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Complete List of Authors:	Nikolaou, Elissavet; Liverpool School of Tropical Medicine, Mitsi, Elena; Liverpool School of Tropical Medicine Ferreira, Daniela; Liverpool School of Tropical Medicine Bartolo, Amanda; Aintree University Hospitals NHS Foundation Trust, ENT/Head & Neck; Mater Dei Hospital, ENT & Audiology Leong, Samuel; University Hospital Aintree, Otorhinolaryngology - Head and Neck Surgery
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Title: Assessing the ideal microwave duration for disinfection of sinus irrigation bottles- a quantitative study

Nikolaou, E.,[1] Mitsi, E.,[1] Ferreira, D.M.[1], Bartolo, A.,[2]and Leong, SC.[2,3]

[1]Department of Clinical Sciences, Liverpool School of Tropical Medicine, UK

[2]Department of Otorhinolaryngology, Aintree University Hospital, Liverpool, UK

[3] Institute of Translational Medicine, University of Liverpool, UK

Correspondence:

Mr. Samuel C Leong MPhil FRCS (ORL-HNS)

Consultant ENT Surgeon and Rhinologist

Department of Otorhinolaryngology – Head and Neck Surgery

Aintree University Hospital NHS Foundation Trust

Liverpool L9 7AL, United Kingdom

Fax: +151 529 5263

Email: lcheel@doctors.org.uk

Short Title: Ideal microwave cycle for bottle disinfection

Abstract

Objectives: Saline irrigation of the nasal cavity and paranasal sinuses by one-way valved sinus containers has a recognised role in the management of chronic rhinosinusitis. However, bacterial recontamination of irrigation bottles through backflow from the sinonasal cavity is a concern in recurrent sinus cavity infections. While patients are encouraged to clean the irrigation bottles regularly, there remains significant concern that the use of contaminated bottles may perpetuate chronic rhinosinusitis. This study assesses the optimal microwave duration to achieve decontamination for each irrigation bottle component part (reservoir, tube, and nozzle) using a standard, commercially available microwave. In addition, the irrigation fluid was also tested for contamination after each microwave cycle.

Study Design: Laboratory-based experimental study.

Participants: No patients were involved in this study.

Main outcome measures: The percentage in vitro decontamination of the bottles' components was determined following 30, 60, 90, 120, 150 seconds of microwave cycles.

Results: Complete decontamination of the bottles was not achieved at any of the tested microwave cycles. Levels of decontamination differed for the different bottle components and the greatest degree of decontamination for all bottle components occurred at 90 seconds. Although higher levels of decontamination were observed at microwave durations exceeding 90 seconds, this was at the expense of thermal degradation and deformation of the reservoir plastic component of the irrigation bottle. Similarly, lowest contamination of irrigation fluid was observed at 120 seconds.

Conclusions: This study highlights the importance of establishing precise decontamination procedures and recommends a microwave cycle of 90 seconds for optimal decontamination.

Introduction

Saline nasal irrigation remains the cornerstone in the management of chronic rhinosinusitis (CRS).[1,2] Although numerous irrigation devices are available commercially, the NeilMed® Sinus Rinse™ (NeilMed® Pharmaceuticals Inc., Santa Rosa, CA, USA) bottle is commonly used in the United Kingdom. Previous studies have demonstrated that low pressure high volume systems like the NeilMed® Sinus Rinse™ are superior to saline sprays or nebulisers for penetration of sinus ostia and lavage of the sinus cavity.[3] The presumed mechanisms of action include physical lavage of inspissated mucus and debris; reduction of inflammatory marker load; and improving mucociliary action.[4,5]

As an adjunct therapy, saline nasal irrigation is routinely recommended before and after endoscopic sinus surgery (ESS).[6,7] Although the cause-and-effect relationship of using a contaminated sinus irrigation device resulting in post-operative infection remain hypothetical, patients are encouraged to wash their devices regularly.[8] Studies have demonstrated significant levels of contamination in irrigation bottles[8,9,10] and irrigation fluid[11] collected from post-operative patients, despite being given detailed instructions on cleaning. Since patient compliance to undertake proper cleaning is a separate but relevant issue, various cleaning techniques have also been assessed to try and simplify the decontamination process.[11, 12, 13, 14]

