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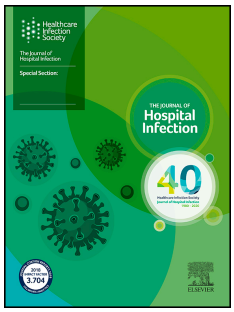

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Higher yield in duodenoscope cultures collected with addition of neutralizing agent.

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Abstract (250 words)

Aim

Microbiological cultures are the gold standard in the monitoring of duodenoscope reprocessing. However, many different sampling and culturing techniques are used making it difficult to compare results. The latest CDC protocol advises the use of a neutralizer to deactivate any remaining disinfectants in the samples. We compare culturing results of duodenoscope samples collected with and without addition of a neutralizer.

Methods

Six duodenoscopes were soiled with gut bacteria in a non-clinical experimental setting and reprocessed afterwards. Samples of the tip and working channel were collected immediately after decontamination or after drying. Dey-Engley (DE) broth was added as a neutralizer to the samples of four duodenoscopes, samples of the other two duodenoscopes were collected without additional DE broth.

Results

Post-decontamination cultures were significantly more often positive for growth of the applied microorganisms in the group of samples collected with DE broth (88.1% vs 20.2%, $p<0.0001$). Post-drying samples were significantly more often positive in the group without use of DE broth (75.7% vs 33.4%, $p<0.001$).

Conclusion

These results show that adding DE broth to samples collected from wet duodenoscopes increases the yield of those cultures. Remaining disinfectants in wet duodenoscopes can lead to false-negative results. This can be overcome by adding a neutralizer, such as DE broth, to the samples. The higher yield post-drying in the group without neutralizer could be due to biofilm formation in these two duodenoscopes, but this was not investigated. Standardization of the sampling method can help to compare both clinical and study results regarding duodenoscope contamination.

Introduction

Duodenoscopes are endoscopic instruments used for minimally invasive diagnosis and treatment of diseases of the pancreas and bile ducts during an endoscopic retrograde cholangiopancreaticography (ERCP). As these endoscopes are made of heat-sensitive materials, sterilization is not possible. Instead they are subjected to an extensive reprocessing protocol including bedside pre-cleaning, manual cleaning, automated decontamination (including cleaning, disinfection and rinsing) and drying after every ERCP procedure. Despite these protocols, up to 15% of reprocessed duodenoscopes can remain contaminated (1), which has caused outbreaks of duodenoscope-associated infections (DAIs) worldwide with both susceptible microorganisms and multidrug-resistant organisms (MDROs) (2).

Culturing is the gold standard to detect duodenoscope contamination. Culturing can be performed when contamination is suspected or in the context of surveillance. However, there is no consensus on the efficacy of periodic surveillance and the appropriate intervals, in case surveillance is utilised. (3). Also, different sampling and culturing techniques are described in national and international guidelines. In 2018, the Centers for Disease Control and Prevention (CDC) revised their sampling and culturing protocol (4). It advised to collect a combined sample of the elevator recess and the instrument channel when investigating the contamination status of endoscopes. The instrument channel should be sampled by a flush-brush-flush technique. A neutralizing solution is added to the combined sample to inhibit the bactericidal effect of remaining disinfectants in the sample. According to the CDC, this protocol was validated by three duodenoscope manufacturers which recovered 65% to 100% of the present microorganisms, however, their methods nor data was not published. No other studies have compared the results of samples collected with and without neutralizers (3). In general, there has been very little comparative research performed on different sampling techniques and outbreak reports show that there is much variety in sampling methods around the world (3).

Many different neutralizing agents are available, some of which can be added to a sampling fluid and others which can be used as a sampling fluid themselves (3). In this article, we share our experiences regarding culturing of duodenoscopes with and without the neutralizing agent Dey Engley broth (DE broth). This broth, which is a growth medium containing several components to neutralise a wide variety of disinfectants, is specifically mentioned in the CDC protocol to be an appropriate neutralizing agent (4). These experiments were conducted as part of a study investigating biofilm formation in duodenoscopes in a non-clinical simulation ERCP setting.

