Disinfection of Rigid Nasal Endoscopes Following In Vitro Contamination With *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*

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**Importance:** If not adequately cleaned, rigid nasal endoscopes (RNEs) have the potential to cause iatrogenic cross-contamination.

**Objective:** To test the efficacy of various disinfection methods in reducing bacterial load on RNEs in vitro.

**Design and Setting:** In vitro model.

**Interventions:** *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* contamination was separately induced on RNEs in vitro. Two experimental sets were completed. The RNEs were disinfected using the following protocols: 30-second scrub with antimicrobial soap (ABS) and water, 30-second scrub with 70% isopropyl alcohol (IA), 30-second scrub with ABS followed by 30-second scrub with IA, 30-second scrub with germicidal cloth, isolated 5-minute soak in an enzymatic soap solution, 5- and 10-minute soaks in ortho-phthalaldehyde, 0.55%, solution (Cidex OPA), and isolated 30-second rinse with tap water. All with 30-second precleaning and postcleaning rinses with tap water. Two sets of experiments (experiment sets A and B) were carried out with a 30-second tap water rinse after inoculation of each RNE. This was followed by immediate cleaning in set A and a 1-hour air-dry delay in set B. Otherwise there were no differences in the disinfection protocols between sets for each method noted.

**Main Outcomes and Measures:** Effectiveness of various disinfection protocols in cleaning rigid nasal endoscopes experimentally inoculated with bacteria commonly found in the upper aerodigestive tract. Positive cultures following disinfection indicated ineffective or incomplete disinfection.

**Results:** Most cleaning methods were effective in eliminating *S aureus*, *S pneumoniae*, and *H influenzae* from the scopes following experimental contamination. Continued growth of *P aeruginosa* was found after all of the disinfection trials in experiment set A with the exception of a 10-minute immersion in Cidex OPA, and in set B except for the 10-minute Cidex OPA immersion and ABS plus IA trials.

**Conclusions and Relevance:** Most cleaning methods used in our trials appear to properly disinfect RNEs after in vitro inoculation with *S aureus*, *S pneumoniae*, and *H influenzae*. However, it appears that disinfectants may be less effective in cleaning rigid scopes experimentally inoculated with *P aeruginosa*. There is a paucity of published data regarding cross-contamination during rigid nasal endoscopy, and these results should guide future studies and to some extent practice to avoid iatrogenic spread of contamination.


For Otolaryngologists, endoscopic examination of the upper aerodigestive tract plays an important role in the management of patients presenting with sinonasal complaints. In particular, rigid nasoendoscopes (RNEs) are often an essential component of the physical examination in both the clinic and inpatient settings for a wide variety of indications, including initial identification of disease in patients experiencing sinonasal symptoms, evaluation of patient response to medical treatment, evaluation and biopsy of nasal masses or lesions, and evaluation and treatment of epistaxis. In a busy tertiary care center, it is not uncommon for the same RNE to be used and reprocessed several times a day between examinations of multiple patients. If not adequately cleaned, these scopes have the potential to cause iatrogenic cross-contamination.
contamination in subsequent patients. The medical literature published during the last decade demonstrates that the risk of endoscopy-related cross-contamination is low and that most known outbreaks are related to inadequate reprocessing practices. Furthermore, the literature citing cases of cross-contamination from rigid endoscopy is chiefly gleaned from other fields such as gastroenterology and pulmonology. However, we believe that the paucity of published data regarding cross-contamination during rigid nasal endoscopy and the lack of standard disinfection protocols for RNEs warrant further study.

During the 1960s, Spaulding devised a 3-tiered classification system for levels of disinfection and sterilization of patient care equipment required to prevent infection: critical, semicritical, and noncritical devices. This classification scheme is accepted by the Centers for Disease Control and Prevention and the US Food and Drug Administration (FDA) and can be described briefly as follows: Critical devices are those that enter sterile tissue or the vascular system and must be sterilized (eg, scalpel, cardiac or urinary catheters). Semicritical devices do not penetrate sterile tissue but come into contact with mucous membranes (eg, rigid nasal endoscope, flexible fiberoptic laryngoscope, bronchoscope) and must, at a minimum, be disinfected at a high level. Noncritical devices do not touch the patient or touch only intact skin (eg, stethoscope) and must be cleaned followed by low-level disinfection.