Some investigators have focused on the efficacy of microwaving the irrigation device to optimise the rate of decontamination.[15] However, the duration of microwave based on manufacturer's recommendation varied between 60 and 120 seconds; 60 to 90 seconds in the UK and 90 to 120 seconds for the United States. Wattage or microwave power are not defined.[16] The discordance in these microwave durations compound effective patients' education and compliance. Furthermore, microwave disinfection has been tested in both unsupervised and supervised patients' settings. It has shown promising results in that complete bacterial disinfection was achieved in the witnessed clinic setting, indicating the lack of patient's compliance to follow instructions when they are unsupervised.[17]

The principal aim of this study was to assess the effectiveness of microwave disinfection of in vitro contaminated NeilMed® Sinus Rinse™ bottles to inform on the optimum microwave duration that would achieve the highest degree of decontamination.

Methods

Study design

The microwave duration was fixed at 30, 60, 90, 120 and 150 seconds. Five bottles were allocated to each of these five groups (see below). For practical purposes, a standard commercially available 700 Watt microwave (Tesco Microwave Oven MT08PN: 261800315297, Tesco PLC., Hertfordshire, United Kingdom) was used in this study. Each microwave cycle was at the maximum power setting (700 W) and the only adjustment made was the duration of the cycle. In terms of total heat energy delivered for each group at 700 Watt (1 Watt = 1 Joule/second), it corresponds to 21000, 42000, 63000, 84000 and 105000 Joules per group's relative seconds tested.

Creation of an inoculation culture

Swabs from two bottles obtained from post-operative patients were used to create an inoculation culture. These swabs were used to inoculate ten brain-heart infusion (BHI) plates (non-selective growth media) and incubated overnight at $37^{o}C$ + 5% CO_2.

Inoculum preparation and in vitro bottle contamination

To prepare the inoculum, the pre-inoculated BHI plates were washed with sterile saline (B. Braun Medical Inc., Sheffield, UK) to 27mL final volume. Each of the 25 bottles were inoculated with 1mL inoculum plus 29mL of sterile saline for five hours in normal room conditions to drain the inoculum. The remaining inoculum was squeezed off and the bottles were left to air dry overnight.

Decontamination of contaminated bottles by microwaving

The contaminated bottles were divided in five groups of five bottles each, totalling 25 bottles. Each group corresponded to different microwave length cycle; Group 1: 30 seconds, Group 2: 60 seconds, Group 3: 90 seconds, Group 4: 120 seconds and Group 5: 150 seconds. For each bottle, a swab of the bottle reservoir, tube and nozzle (Figure 1) was taken before (control) and after microwaving, at full power (700 Watt). After each bottle was microwaved and swabbed, 5mL of sterile isotonic saline was added into the reservoir and swirled gently before the irrigate was poured over a separate culture swab. Swabs were plated immediately onto BHI plates per the four-quadrant streak method (Figure 2a).[18] The plates were incubated for two nights at $37^{o}C$ + 5% CO_2, to account for slow growing organisms.

Quantifying bacterial contaminants

The number of bacterial colonies growing in each quadrant (Q1-Q4), for each plate, was counted at 24 and 48 hours of incubation. Growth was scored on a semi-quantitative scale from no growth to 4+ according to O'Brien et al. (2001) scoring matrix; [19] no growth: no colonies at any of the four quadrants, scant growth:<25 colonies in quadrant Q1,1+ growth: ≥ 25 colonies in Q1 and <25 in Q2, 2+ growth: ≥ 25 colonies in Q2 and <25 in Q3, 3+ growth: ≥ 25 colonies in Q3 and <25 in Q4 and 4+ growth: ≥ 25 colonies in Q4 (Figure 2b).