Methods

Study design

For this study we used data from previous study activities. Between June 2019 and June 2021 we performed a non-clinical simulation ERCP study in the Erasmus Medical Center (Rotterdam, The Netherlands). For the full description of the study methods we refer to the publication of that study (5).

This study was aimed to investigate the formation of biofilm in duodenoscopes exposed to an artificial test soil (ATS 2015, Healthmark Industries Company Inc. Fraser, MI, USA) containing *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Enterococcus faecium* in levels higher than normally found in duodenal fluid (10^8 CFU/ml vs 10^{3-5} CFU/ml) (6, 7). The duodenoscopes were exposed to this test soil during simulation ERCP procedures, after which reprocessing took place. Positive controls of the soiled duodenoscopes confirmed presence of all four microorganisms prior to reprocessing. The study consisted of three phases (figure 1): a pilot phase and the first part of the study in which reprocessing ended with drying in an active drying cabinet (WASSENBURG DRY300D, Wassenburg medical, Dodewaard, The Netherlands) and the second part of the study in which drying was performed using the PlasmaTYPHOON (Pentax Medical, Dodewaard, The Netherlands).

Materials

Throughout the different phases of the study, a total of six unique duodenoscopes were used (Table I). All duodenoscopes were brand new, never used in patients and negative controls prior to the start of the study confirmed them to be free from the microorganisms used in the test soil. In the pilot phase, two duodenoscopes were used, one model ED34-i10T duodenoscope (scope 1) and one DEC ED34-i10T2 (scope 2) duodenoscope (both Pentax Medical, Dodewaard, The Netherlands). In phase 1 of the study, three brand new DEC ED34-i10T2 duodenoscopes were used (duodenoscopes 3, 4 and 5). During this part of the study, it was suspected that a biofilm formed in duodenoscope 3, based on persistent contamination with *P. aeruginosa* despite no longer being exposed to it. Therefore, duodenoscope 3 was no longer used in phase 2 of the study but was then replaced by a new duodenoscope of the same model (duodenoscope 6).

Soiling and reprocessing

A detailed description of the soiling process has been previously described (5). In short, the duodenoscopes were placed in a tripod with the distal tip immersed in a container with 50mL of the test soil. A sterilised reusable biopsy forceps was pushed through the working channel, with the forceps elevator moved up and down five times. Then, the suction and flush valves were pushed alternatingly five times. The biopsy forceps was removed and introduced again, and this was repeated ten times per procedure. The duodenoscopes were immediately pre-cleaned after the procedure and left to dry for 15 minutes. Next, manual cleaning and further decontamination (cleaning, disinfection and rinsing in a validated automated cycle) in an endoscope washer-disinfector (EWD) were performed according to the manufacturer's instructions for use (MIFU). For the pre-cleaning and manual cleaning the detergent Mediclean Forte was used and the disinfectant utilised during decontamination was Neodisher Septo PAC (both Dr. Weigert, Hamburg, Germany). Final rinse water of the EWDs was cultured every three months and was negative for growth during the study period.

Sampling

In the pilot phase, samples were either collected immediately after decontamination or after overnight storage in an active drying cabinet. In phase 1 and 2 of the actual study, samples were collected both after decontamination and after drying since we noticed large differences in culture results collected after

decontamination or drying in the pilot phase. We suspected that retained disinfectants could be an explanation for the differences found in the pilot phase, therefore we altered the sampling method and started using a neutralizer.

During the whole study, sampling consisted of the collection of a separate swab sample (eSwab, COPAN, Brescia, Italy) of the distal tip (including the forceps elevator in the ED34-i10T endoscope) and a combined flush-brush-flush sample (FBF) of the suction and working channel collected at the distal end. The FBF sample consisted of two flushes of 20ml of sterile water and brushing of the channels (CS5522A, Pentax Medical, Dodewaard, The Netherlands). In the pilot phase, no neutralizer was added to the samples. In phase 1 and 2 of the study, DE broth (NutriSelect Plus, Merck KGaA, Darmstadt, Germany) was immediately added to both samples after collection in a 1:1 ratio.

