

# A novel adjunctive cleansing method to reduce colony-forming units on duodenoscopes

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## Bibliography

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**Background and study aims:** Endoscopic retrograde cholangiopancreatography-related infections are of increasing global concern due to the emergence of multidrug-resistant bacteria such as carbapenem-resistant enterobacteriaceae (CRE), with bacterial biofilm production postulated as one cause of persistent infection from such virulent organisms. Because N-acetylcysteine (NAC) has been shown to possess antibacterial and biofilm-disruption properties, we aimed to evaluate if NAC would demonstrate clinical utility in reducing the colony forming units (CFU) at the elevator end of a duodenoscope, one of the hardest areas to clean.

**Patients and methods:** This was a pilot study of 16 procedures involving the use of a duodenoscope. After use, the elevator tip of a duodenoscope was cultured and submerged for 30 minutes, either in 20% NAC (200 mg/mL, intervention) or in sterile

water (control). After 30 minutes, the elevator tip was re-cultured.

**Results:** Submersion of the distal end of a duodenoscope in 20% NAC (200 mg/mL) for 30 minutes resulted in a statistically significant reduction in bacterial colony forming units compared to control (average reduction 41.6% vs 8.8%,  $P=0.001$ ). There was no visible damage and no optical distortion to the duodenoscope after submersion in NAC.

**Conclusions:** In summary, NAC may be a safe, simple, and useful adjunct to currently available methods of duodenoscope reprocessing. Further research may better define NAC's role in duodenoscope reprocessing, either broadly or specifically after procedures suspected to produce a high risk of bacterial contamination (e.g. choledocholithiasis).

## Introduction

Beginning with the first case of carbapenem-resistant enterobacteriaceae (CRE) infection in a North Carolina ICU in 1996 [1], reports of CRE infection have become increasingly frequent with a 4-fold incidence increase in 2001–2011 [2]. This is of grave concern not only because of general ineffectiveness to the carbapenem antibiotics which are the last line of antibiotics, but also the fact that CRE can be transmitted through endoscopic procedures such as endoscopic retrograde cholangiopancreatography (ERCP). The risk of ERCP-transmitted CRE infection has been highlighted in several recent outbreaks at academic medical centers both in the United States and abroad [2, 3]. Once bloodstream infection has occurred, CRE may result in over 40% mortality [4].

In response to CRE outbreaks, all three major duodenoscope manufacturers have submitted updated cleaning instructions to the Food and Drug Administration. The enhanced pre-cleaning steps

add to the complexity of duodenoscope washing, and as such, may be at risk for errors of omission and commission. In addition, some of the current methods of duodenoscope-related infection control (duodenoscope culture and 48-hour sequester, and ethylene oxide gas sterilization) may result in unacceptably increased capital and labor costs [5]. Similarly, liquid and gas-based sterilants have crucial technical and environmental considerations and limitations, including occupational health hazards from ethylene oxide gas, the potential for cosmetic or functional damage, and the ineffectiveness of the gas- and liquid-based chemical sterilants in penetrating biofilm and blood barriers (Centers for Disease Control and Prevention, Vital signs: Carbapenem-resistant enterobacteriaceae [2013]. In Internet: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6209a3.htm> [Accessed August 30, 2016]).

Thus, there is a need for safe, simple, and effective adjuncts to current endoscope cleaning methods. One such potential agent is N-acetyl-

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cysteine, a commonly-used mucolytic agent. Data exist suggesting that N-acetylcysteine (NAC) not only results in biofilm disruption, but also has been shown to be bactericidal against multiple bacterial pathogens including drug-resistant bacteria such as MRSA/VRE [6–8]. To our knowledge, NAC has not previously been used as an adjunct to current duodenoscope cleaning methods.

The objective of the current study was to evaluate the potential efficacy of NAC as an adjunct in reduction of bacterial colony forming units at the elevator tip of a duodenoscope, which has been implicated as a reservoir of persistent bacterial contamination. Our secondary aim was to identify clinical factors that may potentially impact the amount of bacterial contamination of a duodenoscope after routine clinical use.