Determining the percentage (%) decontamination of the bottles and irrigate

To quantify the degree of contamination and to assess the change in contamination after microwaving, a percentage matrix from 0 (no growth) to 100 (4+) was introduced (Figure 2b). The percentage contamination before and after microwaving was used to express the percentage decontamination (((after-before)/before) *100) for each bottle component (nozzle, tube, reservoir) at corresponding microwave length cycle. The results were aggregated per the component parts of the bottles for each group.

Statistical analysis

Data were analysed and compared by using GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA) and considered to be parametric. Statistical significance was determined by one way ANOVA and corrected according to Bonferroni adjustment.

Results

Although there was an incremental increase in decontamination with increasing duration of microwave of all bottle components, none of the groups achieved 100% decontamination after the respective microwave duration (Figure 3). In fact, the percentage of decontamination decreased at 48 hours after microwaving which confirmed the survivability and proliferation of the microbiota, although no statistical significance was observed between the two incubation time points. The bottle tube percentage decontamination is higher compared to bottle nozzle and reservoir, across all time points of microwaving after 24 hours of incubation, whereas bottle reservoir was the least decontaminated (Figure 3).

Low levels of decontamination were achieved after 30 seconds, although it was noted that there was no change to the degree of decontamination in bottle reservoir at this microwave duration. However, a significant increase in decontamination of the bottle reservoir was observed when the microwave duration was increased from 90 to 120 seconds, although there was no significant change at 150 seconds (Figure 3). In contrast, a significant increase in decontamination of the nozzle and tube occurred when the microwave duration was increased from 60 to 90 seconds for both 24h and 48h. Thereafter, the change in the degree of decontamination at microwave durations was marginal (Figure 3).

The level of bacterial contamination of the irrigation fluid followed a similar trend to the decontamination of the bottle reservoir. The level of contamination decreased steadily with increasing microwave duration and reached the lowest after a microwave cycle of 120 seconds (Figure 4).

Although greater decontamination was achieved following longer duration of microwave, warping at the neck of the reservoir was observed in 2 and 3 bottles in Group 4 and 5 respectively. In addition, transference of melted plastic from the reservoir onto the microbiology swab occurred in both groups. This unexpected phenomenon was limited to the bottle reservoir and was not observed in the nozzle or tube.

Discussion

Synopsis of key findings

The manufacturer recommends placing all three components of the NeilMed® Sinus Rinse™ bottle in the microwave for between 60 to 90 seconds in UK (90-120 seconds in USA).[16] This study has assessed the degree of bacterial decontamination of all three bottle components and irrigation fluid at 30 second increments. Interestingly, none of the bottles achieved complete decontamination even at 150 seconds. Significant levels of bacterial contamination persisted after 30 and 60 seconds. In fact, decontamination did not occur in the reservoir after a 30 second microwave cycle. Increased levels of decontamination were achieved at longer microwave durations, typically at 120 seconds.

At all microwave durations, greatest decontamination was observed in the tube followed by the nozzle and reservoir. The degree of decontamination decreased by 48 hours after microwave although these changes were not significant when compared to the degree of decontamination in the preceding 24 hours. The only exception was the degree of decontamination in the tube after 150 seconds' microwave. It is worthwhile mentioning that the bottles started to deform at 120 and 150 seconds suggesting that further assessment of the thermoplastic properties of these bottles is required.

Strengths of the study

Overall, the results suggest the optimum microwave duration to be 90 seconds. This study was designed to be pragmatic and to mimic real-life scenario, hence utilising a standard 700 Watt microwave purchased from a high-street retailer. However, different makes of microwave may

potentially behave differently, in terms of magnetron energy production and size of oven, therefore duration of microwaving may need to be altered. The results of this study corroborate with previously reported data that longer microwave durations resulted in higher degrees of decontamination.[12] However, at 120 and 150 second cycles, we observed plastic deformation because of heat generated by the microwave. This unexpected phenomenon occurred only in the reservoir component of the device and has not been described in previous studies. As this study evaluated the different components of the NeilMed® Sinus Rinse™ irrigation bottle separately, the interesting issue on the thermoplastic properties of the component parts is currently subject to further investigation by this research group in collaboration with polymer engineers at the Liverpool University, United Kingdom.

