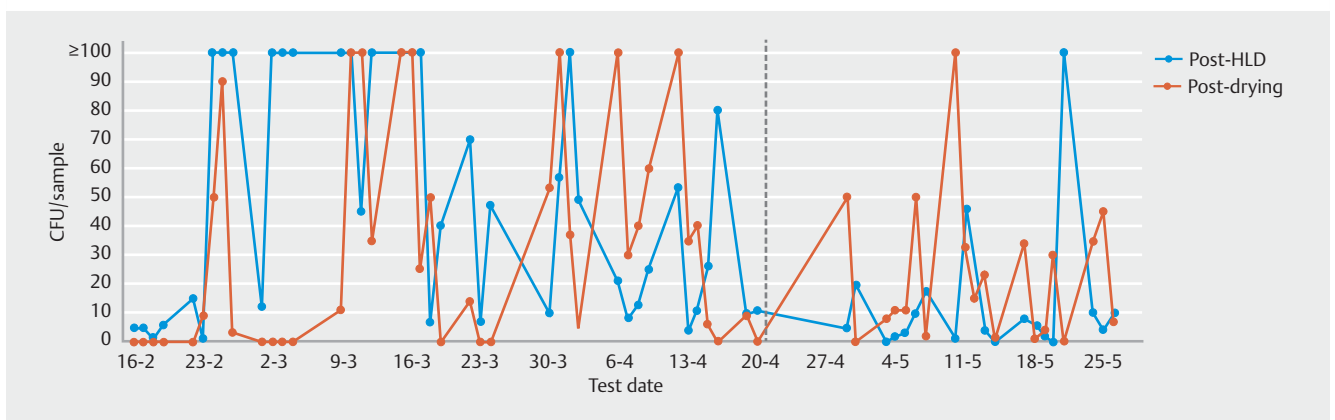
plied gut bacteria. This was reduced to 33.6% after drying with the PlasmaTYPHOON. Of note, most positive post-drying cultures were derived from duodenoscope C in which a biofilm might have been formed, based on the persistent contamination with the same microorganism. In the other two duodenoscopes, contamination fell from 90% post-decontamination to

5% post-drying in duodenoscope A and from 70% to 2.5% in duodenoscope B.

These results confirm the added value of endoscope drying on the elimination of microorganisms previously reported in other research. Saliou et al. found a decrease in gastrointestinal endoscope contamination from 45% to 13% after switching from storage without active drying to storage in cupboards facilitating channel-purge storage [13]. Pineau et al. showed bacterial proliferation in endoscopes stored in a non-controlled environment in contrast with endoscopes stored in drying/storage cabinets [14]. Borescope inspections and cobalt chloride paper tests showed good drying efficacy of the PlasmaTYPHOON in this study. The only droplet found during this study was ten times smaller than the acceptance threshold used by the manufacturer.

Already in 1991, a 10-minute drying step was advised in case the endoscope would be stored overnight [15]. According to the current Dutch guideline [16], endoscopes are allowed to be used without drying in case they are used within 4 hours of reprocessing. Long drying procedures, such as the ninety minutes program of the drying cabinets used in our institution, create situations in which drying between ERCP procedures becomes difficult to accomplish and is skipped due to time pressure in a busy endoscopy unit. However, in this study we noticed that most duodenoscopes were still contaminated with gut microorganisms directly after decontamination which were only removed after drying. The high contamination levels found post-decontamination might suggest ineffective reprocessing procedures or a too large challenge by the amount of contamination used in our study. However, the test soil, including these concentrations of microorganisms, is specifically developed to validate endoscope reprocessing [17]. Not only microbiological safety could be increased, but also workflow, with a fast-drying method such as the PlasmaTYPHOON as demonstrated in this study. This may result in the use of less or even non-contaminated duodenoscopes and better hospital efficiency.

In this study, one of the three duodenoscopes became persistently contaminated with *P. aeruginosa*. No deviations from



► **Fig. 3** Presence of *P. aeruginosa* in post-decontamination and post-drying samples after PlasmaTYPHOON of the channel of duodenoscope C. The line indicates the second phase of the study in which the duodenoscope was no longer exposed to the soil but only reprocessed, dried and sampled on a daily basis. CFU, colony-forming unit.

the study protocol can explain why this only occurred in one (new) duodenoscope. Also, no damages of the working channel could be identified by our borescope inspections. Microscopic damages however, cannot be ruled out as a possible niche for harboring soil and microorganisms. The fact that *P. aeruginosa* was still present in the last four weeks in which duodenoscope C was no longer exposed to this microorganism, suggests the presence of a biofilm. The PlasmaTYPHOON was developed to process and completely dry endoscope channels and not specifically to prevent or remove biofilm. In this study under supra-physiological loads of four types of gut microorganisms, drying with the PlasmaTYPHOON was not able to prevent this persistent contamination and was also not able to remove it during twenty repeated rounds of reprocessing and drying without the endoscope being exposed to new microorganisms. This is in line with findings in outbreaks described by Kovaleva [18] and Qiu [19] in which *P. aeruginosa* contamination could not be removed by standard or intensified reprocessing methods including 13-hour soaking in glutaraldehyde or ethylene oxide sterilization. In both these outbreak cases, contamination was only resolved after replacement of the endoscope channels.

