

The Clinical Significance of Nasal Irrigation Bottle Contamination

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Objectives/Hypothesis: This study aimed to assess the clinical relevance of contamination of nasal irrigation bottles in patients with recalcitrant chronic rhinosinusitis (CRS). Secondary investigations to identify the presence of bacterial biofilms on the inner surface of the bottles and to assess different sterilization methods were also undertaken.

Study Design: Prospective, observational.

Methods: Eleven patients with recalcitrant CRS who were already using nasal irrigation as part of their treatment regimen were examined every 2 weeks for a period of 6 weeks. At each visit, a culture sample was taken from their irrigation bottle and middle meatus, and they were given a new irrigation bottle. Irrigation bottles from six patients were analyzed with scanning electron microscopy (SEM) to detect biofilm formation. Finally, new bottles were inoculated with different strains of *Staphylococcus aureus* and then cleaned with different methods. The bottles were cultured immediately after cleaning and 48 hours later.

Results: Overall, 42 of 43 (97%) bottles collected demonstrated bacterial growth. Concurrent sinonasal and bottle infection with *S. aureus* was seen in 51% of patients during the study. Bacterial biofilms were demonstrated on the inner surface of four of the six irrigation bottles tested. Treatment with Milton's solution (1% NaOCl plus 19% NaCl) and microwaving were found to be effective methods for sterilizing the bottles both initially after the cleaning and 48 hours later.

Conclusions: Patients who irrigate their nose and sinuses commonly contaminate their irrigation bottle, most often with *S. aureus*, which can be in the biofilm form. Simple cleaning methods could reduce contamination of the bottles.

Key Words: Chronic rhinosinusitis, *Staphylococcus aureus*, biofilms, nasal irrigation, bacteria, nasal douche.

Level of evidence: 2c.

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INTRODUCTION

Saline nasal irrigations are now a key component of the medical management of chronic rhinosinusitis (CRS) and are used before and after endoscopic sinus surgery (ESS). A Cochrane review of eight studies evaluating the effectiveness of nasal irrigations concluded that this modality is a worthwhile adjunct therapy.¹ Furthermore, studies have demonstrated that nasal irrigation is the best method for irrigating the paranasal sinuses and that large-volume, low-pressure nasal irrigation is more effective than saline sprays or nebulizers for penetration of sinus ostia and irrigation of the sinus.^{2,3}

Despite their frequent use and proven efficacy, the potential harm of nasal irrigations has only recently been considered. Williams et al., in a nonclinical study, first investigated whether nasal irrigation devices could become contaminated with bacteria. Although no patients were used in the study, simulated flushing with solution still yielded positive cultures of pseudomonas species. They hinted toward a possible role for biofilms in this process.⁴

Welch et al. investigated whether irrigation bottles used in the postoperative period could become infected. Twenty post-ESS patients were enrolled and examined at 1, 2, and 4 weeks after ESS. Overall, 29% of all bottles demonstrated bacterial growth, 25% within 2 weeks and 45% in the last collection period. However, there was a significant dropout rate of 45% in the study, which may have resulted in underreporting of this issue. None of the patients demonstrated signs of infection or had sinus swabs taken.⁵

Thus the question regarding the clinical relevance of nasal irrigation bottle contamination remains

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unanswered. The principle aim of the present study was to assess the clinical relevance of nasal bottle contamination in patients with recalcitrant CRS. Secondary investigations to identify the presence of bacterial biofilms on the inner surface of the bottles and assess different sterilization methods were also undertaken.

MATERIALS AND METHODS

Study Design and Population

The study design was approved by our institution's human ethics committee, and patients were recruited prospectively from the rhinology practice of the senior author (P.-J.W.). Eleven patients with persistent CRS symptoms despite maximal medical (including systemic antibiotic therapy) and surgical treatment who were also using a nasal irrigation bottle at least once per week were recruited. Patients were excluded if they had undergone recent nasal surgery. Informed consent was obtained.

Part 1: Clinical Irrigation Bottle Study

At the initial appointment, the patient's current irrigation bottle was collected, and the patient was provided with a new Sinus Rinse bottle (NeilMed Pharmaceuticals, Santa Rosa, CA). The inner surface of the bottle and residual irrigation fluid were swabbed and cultured in the microbiology laboratories of our institution. During the same appointment, an endoscopically guided middle meatal swab was taken.

