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Do variations in nasal irrigation recipes and storage effect the risk of bacterial contamination?

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Main Article

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Abstract

Objective. Make-at-home nasal irrigation solutions are often recommended for treating chronic rhinosinusitis. Many patients will store pre-made solution for convenient use. This study investigated the microbiological properties of differing recipes and storage temperatures.
Method. Three irrigation recipes (containing sodium chloride, sodium bicarbonate and sucrose) were stored at 5°C and 22°C. Further samples were inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Sampling and culturing were conducted at intervals from day 0–12 to examine for bacterial presence or persistence.
Results. No significant bacterial growth was detected in any control solution stored at 5°C. Saline solutions remained relatively bacterial free, with poor survival of inoculated bacteria, which may be related to either lower pH or lower osmolality. Storing at room temperature increased the risk of contamination in control samples, particularly from pseudomonas.
Conclusion. If refrigerated, pre-made nasal irrigation solutions can be stored safely for up to 12 days without risking cross-contamination to irrigation equipment or patients.

Introduction

Nasal irrigation is a longstanding practice in the management of many inflammatory rhinological conditions, in particular rhinosinusitis. The latest edition of the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2020) advocates the use of nasal irrigation as a primary, secondary and post-surgical treatment for the majority of nasal conditions in both adults and children.[1]

Bacterial colonisation is a key pathophysiological component in patients with acute and chronic rhinosinusitis, both as a primary source of infective inflammation and a driver of immunological and allergic type inflammation.[1] Bacterial biofilms and contamination of nasal irrigation equipment has been well recognised, with staphylococcus and pseudomonas being some of the commonly isolated pathogens among a wide range of species.[2–4] Pseudomonas can be a particular issue following endoscopic sinus surgery.[4,5] As yet, there has been no proven link between nasal irrigation bottle contamination and patient infection or worsening symptoms.[4,6] Nevertheless, various papers have investigated the effectiveness of techniques for decontaminating this equipment with varying results.[7–9]

Make-at-home nasal irrigation recipes are widely advocated to reduce financial burden on patients. In the UK, there are a wide variety of recipes publicly available online. These recommend varying quantities of salt (sodium chloride), bicarbonate of soda and sugar (largely sucrose) in varying volumes of water.[10] Practically, many patients will not make a new nasal irrigation solution for every use, and many will store a pre-made volume of solution over a short period of time.

This study aimed to investigate the effects of recipe components and storage methods of nasal irrigation solution on its microbiology properties and susceptibility to contamination.

Materials and methods

Three nasal irrigation recipes were subjected to testing. The three recipes comprised the following items, which were added to 568 ml (one pint) of cooled boiled water: (1) one level 5 ml measure (one teaspoon) of table salt (sodium chloride); (2) recipe 1 plus a level 5 ml measure (one teaspoon) of bicarbonate of soda; and (3) recipe 2 plus a level 5 ml measure (one teaspoon) of granulated sugar (sucrose).

These recipes reflect the most commonly cited make-at-home recipes publicly available, including as advised by the National Health Service online.[10]

All solutions were mixed in a typical kitchen environment (clean, freshly washed measuring jug) and transported to the testing laboratory in sterilised containers (plastic bottles). Containers were sterilised using a sterilising solution typically used in home food and drink production (Star San Acid Sanitiser, Denver, USA). Solutions were then stored

in two storage environments: 22°C (room temperature) and 5°C (refrigerator temperature).

In addition to this control test, solutions were challenged with two bacterial species: *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). A 0.1 McFarland standard suspension of the organism in sterile distilled water was prepared using a Denischek Plus™. A 1/1000 dilution in sterile distilled water was made from this, and 0.1 ml of this inoculum was introduced to solutions 1, 2 and 3. The aim was to challenge the solutions with approximately 10^3 colony forming units/ml, which would equate to 10^2 colony forming units in 0.1 ml.