## Patients and methods

### Study design and setting

The Kaiser Permanente Institutional Review Board approved this study. We performed a pilot study of 16 cases involving the use of a duodenoscope, from March to October 2015. All cases were performed at Kaiser Permanente, Los Angeles Medical Center. As the largest in-network tertiary referral center for Kaiser Permanente Southern California (KPSC), the endoscopy lab at Los Angeles Medical Center performs approximately 400 ERCP and over 500 EUS procedures annually.

### Cleansing protocol

#### Determination of soak time

Preliminary work to determine optimal NAC soak time was performed using 3 duodenoscopes. During the preliminary testing phase, after a duodenoscope's use, the duodenoscope elevator channel was cultured using a sterile cotton swab prior to any intervention ( $t=0$  min), and plated on commercially-available agar growth plates (Envirotest Media Paddles, QI Medical, Grass Valley, CA). The duodenoscope was then submerged in NAC 20% (200 mg/mL), and cultured in the same fashion at 5-minute intervals for 30 minutes. The optimal soak time, taking into consideration our unit workflow and policy requiring duodenoscope reprocessing within 1 hour of use, was determined to be 30 minutes (Table 1 and Fig. 1). The coefficient of determination for the series ( $R^2$ ) was 0.71–0.83.

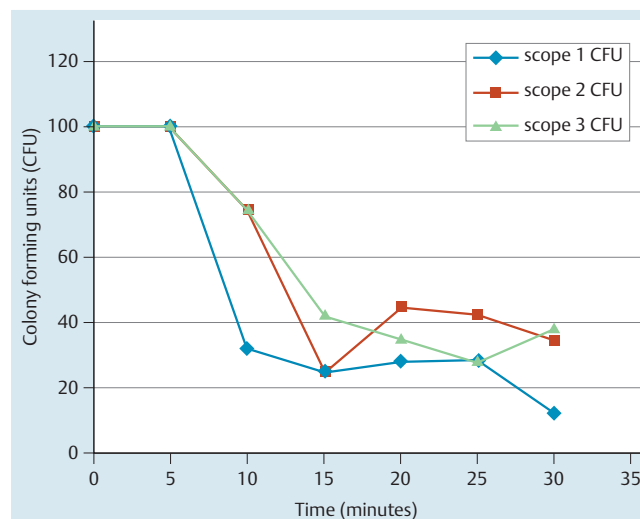
#### Testing phase

After a duodenoscope's use, the scope elevator channel was cultured using a sterile cotton swab ( $t=0$  min), and plated on commercially available agar growth plates (Envirotest Media Paddles, QI Medical, Grass Valley, CA). Subsequently, the duodenoscope tip was submerged either in a container of sterile water, or in a container of sterile NAC 20% (200 mg/mL, Hospira, Lake Forest, IL). After completion of tip submersion, the elevator channel was re-cultured using a second sterile cotton swab ( $t=30$  min), and scope reprocessing commenced in accordance with the most updated instructions available at the time of the study – specifically, those promulgated in the April 2015 Olympus customer letter (Anonymous, FDA Safety Community – New Reprocessing Instructions for Olympus Model TJF-Q180V Duodenoscopes [April 25, 2015]. In Internet: <http://fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm439999.htm> [Accessed April 15, 2015]).

**Table 1** Preliminary soak time evaluation.

Time	Procedure 1 (CFU)	Procedure 2 (CFU)	Procedure 3 (CFU)
0 min (control)	> 100	> 100	> 100
5 min post NAC	> 100	> 100	> 100
10 min post NAC	33	75	75
15 min post NAC	25	25	42
20 min post NAC	28	45	35
25 min post NAC	29	42	28
30 min post NAC	12	35	38

CFU: colony forming unit; NAC: N-acetylcysteine.