The patients were asked to return to the outpatient department every 2 weeks for 6 weeks and thus were seen a total of four times during the study period. At each appointment, the irrigation bottle was exchanged, the old bottle was swabbed, and a middle meatal swab was taken.

Clinical data were also collected from the patients. These data included visual analog symptom (VAS) scoring; quality of life assessment using the 20-Item Sino-Nasal Outcome Test (SNOT-20) questionnaire; and questions to ascertain bottle usage, cleaning methods used during the previous 2 weeks, and antibiotic use during the study period.

Part 2: Scanning Electron Microscopy

Irrigation bottles from six patients in this study were analyzed with scanning electron microscopy (SEM) for the presence of bacteria and evidence of biofilm formation. A randomly selected 8 × 8-mm sample of each bottle was cut and then fixed for 1 hour in 4% paraformaldehyde/1.25% glutaraldehyde in phosphate-buffered saline (PBS) with 4% sucrose (pH = 7.2). The fixed samples were washed for 10 minutes in PBS and 4% sucrose before undergoing postfix in OsO₄ in PBS for 1 hour. Following this procedure, samples were dehydrated progressively in 70%, 90%, and 100% ethanol. Critical point drying then occurred, and prepared samples were mounted and coated with carbon and gold.

The prepared and coated samples were imaged using field-emission SEM (Philips XL30; Philips; Amsterdam, The Netherlands) for secondary electron detection with beam voltage set at 10 kV and magnifications ranging from 50× to 20,000×. Representative images of the inner surface of the bottle were obtained and analyzed for presence of bacteria as well as features indicative of biofilm formation (i.e., grouped bacteria, surrounding matrix, and surface attachment).

Part 3: In Vitro Bottle Cleaning

The effectiveness of five commonly used sterilization techniques were assessed using *Staphylococcus aureus* reference strains as well as clinical isolates of *S. aureus*. Four groups of six NeilMed Sinus Rinse bottles were inoculated with four different strains of *S. aureus* in 30 mL of purified water for 24 hours in normal room conditions. Reference strains ATCC 25923 and ATCC 29213, a clinical *S. aureus* isolate from a study patient, and a methicillin-resistant *S. aureus* strain isolated from another study patient were used. Bottles were then cleaned using five different methods: 1) washed out with cold water, 2) washed out with boiling water, 3) cleaned with detergent, 4) cleaned with Milton's antibacterial solution of 1% NaOCl plus 19% NaCl (Probiotec Ltd, Laverton North, Vic, Australia), or 5) disinfected by washing out the bottle with cold water and placing the microwavable bottle in the microwave for 120 seconds. One bottle was left with inoculated irrigation fluid in it as a control. The bottles were swabbed and cultured immediately after cleaning and then were swabbed again 48 hours after cleaning.

Statistics

Comparative data were collected and analyzed by using SPSS 16.0 software (SPSS Inc., Chicago, Illinois). The data were considered to be nonparametric. The Wilcoxon signed rank test and Mann-Whitney *U* test were used for comparison of the ordinal data we collected in this study.

RESULTS

Demographics

There were 11 patients in this study (six males, five females) with a median age of 63 years (interquartile range, 56–72). Aside from one patient who did not attend the 6-week appointment, the follow-up in this study was complete.

Clinical data recorded demonstrated a significant difference in VAS between the time of the initial test and week 2 ($P = .013$). The SNOT-20 scores approached significance in the same period. There was no significant difference in VAS and SNOT-20 scores between the subsequent weeks. Furthermore, we did not find significant differences in VAS or SNOT-20 scores between those patients with recurrent contamination of their bottles and those without. However the numbers in this study may be too small to demonstrate significant differences. Patients reported using several different methods to clean their bottles, with washing under boiling water being the most common technique, used by six of 11 patients (54%). Two patients washed out their bottles with tap water alone. Other methods used by one patient each were washing in a sanitizing powder, washing with detergent, and washing with Milton's antibacterial solution.

Microbiology

During the study, 43 bottles were collected, and the results of the microbiologic evaluations of the bottle and nose are shown in Table I and Table II. Significantly, 42 of 43 (97%) bottles collected during the study demonstrated bacterial growth of some sort. The various types of bacteria that were cultured from the nasal irrigation bottles can be seen in Table II, the most common being *S. aureus* (29 of 43 bottles [67%]).