Comparison with other studies

This is the first study to assess the level of decontamination based on varying durations of microwave. All reports focusing on the use of microwave, including this study, have demonstrated that this technique does not result in complete decontamination at any microwave duration up to 150 seconds. To document the most effective cleaning method, Keen et al. (2010) examined the success rates of five most commonly recommended cleaning methods; rinsing with cold water, boiling water, detergents, Milton's antibacterial solution, and microwaving. Although contamination still occurred with all cleaning practices, rinsing with boiling water or Milton's solution or microwaving for 90 seconds appeared to reduce the degree of contamination.[12]

Clinical applicability of the study

Clinicians should be involved in the education of patients on the correct use and cleaning of irrigation devices when they are prescribed and reinforce their proper use at subsequent consultations. The present study suggests that the optimal microwave duration is 90 seconds and although longer microwave duration (e.g. 120 seconds) decreased the level of contamination even further, bottles seem to deform. Patient should therefore be encouraged to microwave for at least 90 seconds at maximum power after every use and be vigilant that

device failure, in the form of warping or brittleness, can occur at longer microwave durations. The level of decontamination reduced further 48 hours after microwave. Thus, for patients who do not irrigate daily, they should be encouraged to wash their bottles and microwave before using it.

It must be remembered that the present study has only assessed the NeilMed® Sinus Rinse™ bottle and that there are 25 other irrigation systems available.[20] Further studies are needed to determine if bacterial contamination of these irrigation bottles is clinically relevant. Prospective, clinical studies are required to assess if bacterial contaminants can be cultured from patients' sinuses and whether this results in recurrent disease. If longer microwave durations are required to achieve higher levels of decontamination, the thermoplastic properties of these devices need to be systematically assessed although there may be cost implications to improving the durability of these devices to withstand multiple microwave cycles.

Conclusion

This study has assessed the degree of bacterial decontamination of all three bottle components and irrigation fluid at 30 second increments. Although none of the microwave durations tested in this study resulted in complete decontamination, we recommend a minimum microwave cycle of at least 90 seconds.

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Conflict of interest: Philip Stell Prize winning presentation, Otorhinolaryngological Research Society Spring Meeting, Liverpool 10th March 2017.

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Author Contribution: All authors contribute significantly to study design, data analysis, and interpretation of results as well as to the final manuscript.

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Figure 1: NeilMed Sinus Rinse bottle compartment's tested.

Cultures performed of the irrigation bottle included from left to right; reservoir, tube (inner and outer parts) and nozzle (inner part).

Figure 2: Four Quadrant Streak Method and Growth Scoring

A) Each plate was divided in four quadrants Q1-Q4. Each swab was placed at approximately 2cm over Q1 and spread with a disposable loop using closing parallel streaks. The plate was rotated by 90 degrees and another disposable loop was lightly swept 3 times through the inoculated area and then streaked into Q2. This step was repeated for Q3 and Q4. Colonies in each quadrant were counted after 24 and 48 hours of incubation.

B) Growth was scored on a semi-quantitative scale according to the number of colonies counted in each quadrant. This method was adapted by O'Brien et al. (2001). For each growth score a %contamination score was also given. Lowest growth is representing by no colonies in any of the four quadrants, so no growth score, and thus 0% contamination score. Highest growth is representing by more or equal to 25 colonies at Q4, so 4+ growth score, and thus 100% contamination score.