Culturing

Culturing was largely performed in the same manner in all phases of the study. This included pouring of the 1ml (without neutralizer) or 2ml (including neutralizer) amies medium of the eSwab onto tryptic soy agar and filtration of the FBF sample, after which the filter, 0.22 µm in the pilot phase and 0.45 µm in phase 1 and 2 (both Millipore, Merck KGaA, Darmstadt, Germany), was placed on R2A agar. The samples with neutralizers could not be filtered through 0.22 µm filters, therefore we switched to 0.45 µm filters. All cultures were incubated for three days at 35 °C. The time between sample collection and culturing was aimed to be as short as possible. However, in the pilot phase, sometimes culturing was performed the next day. In the study phase, the interval between sampling and culturing ranged from 15 minutes to four hours. All samples were refrigerated while awaiting culturing.

Outcomes

Per sample we documented whether there was growth of any of the applied microorganisms in the cultures, defined as ≥1 colony forming unit (CFU). Presence of the applied microorganisms was further specified for the four distinct microorganisms. In this study, growth of the applied microorganisms was compared between the samples collected with and without DE broth.

Statistical analysis

Data was collected in SPSS version 25 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Chi-squared tests were performed to assess for different culture yields between the cultures collected with and without DE broth.

Results

In total, 409 sample sets were acquired directly after decontamination and 390 were acquired after drying. Of duodenoscope 1, 50 sample sets were collected after decontamination and 39 after drying. Of duodenoscope 2, 39 sample sets were collected after decontamination and 31 after drying. Duodenoscopes 3 and 6 were sampled sixty times both after decontamination and after drying.

Duodenoscopes 4 and 5 were sampled one hundred times at both moments. The 89 post-decontamination and 70 post-drying sample sets of duodenoscopes 1 and 2 were the only samples collected in this study without addition of DE broth. Of the four applied microorganisms, *P. aeruginosa* was cultured most often. It was found in 275 out of 409 (67.2%) of the post-decontamination channel samples and in 157 out of 390 (40.3%) of the post-drying channel samples. Culture results of all individual duodenoscopes are presented in Table II.

Samples collected without neutralizer

Cultures of the distal tip of duodenoscope 1 were not shown to grow any of the applied microorganisms. Of the channel, 15 samples (30%) collected post-decontamination and 30 samples (76.9%) collected post-drying were positive for growth of any of the four applied microorganisms.

The tip of duodenoscope 2 was positive once (2.6%) after decontamination. All other cultures, both post-decontamination and post-drying of the distal tip were negative. Of the channel, three (7.9%) post-decontamination cultures and 23 (71.9%) post-drying cultures were positive.

Samples collected with neutralizer

Duodenoscope 3 showed three (5%) positive cultures of the distal tip after decontamination and one (1.7%) after drying. Of the channel, we found 57 (95%) positive cultures post-decontamination and 49 (81.7%) post-drying.

Of duodenoscope 4, three (3%) post-decontamination cultures of the distal tip were positive, none of the post-drying samples of the tip were positive. Of the channel, 89 (89%) post-decontamination cultures and 6 (6%) post-drying cultures were positive.

Of duodenoscope 5, four (4%) post-decontamination and one (1%) post-drying sample of the distal tip were positive. Of the channel, 79 (79%) post-decontamination cultures and 8 (8%) post-drying cultures were positive.

Duodenoscope 6 had five (8.3%) positive cultures of the distal tip after decontamination and one (1.7%) after drying. The channel showed 57 (95%) positive cultures post-decontamination and 44 (73.3%) post-drying.

Comparison results with and without neutralizer

No statistical analysis of the distal tip results was possible due to the low number of positive cultures in both groups.

The no-neutralizer results consisted of the FBF results of duodenoscopes 1 and 2. The neutralizer results consisted of all FBF results from duodenoscopes 3, 4, 5 and 6. When all these results were taken together, we found a significant ($p<0.001$) higher yield of the post-decontamination samples collected with a neutralizer (88.1% positive samples) compared to samples collected without neutralizer (20.2% positive samples) (Table III). In the post-drying samples the yield was significantly ($p<0.001$) higher in the samples

collected without neutralizer (75.7%) compared to the samples collected with a neutralizer (33.4%) (Table IV).