Aliquots of 0.1 ml of each solution in each temperature were extracted at days 0, 2, 4, 6, 8, 10 and 12 and streaked on to Columbia agar plates (Biomerieux (Craponne, France) product code 43059). This media was handmade in the laboratory, and each batch was quality control tested to ensure that the media was not contaminated (this may have given us false positives). Each solution was streaked onto 3 plates to ensure validity and incubated for 48 hours in carbon dioxide at 36°C before being examined for microbiological growth. Results are reported as the number of colony forming units. Examiners were blinded to the solution recipes throughout. The time zero result provided a measure of the original inoculum. Results were presented using simple descriptive statistics with comparison of the recipe used, the temperature of storage and the effects on the bacteriological challenge.

No formal ethical approval was required as no patient or human participants were involved. All microbiology investigations and the study protocol were approved and undertaken in line with the local microbiology department processes.

Results

Table 1 displays the number of identifiable micro-organisms within the respective solutions from days 0 to 12. Overall, 378 agar plates were prepared and analysed. Of these, 16 were identified as having contaminants and discounted from analysis.

Of the control solutions stored at 5°C, only 2 of the 60 valid plates demonstrated bacterial growth, and this was not demonstrated in subsequent plates from the same solutions. At day 12, all plates from all control solutions stored at 5°C remained sterile (no bacterial growth), whereas all control solutions stored at 22°C had 150–200 pseudomonal colony forming units by day 12.

In samples challenged with *S aureus* and *P aeruginosa* and stored at 5°C, the number of colony forming units generally decreased from the initial inoculum over the 12 days in all solutions. For solution 1 (sodium chloride only), there were no colony forming units after day 8 for staphylococcus and day 6 for pseudomonas. There was persistent but variable bacterial growth in the vast majority of challenged solutions stored at 22°C.

Discussion

Our study demonstrated that the components of the make-at-home recipe and the storage of this has a wide impact on the potential for bacterial contamination of pre-made nasal irrigation solutions.

Effects of temperature

Control solutions stored in 5°C remained largely sterile at 12 days after mixing. This means that patients who are careful not to cross-contaminate the container can keep any pre-mixed solution ready to use in their home fridge for almost two weeks. Practically, storing solution for this long will not be needed, but this does allow confidence that patients do not have to make fresh solution for every use. Patients could make a quantity sufficient for a few days depending on refrigerator space. For patients with busy daily routines, this added ease may help to improve compliance with regular nasal irrigation at home. There is no published evidence on the effect of performing nasal irrigation with a solution cooled to this extent. However, various studies have demonstrated a benefit of nasal douching at body temperature (37–40°C) compared to room temperature (18–25°C) in terms of mucociliary clearance, symptoms scores and pro-inflammatory markers.[11–13] One group in China compared nasal irrigation at 15°C compared with 25°C in allergic rhinitis patients and found no significant difference in either symptom scores or pro-inflammatory markers.[14] Nevertheless, on the balance of evidence, we would recommend that solution stored at 5°C should be allowed to warm to room temperature (above 15°C) before use until further research on this can be conducted.

Additionally, when stored at 5°C, even solutions challenged with *S aureus* and *P aeruginosa* have a substantial reduction in the number of living bacteria. For solutions with only sodium chloride added, staphylococcus was eradicated by day 8 at worst. This suggests that even when sterility of the storage solution cannot be guaranteed, the environmental temperature will likely protect from a small level of contamination. This potential finding requires significant further research to come to a conclusive recommendation. Additionally, the benefit of storing nasal irrigation equipment (in addition to the solution) in a refrigerator environment for contamination protection may be a target for future research. Various studies have explored the use of microwave decontamination (using heat) to sterilise reusable equipment but found this had to be weighed against the risk of damage or degradation occurring to the plastic components within the equipment.[9] Most plastics used in nasal irrigation equipment will already have a proven ability to withstand prolonged exposure to 5°C without degradation.