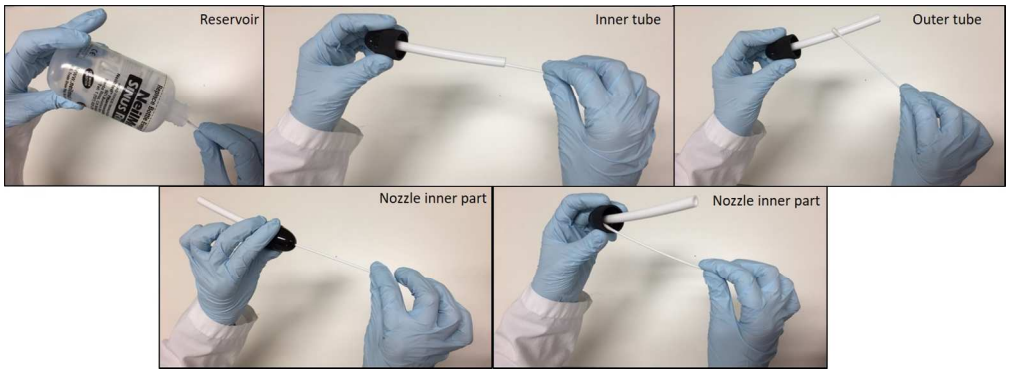
Figure 3: Level of bottle decontamination between 30-150 secs microwaving after 24 and 48 hours' incubation.

Five bottles were used at each time groups (G1:30 secs, G2:60secs, G3:90secs, G4:120secs, G5:150 secs). The % decontamination was calculated for each bottle compartment (tube, nozzle, and reservoir) at each time group after 24 and 48 hours of incubation. Triangular, circular and rectangular points represent the mean % decontamination for bottle tube, nozzle and reservoir respectively from 30 to 150 secs of microwaving time. Vertical bars represent standard errors values. Statistical significance test was analysed by one way ANOVA and

corrected by Bonferroni adjustment; *** denotes P < 0.001 and represents statistical significance for each time point across all bottle's compartments and for each bottle compartment across all time points.

Figure 4: Level of irrigation fluid contamination between 30-150 secs microwaving after 24 and 48 hours' incubation.

For irrigation fluid, 5mL saline was used to wash each bottle at each time groups (G1:30 secs, G2:60secs, G3:90secs, G4:120secs, G5:150 secs) after microwaving. The % irrigation fluid contamination was determined for each bottle at each time group after 24 and 48 hours of incubation. Circular points represent the mean % irrigation fluid contamination from 30 to 150 secs of microwaving time. Vertical bars represent standard errors values. Statistical significance test was analysed by one way ANOVA and corrected by Bonferroni adjustment; *** denotes P < 0.001 and represents statistical significance between time points.



NeilMed Sinus Rinse bottle compartment's tested

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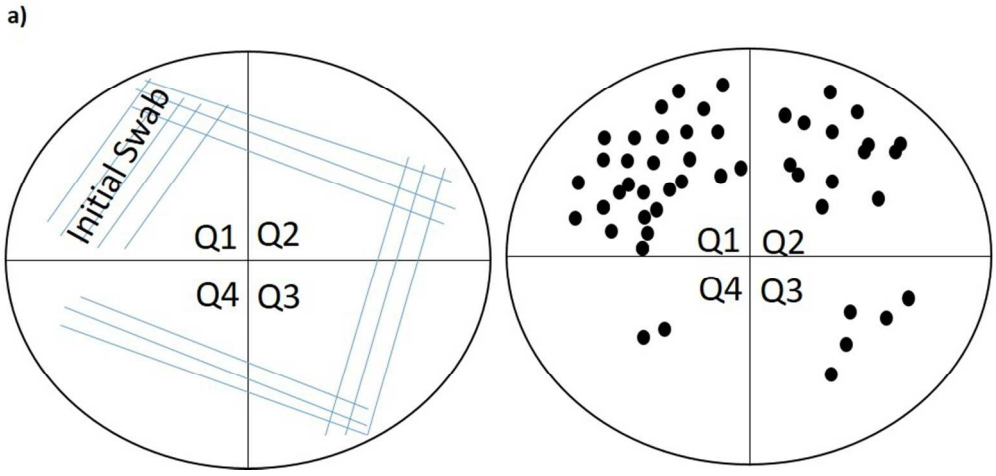