TABLE I.
Microbiology Results From Patients' Bottles and Nasal Swabs.

Patient No.	Week 0		Week 2		Week 4		Week 6	
	Bottles	Nose	Bottles	Nose	Bottles	Nose	Bottles	Nose
1	—	Sa	Sa	Sa	Sa	Sa	Sa, Ss	—
2	Ps, Cl	—	Ss, Ps	—	Sa, Ps, Cl	—	Sa, Cl, Ps	Rf
3	Cl	Sa	Ss, Cl	Sa	Sa, Ss, Ps, Cl	Sa	Sa, Ss, Cl	Sa
4	Ss	Sa	Sa, Ss	Sa	Sa, Ss, Ca	Sa		
5	Ss, Ps, Cl	Rf	Ss, Cl, Sf	—	Ss, Cl	—	Sa	Ea
6	Sa, Ss, Ps, Cl	Sf	Sa, Ps	Sa	Sa, Ps	Sa	Sa, Ps	Sa
7	Sa, Ps	Sa	Sa, Ps	Sa	Sa, Cl	Sa	Ps, Cl	—
8	Sa	Sa	Sa	Sa	Sa, Ss, Cl, Sf	Sa	Ps	Sa
9	Sa, Cl, Ca	Sa, Sp	Sa, Ss	Sa	Sa	Sf	Sa	Sa
10	Sa, Ec	Ec	Sa, Ss, Ps, Cl	Sf	Sa	Sf	Sa	Sf
11	Sa, Ss, Cl	Sa	Sa, Ss, Sf	Sf	Ss, Cl	Sf	Cl	Sf

Ca = *Candida* species; Cl = coliforms; Ea = *Enterobacter aerogenes*; Ec = *Enterobacter cloacae*; Ps = *Pseudomonas* species; Rf = respiratory flora; Sa = *Staphylococcus aureus*; Sf = skin flora; Sp = *Streptococcus pneumoniae*; Ss = coagulase-negative *Staphylococcus*.

Nasal cultures from these CRS patients also demonstrated the growth of a variety of different organisms (Table II). Again the most commonly cultured organism was *S. aureus* (25 of 43 bottles [58%]). *S. aureus* was found concurrently in both nasal swabs and bottles 51% of the time in nine of 11 patients (81%) (Table I). The only other organism that was found concurrently in both nasal swabs and bottles was *Enterobacter cloacae* in both the nasal swab and bottle of one patient.

Four of the 11 patients (36%) used antibiotics during the study, and two of the 11 patients (18%) had finished a course of antibiotics within 4 weeks of study commencement. All patients who had received antibiotic therapy had positive cultures from the nose and bottles even after therapy, including positive cultures for *S. aureus* in the nose and bottle.

In Vitro Cleaning

The results of our in vitro study are shown in Table III. All strains showed growth in the control bottle (i.e.,

TABLE II.
Organisms Found in Cultures of Nasal Irrigation Bottles and Nasal Cultures.

Organism	No. of Bottle Cultures (%)	No. of Nasal Cultures (%) n=43
<i>Staphylococcus aureus</i>	29 (67)	25 (58)
Coliforms	17 (39)	
Coagulase-negative <i>Staphylococcus</i>	17 (39)	
<i>Pseudomonas</i> species	15 (34)	
Skin flora	2 (4)	6 (13)
<i>Candida</i> species	2 (4)	
None		6 (13)
Respiratory flora		2 (4)
<i>Enterobacter aerogenes</i>		1 (2)
<i>Enterobacter cloacae</i>	1 (2)	1 (2)
<i>Streptococcus pneumoniae</i>		1 (2)

water left in bottle) after the initial cleaning. Bottles cleaned with detergent, Milton's solution, and microwave sterilization showed growth of only one or two colonies initially and after 48 hours, a finding that suggests these methods are more effective than cleaning with cold water alone.

S. aureus was the only organism cultured in this component of the study, and none of the waterborne species seen in our clinical study or in previous studies of nasal irrigation contamination⁴ were present. Only one clinical strain from a patient in our study showed growth immediately after cleaning in a bottle cleaned with cold water, boiling water, detergent, and microwave disinfection, suggesting that this strain was particularly virulent.