Because RNEs are considered semicritical devices, high-level disinfection is required, and immersion is currently the most cost-effective and rapid technique used to disinfect RNEs. Though there are a variety of different strategies for achieving high-level disinfection, including both manual and automated endoscope reprocessing, a large variety of types and models of endoscopic equipment exists, and no standardized protocol has been developed by professional organizations within the field of otolaryngology. A review of the current medical literature demonstrates little data to assist clinicians in selecting the best cost-effective method of reprocessing rigid nasal endoscopes. The purpose of this study is to test the efficacy of various manual disinfection methods in reducing bacterial load on RNEs in vitro after inoculation with several common sinonasal microorganisms.

METHODS

The RNE used in our study was a non–channel-containing rigid nasal endoscope (Medtronic). To serve as a negative control and as a method of ensuring sufficient disinfection after each experimental trial, the RNE endoscope was rinsed with tap water for 30 seconds and subsequently immersed in an enzymatic soap solution (Enzol; Johnson & Johnson) for 5 minutes followed by a 20-minute immersion in a solution of ortho-phthalaldehyde, 0.55%, solution (Cidex OPA; Johnson & Johnson).

For the experimental protocols, a clean RNE was placed for 30 seconds in a 0.1 spectrophotometric solution of our respective bacterial cultures (approximately 8-10 microorganisms/mL), spectrophotometric wavelength, 570 nm by Bausch and LombSpectronic 20): Staphylococcus aureus (SA) (ATCC 27660), Streptococcus pneumoniae (SP) (LLUMC Clinical Laboratory Strain), and Pseudomonas aeruginosa (PA) (LLUMC Clinical Laboratory Strain) previously cultured on trypticase soy agar plates with sheep blood, 5% (blood agar plates), and Haemophilius influenza (HI) (LLUMC Clinical Laboratory Strain) previously cultured on GC II Agar with Hemoglobin and IsoVitalex (Becton Dickinson) (chocolate agar plates). The end of the scope was subsequently swabbed with a sterile cotton-tipped applicator. The SA, SP, and PA samples were plated onto a BBL agar plate (Becton Dickinson), and the HI sample was plated onto a chocolate agar plate. These served as our positive controls.

Two sets of experiments (experiment sets A and B) were carried out with a 30-second tap-water rinse after inoculation of each scope. This was followed by immediate cleaning in set A and a 1-hour air-dry delay in set B. Otherwise there were no differences in the disinfection protocols between sets for each method noted. The experimental disinfection protocols included a 30-second scrub with antimicrobial soap (ABS) (Equate; Vi-Jon Laboratories), 30-second scrub with isopropyl alcohol, 70% (IA) (Aron), 30-second ABS-and-water scrub followed by 30-second IA scrub, 30-second scrub with germicidal cloth (Steris), 5-minute immersion in enzymatic soap solution, and 5- and 10-minute immersions in Cidex OPA. The same experimental protocols were also carried out with immersion into a 0.1 spectrophotometric solution of HI (ATCC 10211) previously cultured on chocolate agar plate, which was placed onto Chocolate II Agar reagent (Becton Dickinson). All plates were placed in a 37°C incubator (HI in an atmosphere containing 5% carbon dioxide) and examined at 24 and 48 hours, and colony counts were performed. Each different disinfection protocol with each organism was performed 4 separate times for both experiment sets A and B.

RESULTS

**STAPHYLOCCUS AUREUS**

Negative controls were always negative and positive controls were always positive. Positive control plates of SA, PA, SP, and HI were always too numerous to count (TNTC). In experiment set A, SA growth averaged 4 colony-forming units (CFUs) after isolated 30-second tap-water rinse on 3 of 4 plates (Table 1), and in experiment set B, it averaged 2 CFUs after 30-second ABS scrub on 1 of 4 plates (Table 2). No growth was achieved with the remaining disinfection trials.

**STREPTOCOCCUS PNEUMONIAE**

In experiment set A, SP growth demonstrated 3 CFUs after 30-second tap-water rinse on 1 of 4 plates (Table 1), and in experiment set B, it averaged 1 CFU after 30-second germicidal cloth scrub on 1 of 4 plates (Table 2). No growth was achieved with the remaining disinfection trials.