**Fig. 1** Preliminary work – duodenoscope culture results (determination of soak time).

## Materials and methods

The duodenoscopes used in the study represent three models across two endoscope vendors (Olympus TJF-160VF; Pentax ED-3470TK, ED-3490TK).

### Bacterial culture

The agar plates were incubated at 32° C to 35° C for 48 hours to 72 hours (Quincy Lab Model 10-140, Chicago, IL), and the CFU were counted in a semi-quantitative fashion by the lead pharmacist responsible for workspace sterility at LAMC's compounding pharmacy. The most optically dense portion of the agar plate was identified, and a 0.5×0.5-inch square was counted under a standard gooseneck 5×magnification lamp. Colony forming units above 100 were listed as > 100. To minimize potential bias, bacterial culture interpretation was performed in a blinded fashion.

### Data analysis

The primary outcome of interest was the change in number of colony forming units (CFU) at the elevator channel 30 minutes after soak time. Change in CFU from baseline was compared across study groups using analysis of covariance (ANCOVA). We used the ANCOVA analysis to test the relationship between use of NAC vs. placebo with CFU at 30 minutes as the dependent variable and CFU at 0 minutes as a covariate.

Secondary analyses included the potential impact of procedural indication on baseline scope CFU, and the impact of NAC submersion on scope functionality, as assessed by visible cosmetic or

Case number	NAC/control	Make of scope	Indication	Ductal cannulation	CFU at t=0 min	CFU at t=30 min
1	NAC	Pentax	CBD stone	Yes	>100	35
2	NAC	Pentax	Pancreatic cancer	Yes	13	8
3	NAC	Pentax	Cholangiocarcinoma	Yes	14	8
4	NAC	Olympus	Biliary dilation	No	>100	37
5	NAC	Pentax	Pancreatic cyst	No	59	49
6	NAC	Pentax	Pancreatic cyst	No	>100	55
7	NAC	Pentax	CBD stone	Yes	>100	73
8	Control	Pentax	CBD stone	Yes	>100	>100
9	NAC	Olympus	Pancreatic cyst	No	80	25
10	NAC	Olympus	Pancreatic cyst	No	38	29
11	Control	Olympus	Pancreatic cyst	No	>100	>100
12	NAC	Olympus	Biliary colic	No	>100	75
13	Control	Olympus	Biliary colic	No	>100	69
14	Control	Pentax	Cholangiocarcinoma	Yes	>100	78
15	Control	Pentax	CBD stone	Yes	>100	>100
16	Control	Pentax	CBD stone	Yes	>100	>100

CBD: common bile duct; CFU: colony forming unit; NAC: N-acetylcysteine.

physical damage to the duodenoscope, as well as evidence of any critical scope functional failures (e.g. failure on leak testing).

## Results

Sixteen duodenoscopes were included in this study: 6 in the control arm and 10 in the intervention arm. Duodenoscopes were used for a variety of indications, including ERCP for choledocholithiasis (CBD stone), pancreaticobiliary malignancy, and detailed ampullary inspection if during an EUS exam, the ampulla was poorly visualized on the echoendoscope's oblique-facing viewfinder (i.e. no ductal cannulation). The baseline CFU varied by indication (Table 2); however, cases involving choledocholithiasis consistently resulted in CFU > 100 at baseline.

The change in CFU based on intervention is presented in Table 2. CFU consistently dropped after 30-minute submersion in NAC compared to the control group (average reduction 41.6% vs 8.8%) (Fig. 2, boxplot). We found that there was a significant effect on using NAC vs. placebo on the duodenoscope on CFU at 30 min after removing the variance accounted for by CFU at 0 min ( $P=0.001$ ). After submersion in NAC, all scopes continued to pass leak testing, without visible damage and without optical distortion.

## Discussion

In this pilot study, we have demonstrated that simply submerging a duodenoscope elevator tip into N-acetylcysteine (NAC) for 30 minutes immediately following usage results in demonstrable CFU reduction prior to any standard cleansing procedures. Because NAC has both bactericidal and biofilm disruption properties, this adjunctive measure – performed in addition to any standard reprocessing step – may be of significant clinical utility as a method to enhance the scope cleansing process. This is most important particularly with regards to the elevator channel, which is arguably the most challenging duodenoscope location to clean.