Prior biological research suggests that staphylococcus species may have a greater tolerance to cold. This is because of an ability to rapidly select 'small colony variants' that have a thicker cell wall and therefore have a greater long-term cold tolerance.[15] This phenomenon was not demonstrated in our study. Pseudomonas is recognised to have reduced growth at low temperatures,[16] and this correlates with our findings.

When stored at room temperature, solution 1 did not allow significant growth of staphylococcus when challenged directly, but all other samples (challenged or control) showed significant bacterial growth. There were colony forming units of pseudomonas present in all control solutions at day 4 and beyond. This growth quickly rose to levels comparable with samples directly challenged with pseudomonas and could represent a clinically significant route of cross-contamination to the irrigation equipment and the patient. The source of this contamination was likely at the solution mixing stage prior to day 0 and reflects the real-world risk of contamination during this step. Our findings therefore do not provide conclusive evidence that any recipe solution can be stored safely at room temperature without contamination. If patients do not have access to refrigerated storage, then all nasal irrigation

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Table 1. Number of colony forming units on microscopy by day of extraction, solutions used and storage

	Solution	Repeat	Colony Count (colony forming unit) Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
5 Degrees									
Control	Solution 1	1	0	0	0	0	0	0	0
		2	0	0	1(s)	0	0	0	0
		3	0	<50(s)	0	0	0	0	0
	Solution 2	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	Solution 3	1	(f)	0	0	0	(f)	0	0
		2	0	0	0	0	0	0	0
		3	(f)	0	0	0	0	0	0
Staphylococcus*	Solution 1	1	150-200	150-200 +(f)	50-100	2	0	0	0
		2	200-250	150-200	50-100 +(f)	<50	0	0	0
		3	100-150 +(f)	150-200	50-100	5 + 1(f)	0	0 +(f)	0
	Solution 2	1	150-200	150-200	100-150	50-75	50-75	25-50	25-50
		2	150-200	150-200	100-150	50-75	50-75	25-50	50
		3	150-200	150-200	100-150	50-75	50-75	25-50	<50
	Solution 3	1	150-200	50-100	50-100	50-75	50-75	25-50	25-50
		2	150-200	50-100	50-100	50-75 +(f)	<50	25-50	25-50
		3	150-200	50-100	50-100	50-75	<50	25-50	25-50
Pseudomonas*	Solution 1	1	50-100	50-100	2	1	0	0	0
		2	50-100	50-100	0	0	0	0	0
		3	50-100	50-100	1	0	0	0	0
	Solution 2	1	50-100	50-100	50-100	<50	<50	50-75	<50
		2	50-100	50-100	50-100	<50	<50	25-50	<50
		3	50-100	50-100	50-100 +(f)	50-75	<50	25-50	<50
	Solution 3	1	50-100	50-100	50-100	50-75	25-50	25-50	25-50
		2	50-100	50-100	50-100	50-75	25-50	25-50	25-50
		3	50-100	50-100	50-100	300	50-75	25-50	25-50
Room Temperature									
Control	Solution 1	1	0	(f)	150-200 (p)	150-200 (p)	150-200 (p)	100-150 (p)	150-200 (p)
		2	0	(f)	150-200 (p)	150-200 (p)	150-200 (p)	100-150 (p)	150-200 (p)
		3	0	0	150-200 (p)	150-200 (p)	150-200 (p)	100-150 (p)	150-200 (p)
	Solution 2	1	0	0	1 (s)	50(p)	50-100(p)	150-200(p)	150-200(p)
		2	0	25-50(s)	1(s)	50(p)	50-100(p)	150-200(p)	150-200(p)
		3	0	0	1(s)	50(p)	50-100(p)	150-200(p)	150-200(p)
	Solution 3	1	0	50-100(p)	50-100(p)	100(p)	100-150(p)	150-200(p)	150-200(p)
		2	0	50-100(p)	50-100(p)	150(p)	100-150(p)	150-200(p)	150-200(p)
		3	0	50-100(p)	50-100(p)	150(p)	100-150(p)	150-200(p)	150-200(p)
Staphylococcus*	Solution 1	1	150-200	(f)	0	0	0	0	0
		2	150-200	(f)	0	0	1 (f)	0	0
		3	150-200	(f)	0	0	0	0	0
	Solution 2	1	150-200	200-250	150-200	150-200	150-200	100-150	150-200
		2	200-250	200-250	150-200	150-200	150-200	100-150	150-200
		3	200-250	200-250	150-200	150-200	150-200	100-150	150-200
	Solution 3	1	200-250	100-150	100-150	75-100	75-100	25-50	75-100
		2	150-200	100-150	100-150	75-100	75-100	25-50	75-100
		3	150-200	100-150	100-150	75-100	75-100	25-50	75-100
Pseudomonas*	Solution 1	1	50-100	100-150	100-150	100	100	100-150	100-150
		2	50-100	100-150	100-150	100	100	100-150	100-150
		3	50-100	100-150	100-150	150	100	250	100-150
	Solution 2	1	50-100	200-250	200-250	150-200	150-200	100-150	150-200
		2	50-100	200-250	200-250	150-200	150-200	100-150	150-200
		3	50-100	200-250	200-250	250-300	150-200	150-200	150-200
	Solution 3	1	50-100	200-250	200-250	100	100	150-200	100-150
		2	50-100	200-250	200-250	100	100	150-200	100-150
		3	50-100	200-250	200-250	75	100-150	100-150	100-150