Biofilms

Six randomly selected patient bottles underwent SEM analysis for the presence of bacteria and biofilm formation. For one patient, extensive biofilm formation on the inner surface of the bottle was demonstrated, with encased bacteria surrounded by a dense exopolysaccharide matrix and attached to the surface (Fig. 1). For three patients, individual bacteria were identified on the imaged surface. These bacteria were either free on the surface or attached (see Fig. 2), suggesting that bacteria existed in different stages of the biofilm lifecycle.

DISCUSSION

In this study of 11 patients with stable treatment-resistant CRS, we identified significant rates of bacterial growth in the irrigation bottles that these patients were using to manage their CRS. In 42 of 43 bottles (97%) collected, bacterial growth of some kind was demonstrated. There was a high rate (81%) of concurrent sinonasal and bottle infection with *S. aureus*. Furthermore, we demonstrated that bacteria can form biofilms on the inner surface of the irrigation bottles provided to patients; this

TABLE III.
In Vitro Cleaning Results.*

	Reference Strain 29213		Reference Strain 25923		Staphylococcus aureus Patient Isolate		MRSA Patient Isolate	
	T0	T48	T0	T48	T0	T48	T0	T48
Water left	+++	1 col	+++	—	+++	3 col	+++	5 col
Cold water	2 col	—	—	—	+++	1 col	++	—
Boiling water	—	—	—	—	+++	—	—	—
Detergent	—	—	—	—	1 col	—	—	2 col
Milton's	—	—	—	—	—	—	—	1 col
Microwavable bottles	1 col	—	—	—	1 col	—	—	—

*Bottles were cultured after the initial cleaning (T0) and then 48 hours later (T48).
++ = growth; +++ = heavy culture; col = number of colonies; MRSA = methicillin-resistant *S. aureus*.

phenomenon may be important in the recurrent sinonasal infections we have observed in this study.

Although nasal irrigation has been shown to be effective in the management of CRS, infection of the bottle with bacteria may actually play a role in potentiating recalcitrant infections. It has been noted that many patients with CRS have grown *S. aureus* and *Pseudomonas* species in their sinonasal cavities. We have observed these species in irrigation bottles, possibly implicating contaminated irrigation fluid in the genesis of these infections.⁶

It is clear that nasal irrigation bottles can easily become infected, even without patient use,⁴ and our study has shown that the bottles can harbor a large variety of bacteria. In our study of a stable group of CRS patients during a long testing period, *S. aureus* was grown in 67% of bottles, with waterborne organisms such as coliforms and *Pseudomonas* species cultured in 41% and 34%, respectively. However, these waterborne species were rarely seen in the nasal cultures. The most commonly cultured organism from the nose was *S. aureus*, in 58% of patients. There was also a high frequency of concurrent sinonasal and bottle infection with *S. aureus*, occurring simultaneously in 81% of patients. It is worth noting that even after bottles were exchanged,

growth of similar bacteria was seen in subsequent bottles, again suggesting a dynamic flow of bacteria between nose and bottle.

Bacterial biofilms are known to avidly adopt the biofilm form when they become associated with inert surfaces, resulting in firm bacterial adhesion.⁷ Furthermore, they have also been demonstrated on the sinonasal mucosa of CRS patients⁸ and have been shown to be present particularly in CRS patients who have required revision surgery.⁹ Bacteria existing in this form are able to evade host defenses, and the genotypic changes assumed in adopting this mode of growth reduce their susceptibility to antibiotic therapy.¹⁰ The biofilm status of the patients in this study was not specifically known. Given previous biofilm frequencies,⁹ it may be reasonable to suggest that at least some of the patients tested using SEM had biofilms present on their sinus mucosa. As such, reflux of biofilm-forming bacteria into the irrigation bottle may facilitate attachment to the inert inner surface of the bottle. Bacteria were found to be in varying stages of the biofilm lifecycle from initial single-bacteria attachment (Fig. 2) to robust biofilm formation with extensive exopolysaccharide matrix surrounding the sessile bacteria (Fig. 1). The final stage of the biofilm lifecycle is the release of planktonic bacteria.