Discussion

This non-clinical experimental study is the first to describe the added value of DE broth to the yield of FBF samples collected of wet duodenoscopes. No added effect of DE broth was found on FBF samples collected after drying of the duodenoscopes. Due to a low number of positive swab samples collected from the distal tip, a comparative analysis of these samples collected with and without DE broth was of no added value.

Culturing is currently the only method to monitor the efficacy of endoscope reprocessing and to check for endoscope contamination (8). However, there is much discussion regarding the optimal sampling and culturing methodology. National and international guidelines advise the use of different sampling fluids, different culturing techniques and different intervals for surveillance measurements. The addition of DE broth to the samples has so far only been recommended by the CDC (4), however, no comparative studies have yet been executed to prove the added value of this neutralizer. The ESGE-ESGENA guideline from 2007 advises a sampling fluid of sterile saline 0.9% and states that neutralizers can be added (9). A French guideline recommends to use a sterile neutralizing sampling fluid instead of adding a neutralizer afterwards (10).

This study shows that adding DE broth to FBF samples collected directly after decontamination increases the culture yield of those samples. This can be explained by the neutralizing effect of DE broth on retained disinfectants that can be transferred from the duodenoscopes into the samples. No higher yield was found in samples collected with DE broth after drying, possibly because there was no remaining disinfectant in completely dried endoscopes. DE broth is not the only neutralizing agent that can be used for this purpose. Apart from neutralizers that can be added to a sampling fluid like water or saline, there are some sampling fluids that already contain a neutralizer, such as neutralizing pharmacopeia diluent (NPD) which is used in some studies (11, 12). Cattoir et al. found no higher yield of cultures collected with NPD compared to physiological saline (13). There are no studies comparing culturing results of samples collected with and without addition of a neutralizer (3). It would be interesting to investigate whether the final rinse water of EWDs contains disinfectants and if there are differences in the final rinse water between various EWDs and disinfectants.

Apart from the use of neutralizers, sampling and culturing methods vary between studies due to different choices in sampling fluid, use of a flush-only or FBF technique, concentration of the sample through centrifugation or filtration and the duration of the incubation period (14). These different methods presumably contribute to the large dispersion in contamination rates found between different studies, with endoscope contamination rates ranging from 0% (15) to 75% (16). The choice of sampling and culturing method might also be an explanation why in some outbreak investigations the causative microorganism could not be found in cultures from the implicated duodenoscopes (17-21). This is also

demonstrated by the outbreak described by Aumeran et al., in which the causative microorganism could only be identified on the implicated duodenoscope after changing the sampling method from only a flush with a sterile saline solution to a brush with a neutralizing solution (22).

We believe that due to the fact that no neutralizer was used, many of the post-decontamination cultures of duodenoscope 1 and 2 showed false-negative results. It is unlikely that these duodenoscopes were so often contaminated only after drying but not immediately after decontamination. This is further substantiated by the observation that the other four duodenoscopes all had higher contamination rates after decontamination than after drying. This is in line with studies showing a reduction in contamination rate after effective drying (23-25). Cultures of the connection tubes of the drying cabinet were collected after the pilot phase, but did not show growth of any of the studied microorganisms. The striking difference in post-decontamination and post-drying culture results during the pilot phase made us reconsider the sampling methods and resulted in the modification of the sampling protocol before the start of phase 1 and 2 of the actual study.

The high contamination levels in the post-decontamination samples of the neutralizer group indicate that our reprocessing method is ineffective in removing these microorganisms. The fact that in two of these duodenoscopes were predominantly negative in the post-drying cultures, demonstrates the importance of drying. This could mean that the rinsing phase in our EWDs fails to flush all remaining microorganisms. Another possibility is that the sampling technique failed in these duodenoscopes more often. It is well recognised that bacteria from biofilms, are difficult to extract from channels with regular sampling methods, and could lead to false negative culture results (26).