*Colony forming unit species only specified when differing from the challenged species.
Day 0 represents the initial inoculum for staphylococcus and pseudomonas challenged samples.
Solution 1 = sodium chloride; solution 2 = sodium chloride + sodium bicarbonate; solution 3 = sodium chloride + sodium bicarbonate + sucrose.
(s) = Staphylococcus epidermidis colony forming units; (f) = fusarium colony forming units; (p) = pseudomonas colony forming units

<5 CFU	5-50 CFU	50-100 CFU	100-150 CFU	150-200 CFU	200-250 CFU	>250 CFU	Contaminated culture plate

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solutions should be mixed on the day of use to limit contamination.

Effects of the recipe components

Across all challenged solutions, there appeared to be significant difference in the profile of bacteria growth between solution 1 and solutions 2 and 3. This could be the result of two factors: pH and/or osmolality.

The main advocated reason for the addition of sodium bicarbonate is to affect the pH of the solution, with alkaline pH being shown *ex-vivo* to improve mucociliary function.[17,18] Bacterial biofilms have also been demonstrated to produce an alkaline microclimate to advantage their growth and limit the body's ability to mount effective local inflammatory responses.[19] Therefore, the alkaline pH may be giving an advantage to microbiological growth, or at least the bacteria have a reasonable biological tolerance to this.

Osmolality may also have an effect. The solution recipes used were primarily chosen to reflect the most common publicly available make-at-home recipes. None of these produce an isotonic solution (as recommend by the European Position Paper on Rhinosinusitis and Nasal Polyps).[1,10] All solutions produce hypertonic solutions (331.4 mosmol/l, 532.6 mosmol/l and 550.5 mosmol/l, respectively), with solution 1 producing the closest to an isotonic solution. The effects of osmolality of a solution of bacterial growth has been researched previously, with other hyperosmolar agents (such as honey) being advocated in management of biofilms as they effect the water regulation of microbiomes.[19] The demonstrated effect of solution 1 having less bacterial growth appears to contradict this evidence, and conversely we have observed greater bacterial propagation in hyperosmolar solutions.