Four Quadrant Streak Method and Growth Scoring

264x126mm (96 x 96 DPI)

b)

Growth Score	Quadrant No Colonies	% Contamination
No growth	0	0
Scant	Q1 < 25	20
1+	Q1 ≥ 25 and Q2 < 25	40
2+	Q2 ≥ 25 and Q3 < 25	60
3+	Q3 ≥ 25 and Q4 < 25	80
4+	Q4 ≥ 25	100

Four Quadrant Streak Method and Growth Scoring

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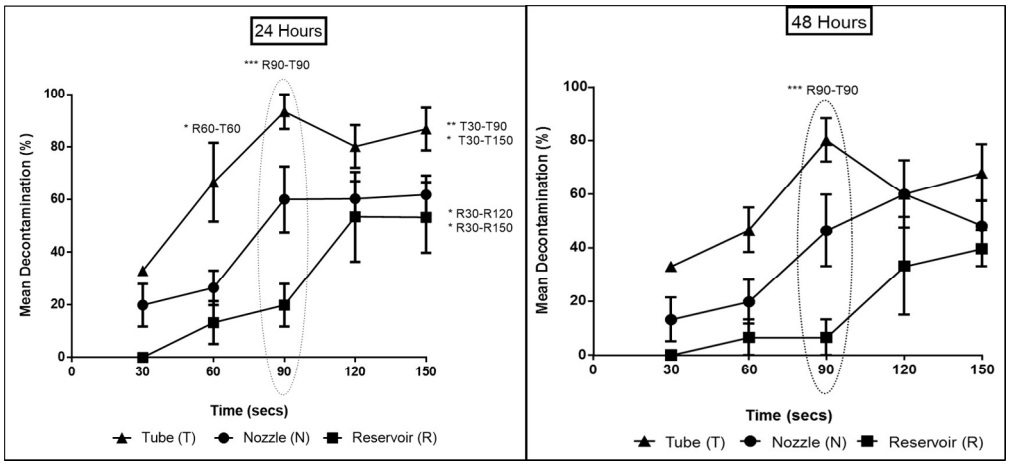

Level of bottle decontamination between 30-150 secs microwaving after 24 and 48 hours' incubation.

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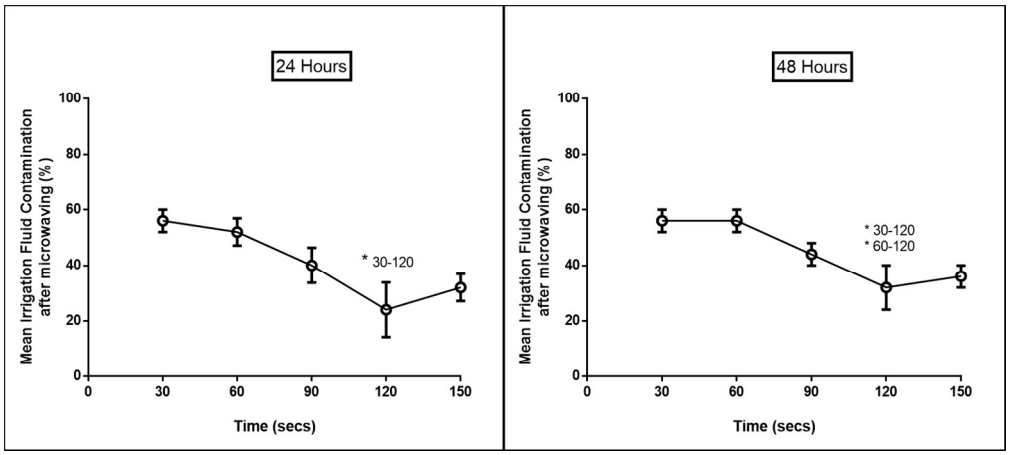

Level of irrigation fluid contamination between 30-150 secs microwaving after 24 and 48 hours' incubation.

431x191mm (96 x 96 DPI)