In the duodenoscopes with high contamination percentages post-drying, we noticed that once an endoscope became positive for *P. aeruginosa* it remained largely positive during follow-up throughout the study, although with a few incidental negative samples. This seems to support the hypothesis of biofilm formation having occurred, which is difficult to remove once present (26). However, we have no definite proof of biofilm being present inside the duodenoscopes as this would require removal of the inner channels. We have no explanation why *P. aeruginosa* was overrepresented in the cultures. In the positive controls collected immediately after soiling and cultures directly collected from the soil, the four microorganisms were present in similar amounts. Biofilms mostly consist of multiple bacterial species (27). A theory could be that the *P. aeruginosa* strain used in the soil was more virulent than the other three microorganisms and outcompeted them. Another explanation could be that recovery bias caused under detection of the other microorganisms as some microorganisms might be more easily damaged during the sampling and culturing process. However, the sampling and culturing techniques are also used in clinical surveillance where in positive controls it has been proven that multiple species can be detected at once. The larger filter used in the study phase did not seem to impact the growth of *P. aeruginosa* as it was often found in the pilot and study phase. The other three microorganisms were rarely found during the pilot phase, but were recovered to some extent in the duodenoscopes used in the study phase. This could be the result of the neutralizer, but an effect of a larger filter cannot be excluded.

Alfa and Singh (3) report that only 19% of clinical studies on endoscope sampling used a neutralizer. The only study using DE broth is a study performed by Ofstead et al. (28) in which the drying efficacy of

different endoscopes in three endoscopy clinics was tested. They found that their drying methods were ineffective and 71% of the endoscopes harboured microorganisms. It is possible that studies with low contamination levels and outbreaks in which the causative microorganisms could not be found in duodenoscopes were based on false-negative cultures due to the absence of a neutralizing agent in the sampling method. We support the recommendation of Alfa and Singh (3) to use the CDC sampling and culturing protocol (4) in studies to increase the comparability of study results.

Limitations

The main limitation of this study is that it was not designed with the intention to investigate the difference between culture results of samples collected with and without neutralizer. Therefore, there was no sample size calculation performed for this purpose and some of the methods varied between the different study phases. This resulted in a lower number of samples collected without DE broth. Also, in the pilot phase, samples were collected either after decontamination or after drying, while in the first and second study phase, sample sets were collected at both moments after every round of reprocessing. Ideally, samples with and without DE broth would have been collected from the same endoscopes for a better comparison. Internal damage in used endoscopes, for example as induced by the passage of (metal) accessories through channels, can compromise cleaning and disinfection to different extents in individual endoscopes. Also, we only used six duodenoscopes, making statistical analyses difficult, especially since the measurements per duodenoscope are dependent on each other. Duodenoscope 1 was a different model than the other five duodenoscopes. This might have influenced the different results after decontamination between duodenoscope 1 and 2. In future studies we recommend to use identical endoscopes. Due to the non-clinical setup of the study with exposure of the duodenoscopes to large amounts of gut pathogens, the contamination rate of the used duodenoscopes is not comparable to that of clinically used duodenoscopes. However, this was beneficial for this study purpose since it provided a high contamination rate in both groups, making it possible to evaluate the added effect of DE broth to the yield of those cultures.

Conclusion

The results of this non-clinical experimental study show that adding DE broth to FBF samples collected from wet duodenoscopes increases the yield of those cultures. When samples are collected from wet duodenoscopes, the effect of any remaining disinfectants should be accounted for as they can lead to false-negative results. This can be overcome by the addition of a neutralizer such as DE broth. Controlled and preferably clinical studies are needed to support the findings of this study and investigate its applicability in daily practice.