Osmotic fluid shift is also a well theorised method of therapeutic action on the nasal mucosa itself. The latest guidelines on treatment for allergic rhinitis (International Consensus on Allergy and Rhinitis: Allergic Rhinitis 2018)[20] recognise some studies demonstrate a benefit in the use of hypertonic saline to draw fluid out of nasal mucosa and thus reduce oedema. They conclude that this might be preferentially advocated for in the treatment of allergic rhinitis in children. This consensus statement contradicts the European Position Paper on Rhinosinusitis and Nasal Polyps,[1] which recommends against hypertonic saline solution (because of the side-effect profile). Either way, isotonic saline is unlikely to be advocated for based purely on this observed bactericidal effect *in vitro*, but this study may encourage further research. Repeat experimentation with a hypotonic and isotonic saline solution would be helpful to determine if pH or osmolality is the causative factor in limiting bacterial growth in solution 1.

The presence of granulated sugar (sucrose) in nasal saline irrigation solution appears to cause earlier propagation of bacteria within control solutions stored at room temperature. In samples at room temperature that were challenged directly with bacteria, the sucrose containing solutions had comparably less bacterial growth at the end of 12 days than solutions without. This may be related to a relatively high metabolic activity at the start of the experiment, which used up the available glucose meaning the solution subsequently had a nutritional deficit for the number of colony forming units.

Although not a common additive,[10] sugar is advocated by some to improve the taste of saline irrigation and therefore the patient compliance. Our study suggests that sugar has no greater effect on bacterial growth in stored nasal irrigation solution beyond a standard salt and bicarbonate of soda solution (within 12 days).

Limitations of study

Three specific limitations exist within this study. First, the inoculation density introduced was variable, particularly with those challenged with staphylococcus. Our methods explain how we attempted to control this; however, with this technique, there is invariably going to be some variation. The fact that the density is not known until 48 hours after the inoculation means that there was no ability to correct this once introduced. The effect this has on the results are likely to be minimal.

Second, there was contamination in several cultures. This has not affected the overall ability of the study to draw conclusions, and indeed efforts were made in study design to account for this by duplicating plates. Only one sample was contaminated for all three plates and therefore unable to give any useable results (solution 1 challenged with staphylococcus at room temperature on day 2). There is no indication that any of the stored solutions were contaminated during initial preparation or extractions (subsequent samples from the same storage container were clear of environmental contamination bacteria), but rather this contamination occurred during the plate preparation and culturing stages. Therefore, results from subsequent extraction in the same sample appear trustworthy.

- Storing an irrigation solution at refrigerated temperatures can maintain sterility of solutions for at least 12 days and will reduce the chance of bacterial propagation if contamination occurs
- When stored at higher temperatures, there is a reasonable chance of contamination causing prolonged colonisation
- If it is not possible to store solution under refrigeration, it is recommended that nasal irrigation solution is used on the day of mixing

Finally, the decision to store samples in sterilised containers may not reflect real-world conditions. This decision was taken to reduce the number of potential confounding factors that could limit the ability to draw conclusions on the primary objectives (namely the effect of storage temperature and solution components). If we used unsterilised containers, the solutions may have been stored in non-standardised conditions making direct comparison impossible. This may not reflect how patients may wish to store their irrigation solutions; however, the method of sterilisation was with make-at-home acid sanitiser, which is commonly used for home brewing and other food and drink production. Therefore, it is easily feasible that this could also be employed by patients should they or their clinicians wish to ensure identical conditions to this study.

Conclusion

Our study demonstrated that nasal irrigation solutions made with any recipe including salt, sodium bicarbonate or sugar can be stored at refrigerator temperature for up to 12 days without significant growth of bacteria. Refrigerating nasal irrigation solution will also limit growth when directly contaminated with staphylococcus and pseudomonas but will not eradicate it. Storing solution at room temperature can allow bacterial growth in any solution, particularly pseudomonas, from as early as day 2 after mixing (particularly if

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sugar has been added). Saline solutions appear to have a bactericidal effect at any storage temperature, but it is not clear if this is related to low osmolarity or relatively higher pH. Decisions on recommendations for patients will have to be made in conjunction with considering the desired biochemical and immunological properties of nasal irrigation solutions.

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Competing interests. None declared

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