The risk of ERCP-transmitted infections is not new, and has been previously reported in the literature [8–10]. However, persistent bacterial contamination of endoscopes, despite adherence to accepted reprocessing techniques, is an increasingly recognized

Table 2 CFU results.

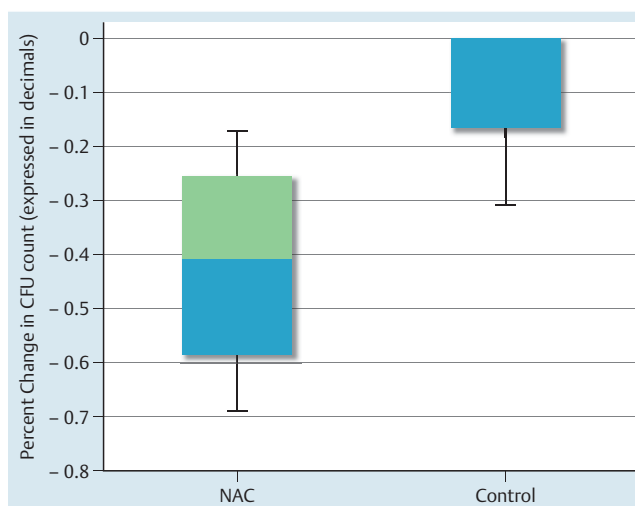


Fig. 2 Box plot of results (testing phase). The CFU reduction in the NAC arm was significantly more than the CFU reduction in the control arm (41.6% vs 8.8%,  $P=0.001$ )

phenomenon [11,12]. Currently available methods of scope reprocessing are problematic. During recent ERCP-related CRE outbreaks at academic referral hospitals in Washington state and California, persistent duodenoscope contamination required extraordinary measures such as mandatory scope sequestering and culture, as well as ethylene oxide gas sterilization, to arrest the outbreak. Unfortunately, there are economic, workplace safety, and bacteriologic limitations to such approaches, the most important of which is the failure of liquid- and gas-based sterilants to penetrate bacterial organisms in the presence of bacterial protective material such as serum and salt (Centers for Disease Control and Prevention, Rutala WA, Weber DJ, and the Healthcare Infection Control Practices Advisory Committee [HICPAC], Guideline for disinfection and sterilization in healthcare facilities, 2008 [2008]. In Internet: [http://www.cdc.gov/hicpac/pdf/Disinfection\\_Sterilization/Pages1\\_2Disinfection\\_Nov\\_2008.pdf](http://www.cdc.gov/hicpac/pdf/Disinfection_Sterilization/Pages1_2Disinfection_Nov_2008.pdf) [Accessed August 30, 2016], 8 and 13).

One possible explanation for such failures is the bacterial formation of biofilm. Once free-floating (planktonic) bacteria are able to take root and adhere to a surface, biofilm production begins,

which is a complex mixture of proteins and exopolysaccharide (EPS) matrix [11, 14, 15]. This ultrastructure is highly advantageous to bacterial colony survival; not only can it result in up to 1000-fold antibacterial resistance, but also may serve as a reservoir through which bacteria can intermittently be released back into circulation as a planktonic form. This has gained urgency due to the emergence of multidrug resistant organisms such as duodenoscope-transmitted CRE, as this may result in over 40% mortality.

A modern duodenoscope is an intricate medical device. Due to narrow-lumen channels, complex elevator mechanisms, and more recently, attempts at sealing off the elevator wire mechanism, there are blind spots that make it exceptionally difficult to consistently provide effective cleansing, especially since microscopic deposits of biomatter as small as 122µm×90µm have been shown to harbor bacterial microorganisms [3, 12]. This phenomenon was observed during the Washington state outbreak of duodenoscope-associated CRE infections, wherein 3% of duodenoscopes continued to harbor pathogenic organisms after being reprocessed to specifications that met or exceeded manufacturer-reprocessing recommendations [16].