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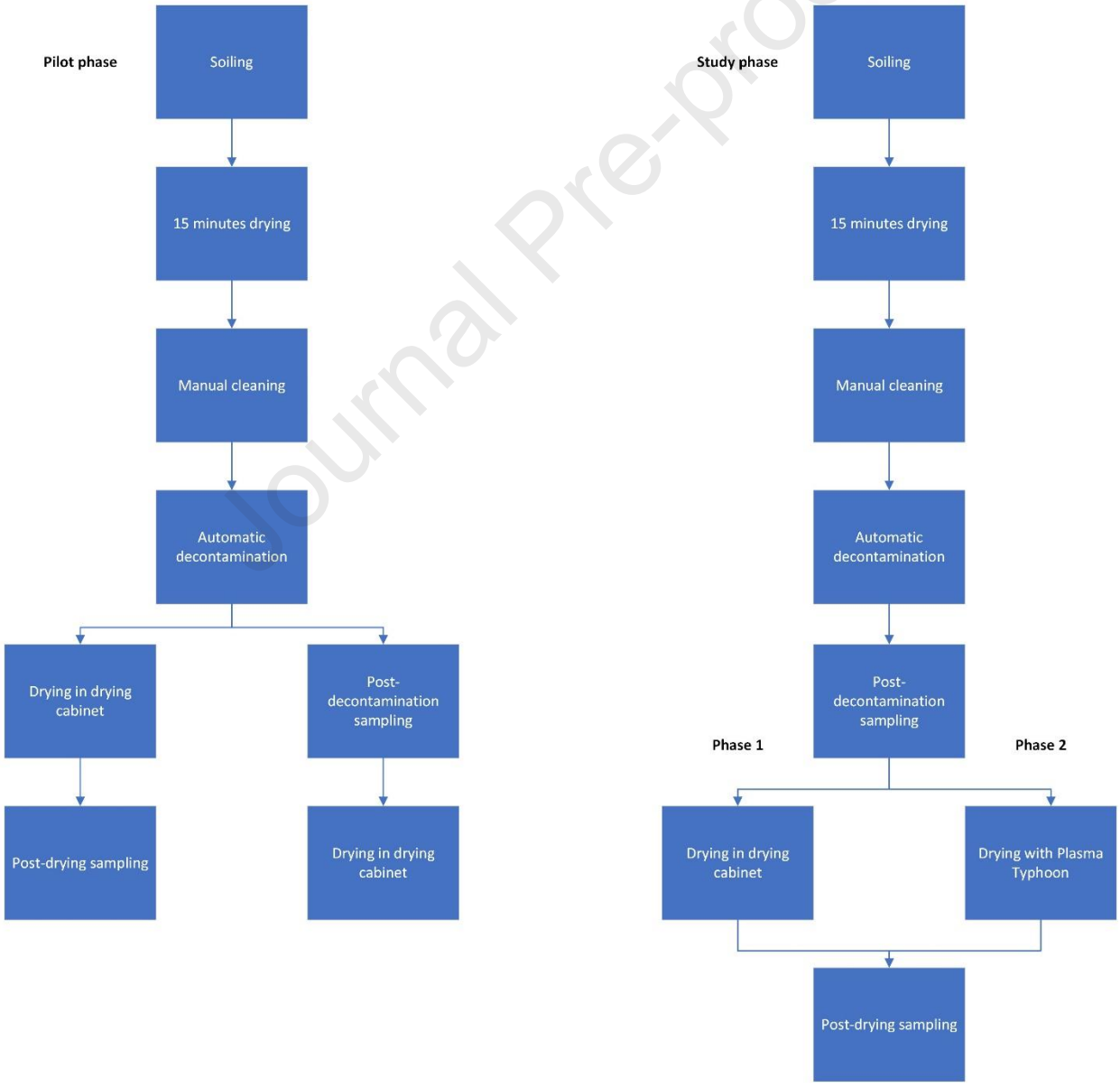
Tables and figures

	Study phase	No. of cultures post-decontamination	No. of cultures post-drying	Sample collecting method
Duodenoscope 1	Pilot	50	39	Without neutralizer
Duodenoscope 2	Pilot	39	31	Without neutralizer
Duodenoscope 3	Phase 1	60	60	With neutralizer
Duodenoscope 4	Phase 1 & 2	100	100	With neutralizer
Duodenoscope 5	Phase 1 & 2	100	100	With neutralizer
Duodenoscope 6	Phase 2	60	60	With neutralizer

Table I. Use of the six different duodenoscopes throughout the three phases of the study.

Figure 1: flowchart soiling, cleaning, decontamination and sampling during the different study phases.