**PSEUDOMONAS AERUGINOSA**

In experiment set A, PA growth averaged 8 CFUs after 30-second ABS scrub on 2 of 4 plates, 1 CFU after 30-second germicidal cloth scrub on 1 of 4 plates, 2 CFUs after 30-second IA scrub on 2 of 4 plates, 12 CFUs after isolated 30-second tap water rinse on 4 of 4 plates, 1 CFU after ABS plus IA scrub on 1 of 4 plates, 4 CFUs after 5-minute immersion in enzymatic soap solution on 2 of 4 plates, and 7 CFUs after 5-minute immersion in Cidex OPA on 1 of 4 plates. No growth was achieved with 10-minute immersion in Cidex OPA (Table 1). In experi-
Table 1. Results of the Various Disinfection Techniques in Experiment Set A

<table>
<thead>
<tr>
<th>Disinfection Technique</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pneumoniae</th>
<th>Pseudomonas aeruginosa</th>
<th>Haemophilus influenzae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antimicrobial soap scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 8 CFUs in 2 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>IA scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 2 CFUs in 2 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Antimicrobial soap and water scrub, 30 seconds, followed by IA scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 1 CFU in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Germicidal cloth scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 1 CFU in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Immersion in enzymatic soap solution, 5 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 4 CFUs in 2 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Immersion in Cidex OPA, 5 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 7 CFUs in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
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<tr>
<td>Immersion in Cidex OPA, 10 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Isolated tap water rinse, 30 seconds</td>
<td>+ For 4 CFUs in 3 of 4 plates</td>
<td>+ For 3 CFUs in 1 of 4 plates</td>
<td>+ For 12 CFUs 3 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
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</tbody>
</table>

Abbreviations: Cidex OPA, ortho-phthalaldehyde, 0.55%, solution (Johnson & Johnson); CFU, colony-forming unit; IA, isopropyl alcohol, 70%; +, positive findings on culture; -, negative findings on culture.

The set A protocol did not include a 1-hour air drying period prior to disinfection; bold typeface indicates disinfection protocols in which all culture results were negative.

Table 2. Results of the Various Disinfection Techniques in Experiment Set B

<table>
<thead>
<tr>
<th>Disinfection Technique</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pneumoniae</th>
<th>Pseudomonas aeruginosa</th>
<th>Haemophilus influenzae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
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<td>0</td>
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<tr>
<td>Negative</td>
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<tr>
<td>Antimicrobial soap scrub, 30 seconds</td>
<td>+ For 2 CFUs in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>IA scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Antimicrobial soap and water scrub, 30 seconds, followed by IA scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Germicidal cloth scrub, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 1 CFU in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Immersion in enzymatic soap solution, 5 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 1 CFU in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Immersion in Cidex OPA, 5 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 2 CFUs in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
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<tr>
<td>Immersion in Cidex OPA, 10 minutes</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
<tr>
<td>Isolated tap water rinse, 30 seconds</td>
<td>No growth in 4 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
<td>+ For 4 CFU in 1 of 4 plates</td>
<td>No growth in 4 of 4 plates</td>
</tr>
</tbody>
</table>

Abbreviations: Cidex OPA, ortho-phthalaldehyde, 0.55%, solution (Johnson & Johnson); CFU, colony-forming unit; IA, isopropyl alcohol, 70%; +, positive findings on culture; -, negative findings on culture.

The set B included a 1-hour air drying period prior to disinfection; bold typeface indicates disinfection protocols in which all culture results were negative.

In both experimental sets A and B, no growth was achieved with any of the disinfection trials (Tables 1 and 2).

HAEMOPHILUS INFLUENZAE

Preventing endoscopic cross-contamination of infectious materials between patients is of primary impor-
tance in clinical practice. Therefore, practitioners, medical assistants, and others working in an outpatient otolaryngology setting should be trained in and adhere to accepted recommendations for infection prevention and control.15,16 Because there is currently no consensus among the otolaryngologic community regarding a standard protocol for disinfection of RNEs, we believe that it is sensible to obtain the most current information regarding these techniques from the FDA website and independent peer-reviewed publications.17,18