NAC is an N-acetyl derivative of a naturally-occurring amino acid, L-cysteine (FDA package insert). This molecule's mucolytic properties are derived from its free sulfhydryl group, which breaks disulfide bonds in mucus resulting in lowered viscosity. Although it is not an antibiotic, NAC possesses both antibacterial and biofilm-disruptive properties, and shows potential of detaching already-attached bacteria on stainless steel surfaces (Centers for Disease Control and Prevention, Rutala WA, Weber DJ, and the Healthcare Infection Control Practices Advisory Committee [HICPAC], Guideline for disinfection and sterilization in healthcare facilities, 2008 [2008]. In Internet: [http://www.cdc.gov/hicpac/pdf/Disinfection\\_Sterilization/Pages1\\_2Disinfection\\_Nov\\_2008.pdf](http://www.cdc.gov/hicpac/pdf/Disinfection_Sterilization/Pages1_2Disinfection_Nov_2008.pdf) [Accessed August 30, 2016] and 17–19). NAC has several advantages as an inexpensive, nontoxic, widely available agent used across multiple medical disciplines including pulmonology and gastroenterology. It carries an excellent safety profile. As of January 2016, a 10-year search of the FDA MAUDE database (Food and Drug Administration Center for Devices and Radiologic Health) was performed to determine potential complications of a specific technology, using search terms “NAC,” “acetylcysteine,” “duodenoscope,” “endoscope,” “ERCP,” & “mucomyst” in various combinations. No records exist of endoscope damage resulting from NAC use.

There were some limitations to the current study. First, the distal elevator tip was cultured using a sterile cotton swab, to facilitate plating onto agar plates. There is a possibility that the physical act of culturing may have manually debrided some of the inoculum from the elevator channel. However, we believe this did not negatively impact the results, as all 16 scopes in this study were cultured in the same fashion.

Second, the CFU count was semi-quantitative, performed by a lead pharmacist in charge of workspace sterility at our center's compounding pharmacy. Colony counts above 100 CFU were recorded as >100 CFU. Thus, it is possible the magnitude of CFU reduction could have been greater in the NAC controls.

Third, our study was not designed to differentiate between “high concern” and “low concern” organisms as proposed by the Centers for Disease Control (CDC). Nonetheless, background literature has shown that NAC carries bactericidal/biofilm disruption activity against clinically significant enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* [20].

There are potentially significant clinical implications to the current study findings. It is now appreciated that a comprehensive, multimodality approach (including proper pre-cleaning, brushing, high-level disinfection, and drying) is required for consistent, effective duodenoscope reprocessing. However, the current methods of duodenoscope reprocessing are complex, require properly timed steps and repetitions, and demand significant attention from multiple individuals responsible for the duodenoscope's reprocessing to prevent errors of omission or commission. The ability for a single chemical agent to show significant bactericidal activity without the need for repetitive and cumbersome maneuvers can result in a substantial workflow improvement as well as a crucial enhancement to patient safety. Unlike pre-cleaning steps that require specialized adaptors and carefully timed and numbered steps, submersion of the elevator tip is a “set and forget” intervention, with a lower likelihood of incorrect performance. At a minimum, it is conceivable that NAC submersion may add an additional measure of safety if instituted alongside the currently accepted steps of duodenoscope pre-cleaning, as a method of “jump starting” the cleaning process through its bactericidal and biofilm disruption properties.

## Conclusion

In summary, in this pilot study, NAC appeared to be a safe and potentially effective adjunct to current methods of duodenoscope precleaning. ERCP procedures involving choledocholithiasis appear to consistently yield elevated CFU at baseline. Further research is warranted to validate the current study findings and refine the potential role of NAC as an adjunctive agent in the reprocessing of duodenoscopes.

**Competing interests:** None

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