	P. aeruginosa, n (%)	*K. pneumonia*, n (%)	*E. coli*, n (%)	*E. faecium*, n (%)	Any indicator microorganism, N (%)
Duodenoscope 1 (without neutralizer)					
Tip after decontamination (N=50)	0	0	0	0	0
Tip after drying (N=39)	0	0	0	0	0
Channel after decontamination (N=50)	14 (28.0%)	1 (2.0%)	0	0	15 (30.0%)
Channel after drying (N=39)	30 (76.9%)	0	0	1 (2.6%)	30 (76.9%)
Duodenoscope 2 (without neutralizer)					
Tip after decontamination (N=39)	0	0	0	1 (2.6%)	1 (2.6%)
Tip after drying (N=31)	0	0	0	0	0
Channel after decontamination (N=39)	3 (7.9%)	0	0	1 (2.6%)	3 (7.9%)
Channel after drying (N=31)	23 (71.9%)	0	0	0	23 (71.9%)
Duodenoscope 3 (with neutralizer)					
Tip after decontamination (N=60)	3 (5.0%)	1 (1.7%)	1 (1.7%)	0	3 (5.0%)
Tip after drying (N=60)	1 (1.7%)	0	0	0	1 (1.7%)
Channel after decontamination (N=60)	56 (93.3%)	24 (40.0%)	9 (15.0%)	0	57 (95.0%)
Channel after drying (N=60)	49 (81.7%)	3 (5.0%)	1 (1.7%)	0	49 (81.7%)
Duodenoscope 4 (with neutralizer)					
Tip after decontamination (N=100)	3 (3.0%)	1 (1.0%)	1 (1.0%)	0	3 (3.0%)
Tip after drying (N=100)	0	0	0	0	0
Channel after decontamination (N=100)	76 (76.0%)	39 (39.0%)	25 (25.0%)	11 (11.0%)	89 (89.0%)
Channel after drying (N=100)	4 (4.0%)	0	0	2 (2.0%)	6 (6.0%)
Duodenoscope 5 (with neutralizer)					
Tip after decontamination (N=100)	2 (2.0%)	2 (2.0%)	0	1 (1.0%)	4 (4.0%)
Tip after drying (N=100)	0	0	0	1 (1.0%)	1 (1.0%)

Channel after decontamination (N=100)	69 (69.0%)	35 (35.0%)	35 (35.0%)	5 (5.0%)	79 (79.0%)
Channel after drying (N=100)	7 (7.0%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	8 (8.0%)
Duodenoscope 6 (with neutralizer)					
Tip after decontamination (N=60)	3 (5.0%)	1 (1.7%)	2 (3.3%)	1 (1.7%)	5 (8.3%)
Tip after drying (N=60)	0	0	0	1 (1.7%)	1 (1.7%)
Channel after decontamination (N=60)	57 (95.0%)	6 (10.0%)	2 (3.3%)	1 (1.7%)	57 (95.0%)
Channel after drying (N=60)	44 (73.3%)	0	0	0	44 (73.3%)

Table II. Growth of the four applied microorganisms per sampling moment and sample site of each duodenoscope. Growth was defined as ≥1 CFU per microorganism.

	Flush-brush-flush negative	Flush-brush-flush positive	Total
Samples collected without addition of DE broth	71 (79.8%)	18 (20.2%)	89
Samples collected with addition of DE broth	38 (11.9%)	282 (88.1%)	320
Total	109 (26.7%)	300 (73.3%)	409

Table III. Post-decontamination flush-brush-flush results of all duodenoscopes combined. P<0.001

	Flush-brush-flush negative	Flush-brush-flush positive	Total
Samples collected without addition of DE broth	17 (24.3%)	53 (75.7%)	70
Samples collected with addition of DE broth	213 (66.6%)	107 (33.4%)	320
Total	230 (59.0%)	160 (41.0%)	390

Table IV. Post-drying flush-brush-flush results of all duodenoscopes combined. P<0.001

Conflict of interests

JK: Grant recipient Pentax Medical and Boston Scientific

MV: Grant recipient 3M, Pentax Medical and Boston Scientific

MB: Grant recipient 3M, Pentax Medical, Boston Scientific, Mylan and Interscope. Consultant Boston Scientific and Cook Medical.