The results of our study show that disinfecting RNEs inoculated with SA, SP, or HI with either ABS, IA, germicidal cloth, a 5-minute immersion in enzymatic soap solution, or a 5- or 10-minute immersion in Cidex OPA was mostly effective in disinfecting RNEs sufficiently to result in negative cultures. We chose not to complete longer immersions in Cidex OPA because all cultures with this agent were negative at 10 minutes. In contrast to SA, SP, and HI, we observed that PA, an organism with tenacious adherence properties, proved to be more resistant to bactericidal techniques than the other microbes we tested. Interestingly, there was a marked reduction in positive PA cultures in experiment set B. Allowing the inoculated scopes to air dry for 1 hour prior to disinfection to simulate clinical practice may have decreased the bacterial load compared with the trials in experiment set A. With insufficient time for PA to maximally adhere or form a biofilm in an ideal environment, air drying the scope between cleanings could feasibly reduce the chances of nosocomial PA infection when using scopes with multiple consecutive patients.19

Based on the results of this and previous studies, we do not advocate using ABS, IA, or germicidal cloth alone if a more complete high-level disinfection is possible. In our clinic, we typically use, at minimum, a 5-minute enzymatic soap immersion followed by a 20-minute immersion in Cidex OPA. Our results are in alignment with the 2008 FDA guidelines for endoscope disinfection,15 in which a soak in Cidex OPA for 12 minutes at 20°C is an approved disinfection method. The other methods used in this study, however, are nonstandard by FDA guidelines for high-level disinfection and were studied because the microbicidal efficacy of these chemical disinfectants on RNEs is not well characterized in the literature. Though this study demonstrates that the involved disinfection techniques were mostly effective in preventing positive growth with SA, SP, and HI, the shorter, simpler disinfection techniques should not be routinely used in clinical practice because they are not sufficient to achieve a comprehensive high-level disinfection for RNEs. Furthermore, approved automated endoscope reprocessors have been shown to be effective in disinfection of semicritical devices but were not used in this study.

We chose to test 4 clinically relevant microorganisms known to be ubiquitous in the nasopharyngeal tract and sinuses. Furthermore, as in our group’s previous study with flexible fiberoptic laryngoscopes,24 we opted to inoculate the RNE for 30 seconds with each microorganism because that is the maximum duration for the vast majority of procedures performed by otolaryngologists using an RNE. Because this study did not include all of the possible bacteria, fungi, or viruses that may be found within the upper aerodigestive tract, it is somewhat limited. Also it cannot be known whether transmission of the organisms included in this study would result in actual disease, a variable that would theoretically be specific to both the patient from whom the organism came and the patient being infected through accidental cross-contamination.

Another potential limitation of our study is the relatively small number of trials for each of the experiments and organisms (a total of 8 trials were performed for each of the 8 disinfection techniques for all 4 organisms). This allows for an increased probability that some of the results are owing to chance. Similar studies should be repeated with a greater sample size and statistical analysis to minimize potential errors.

As has been suggested in previous studies, further investigations are warranted for the analysis of organisms that are notoriously difficult to eradicate, such as Mycobacteria and viruses.14 In this study, we chose HI as a target organism for our disinfection experiments. Because it is a relatively fastidious organism that can be challenging to culture, it is feasible that errors in the culturing of this organism could limit the interpretation of the results. Future studies including automated endoscope reprocessing or another rapid sterilization method would also be desirable. It should also be noted that entrapment of organisms in mucus or crusts that may adhere to the RNE during endoscopy of a patient, which was not duplicated in these studies, could further limit the applicability of our results. The disinfectant methods herein described did not include scrubbing or removal of gross debris.

In conclusion, a 30-second predisinfection rinse using running tap water is important for decreasing bioload on contaminated RNEs. Thirty-second ABS-and-water scrub, 30-second IA treatment, 30-second ABS-and-water scrub followed by 30-second IA scrub, 30-second germicidal cloth scrub, 5-minute immersion in enzymatic soap solution, and 5- and 10-minute immersions in Cidex OPA were all mostly effective at disinfecting a RNE contaminated in vitro with SA, SP, and HI. However, it appears that disinfectants may be less effective in cleaning rigid scopes experimentally inoculated with PA.
Conflict of Interest Disclosures: None reported.
Previous Presentation: This study was presented as an oral presentation at the Combined Otolaryngology Spring Meetings, American Head & Neck Society; April 10, 2013; Orlando, Florida.
Additional Contributions: The authors would like to thank Bill Keeler in the Loma Linda University Department of Microbiology for his valuable ideas and suggestions and for his guidance in the lab throughout this project.

REFERENCES
6. Nelson DB. Hepatitis C virus cross-infection during endoscopy: is it the “tip of the iceberg” or the absence of ice? Gastrointest Endosc. 2007;65(4):589-591.