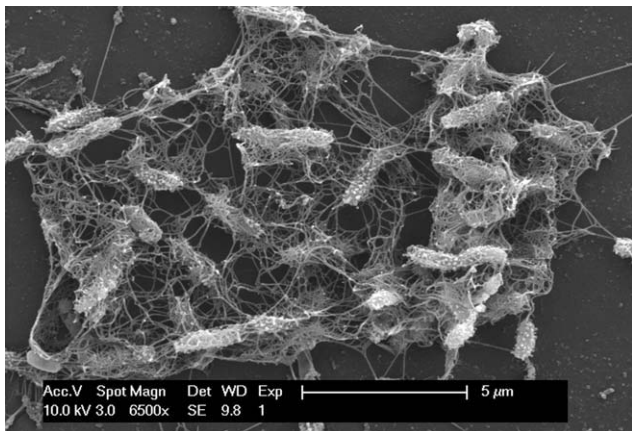


Fig. 1. Scanning electron microscopy image showing active biofilm formation detected on the surface of a nasal irrigation bottle.

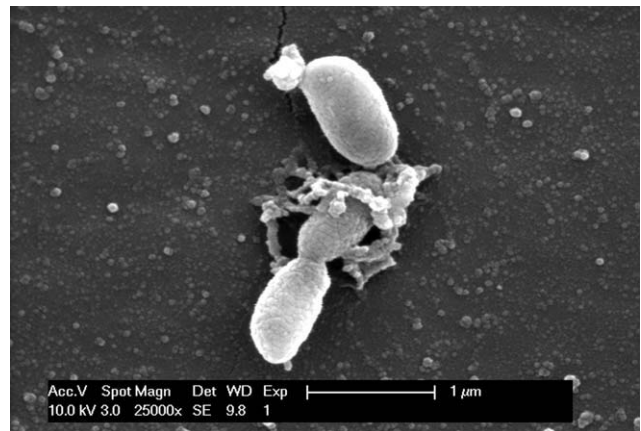


Fig. 2. Scanning electron microscopy image identifying individual bacteria attaching onto the surface of the bottles.

Surface biofilms thus may provide a nidus for the release of planktonic bacteria into the irrigant, which is then expelled into the nasal cavity.

The dynamics of bacterial movement between nose and bottle cannot be definitively determined from the present study. However, our results imply that bacteria can move in both directions. At the time of initial consultation, patients 1, 3, and 4 had negative cultures for bacteria in their irrigation bottles but positive cultures for *S. aureus* in their nose. However, at subsequent time points, they had positive cultures for *S. aureus* from both nose and bottle, a finding that suggests the *S. aureus* originated in the nose and then infected the bottle. Conversely, patient 6 did not have a positive culture for *S. aureus* in the nose initially, despite growth in the irrigation bottle. The *S. aureus* subsequently grew in both nose and bottle culture, a finding that suggests the *S. aureus* in this case originated in the bottle and reinfected the nasal cavity. This is an important point to consider because it suggests bottle contamination can cause sino-nasal infections and therefore is clinically relevant.

The questionnaire results from this study tell us that patients frequently use their bottles for prolonged periods of time and clean them intermittently at best, with the VAS and SNOT-20 scores showing patients' symptoms improved after receiving a new bottle. This practice leaves them susceptible to introducing organisms into their sinonasal cavity, contributing to persistent sinus infection. However, our in vitro sterilization results have demonstrated that nasal irrigation bottles can be simply and successfully sterilized. Boiling water, detergent, Milton's solution, and microwave disinfection were all effective in sterilizing the bottles initially after cleaning, and they remained sterile 48 hours later. These results reinforce the understanding that patients need to be educated on the hygienic use of their nasal irrigation bottles. We would recommend that bottles be easy to open and regularly sterilized, preferably before each use. Simple methods, such as those used in our study, are effective for this purpose. Regular changing of nasal irrigation bottles could be of benefit as well. The newly available microwave-safe bottles provide a quick, convenient method of cleaning the bottle after every use.

CONCLUSION

Bacterial contamination of nasal irrigation bottles is a clinically relevant problem, which may be contribut-

ing to recalcitrant infections. We have demonstrated high rates of irrigation bottle contamination with a range of bacteria, which can form biofilms on the inner surface of the bottle. The clinical consequences of this phenomenon were demonstrated by concurrent bottle and nasal infection with *S. aureus* in more than half of our patients. Irrigation bottles can be successfully sterilized by using simple techniques, and the importance of frequent bottle sterilization should be strongly impressed on all patients using this treatment modality.

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