In this study we focused on the growth of four indicator microorganisms. *E. coli* and *E. faecium* were least often cultured. This could be due to a less sensitive culturing method for these microorganisms. However, they were all cultured in high loads in the positive controls and are also found in low levels in clinical cultures using the same methods. We did not account for microorganisms entering a viable but non-culturable state (VBNC). Microorganisms enter this state, in which they no longer replicate but remain metabolically active, due to stressful environmental conditions, such as reprocessing or drying. At a later moment they can return to a reproductive state. This was found by Johani et al. to result in false-negative culture results [4]. Alfa et al. showed that *P. aeruginosa* was able to survive in the VBNC state for at least 26 weeks [20].

In comparison to duodenoscope 1 in our previous study [10], the *P. aeruginosa*-positive cultures from duodenoscope C had lower CFU levels. Because it only concerns two duodenoscopes, we cannot prove that this difference in load of *P. aeruginosa* is attributable to the use of the PlasmaTYPHOON. Another possible explanation is that we noticed high levels (≥ 100 CFU) of environmental microorganisms such as *Microbacterium* and *Methylobacterium* species only in cultures of duodenoscope C which might have competed with *P. aeruginosa* while growing in a biofilm inside the channel leading to lower *P. aeruginosa* levels in the cultures. Especially *Methylobacterium* sp. are known for their strong biofilm producing ability, these microorganisms can be often found in water supplies [21]. Environmental microorganisms were also shown to be often present in reprocessed endoscope channels [4, 22]. Although environmental microorganisms are considered low risk microorganisms when found in endoscopes, if they form biofilms, other (high risk) microorganisms might adhere to these biofilms as well.

Limitations

This study was designed as a proof-of-principle study to investigate the effect of the PlasmaTYPHOON on duodenoscope contamination in a controlled non-clinical ERCP simulation setting exposing the duodenoscopes to supra-physiological loads of gut microorganisms. Although the PlasmaTYPHOON was not developed to prevent or remove biofilm, however, but created to improve the drying efficacy, we deemed it of interest to investigate the effect on duodenoscope contamination since biofilm is mainly developed in wet environments. Only three new duodenoscopes were used in this experiment for which reason no statistical analyses could be performed. Nevertheless, our results indicate that the PlasmaTYPHOON is an effective fast-drying method capable of abolishing nearly all remaining microorganisms after decontamination provided no biofilm has developed, even when using a supra-physiological concentration of bacterial load. Endoscope A and B have been used in the previous part of this study where they have already been contaminated. Any remaining microorganisms might have influenced growth of biofilm or accumulation of other microorganisms in the current study.

Another limitation is that this experiment was not controlled in the sense that we were unable to make a direct comparison with drying in a regular drying cabinet or no active drying at all. As a result, we cannot exclude that the low levels of contamination found directly after decontamination in duodenoscope A and B would also have disappeared spontaneously by the passing of time after overnight storage without drying. For instance, due to desiccation or remaining disinfectants in the channels.

The air/water channels were not investigated by culturing or borescope inspection. Therefore, we do not know whether they remained contaminated as well and whether droplets were residing in these channels. If droplets remained present in these channels after drying, this might have caused a humid environment inside the PlasmaBAG, which could stimulate biofilm formation in other channels as well. Nerandzic et al. [23] found that especially the air/water channels are difficult to dry.

This experimental set-up withholds from drawing definite conclusions for the efficacy of the PlasmaTYPHOON in daily clinical practice. Although a promising tool to contain and reduce endoscope contamination based on the current results, clinical studies are indicated to confirm its merits in daily clinical practice, preferably by showing not only a reduction in endoscope contamination but also in patient colonization and infection.

Conclusions

The PlasmaTYPHOON is a fast and effective drying method that can help to minimize the use of wet endoscopes prone to be contaminated with gut microorganisms provided a biofilm has not developed. Consequently, its use does not obviate the need for regular microbiological surveillance of endoscopes to timely detect persistent contamination. Further research is needed to investigate the merits and efficacy to contain duodenoscope

contamination in a clinical setting, specifically in comparison with the use of conventional drying cabinets.

Competing interests

Dr. Kwakman has received grants from Pentax Medical and Boston Scientific. Dr. Vos has received grants from 3 M, Pentax Medical, and Boston Scientific. Dr. Bruno has received grants from 3 M, Pentax Medical, Boston Scientific, Mylan, and Interscope and is a consultant for Boston Scientific and Cook Medical.

Funding

Pentax Medical

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