

Homemade Hypertonic Saline: Essential Treatment Can Be Available and Affordable

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ABSTRACT: Background: Nebulized hypertonic saline (HS) treatment is unavailable to large populations worldwide.

Objectives: To determine the bacterial contamination and electrolyte concentrations in homemade (HM-HS) vs. pharmacy made (PM-HS).

Methods: We conducted three double-blind consecutive trials: 50 boiled-water homemade 3%-HS (B-HM-HS) bottles and 50 PM-HS. The bottles were cultured after 48 hours. Electrolyte concentrations were measured in 10 bottles (5 per group). Forty bottles (20 per group) were distributed to volunteers for simulation of realistic treatment by drawing 4 ml HS three times daily. From each bottle, 4 ml samples were cultured after 1, 5, and 7 days. Volunteers prepared 108 bottles containing 3%-HS, sterilizing them using a microwave oven (1100–1850W). These bottles were cultured 24 hours, 48 hours, and 1 month after preparation.

Results: Contamination rates of B-HM-HS and PM-HS after 48 hours were 56% and 14%, respectively ($P = 0.008$). Electrolyte concentrations were similar: $3.7\% \pm 0.4$ and $3.5\% \pm 0.3$, respectively ($P = \text{NS}$). Following a single day of simulation B-HM-HS bottles were significantly more contaminated than PM-HS bottles: 75% vs. 20%, respectively ($P < 0.01$). By day 7, 85% of PM-HS bottles and 100% of B-HM-HS bottles were contaminated ($P = 0.23$). All 108 microwave-oven prepared bottles (MICRO-HS) were sterile, which was significantly better than the contamination rate of B-HM-HS and PM-HS ($P < 0.001$). Calculated risk for a consecutive MICRO-HS to be infected was negligible.

Conclusion: Microwave preparation provides sterile HS with adequate electrolyte concentrations, and is a cheap, fast, and widely available method to prepare HS.

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KEY WORDS: hypertonic saline, bronchiolitis, culture, sterilization, microwave oven

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Nebulized hypertonic saline (HS) in various concentrations (3%–7%) is suggested for treatment of most airway diseases including acute viral bronchiolitis, cystic fibrosis, bronchiectasis, chronic bronchitis, and occasionally even asthma [1,2]. Thus, when indicated, HS should be an available and affordable treatment option. Children with acute viral bronchiolitis may especially benefit from nebulized HS [3–6]. Although there is some recent controversy regarding the specific outcomes and populations, nebulized HS 3% combined with β -agonists has previously been proven to be effective in acute viral bronchiolitis [3–6]. Potential effective treatment for acute viral bronchiolitis, the most frequent respiratory disease causing hospitalization in young infants, should be available for all pediatric patients worldwide. However, due to low availability globally and a relative high cost of commercial medicines, only a small percentage of these pediatric patients eventually receive appropriate treatment [7]. For example, a pack of HS 3% ampules for inhalation [PARI Respiratory Equipment, Inc., Midlothian, VA] costs US\$64 in the United States. In Israel, until 2015, in some private pharmacies the price of HS 3% was 150 ILS (~US\$42) or even higher, with limited availability of all concentrations. Even in a Western country like Israel, in 2013, only hospitalized pediatric patients (accounting for only 1% of the children with acute viral bronchiolitis) had timely access to HS 3% [8]. This dismal scenario is far worse in Third World countries where affordability is a serious issue for the majority of the population [9–12]. It is therefore pertinent to improve access to these medications that are easy to prepare, consisting of only water and sea salt, components that are both cheap and widely available.

Our objectives for these consecutive studies were to:

- Determine if homemade boiled HS solution (B-HM-HS) was comparable to pharmacy made HS (PM-HS), in terms of baseline microbial contamination rates and electrolyte concentration

- Assess the incidence of microbial contamination of the solutions (B-HM-HS vs. PM-HS) in actual settings simulating a course of treatment
- Evaluate the contamination rate of HS prepared by using a microwave oven (MICRO-HS) as compared to B-HM-HS and PM-HS without any additional manipulation

PATIENTS AND METHODS

PATIENTS (VOLUNTEERS)

The volunteers were parents (n=20) or grandparents (n=3) of children at ages similar to those who typically require such treatment for acute viral bronchiolitis. The preparations were made in their home kitchens in the midst of their daily routine, as in reality. We chose tools and ingredients that are available or are found in most homes, such as baby bottles, a microwave oven, boiling water, and sea salt.

CONSECUTIVE TRIALS

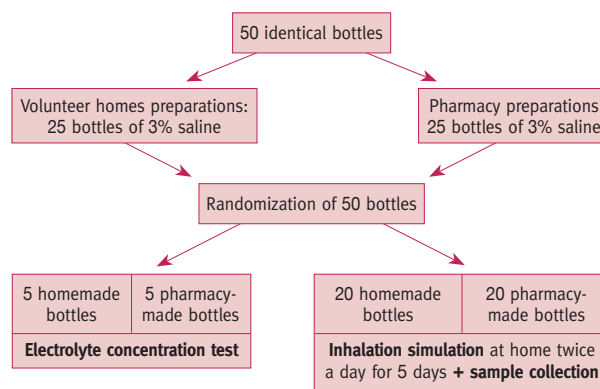
In the first double-blind-controlled study 50 homemade (HM-HS) 3% bottles and 50 pharmacy-made (PM-HS) 3% bottles were cultured 48 hours after preparation. The PM-HS was concocted at the hospital pharmacy by an experienced pharmacist at Wolfson Medical Center who prepares these solutions on a regular basis. The pharmacist added 7.5 grams of Merck NaCl salt [Merck KGaA, Darmstadt, Germany] to 250 cc of sterile distilled water. This mixture was carefully prepared under a fume hood while the pharmacist was wearing gloves and a laboratory coat.

B-HM-HS saline was prepared using cooking salt, which is sea salt [Salt of the Earth Ltd. Atlit, Israel] by the volunteers in their homes in a realistic setting, without wearing gloves; however, after receiving both verbal and written (in their native language) detailed explanation of the procedure for preparing the solution [Supplementary material 1], the importance of washing hands and tools with soap and boiling the water to ensure sterility were emphasized. The volunteers prepared boiling home-made HS (B-HM-HS) by boiling water for 15 minutes and adding 7.5 grams NaCl to 250ml of boiling water, which forms exactly 3% HS, and pouring it into bottles.

After the preparation, all of the bottles (both home-made and pharmacy-made) seemed identical and were randomized in the pharmacy using a key-code completed by the statistician for two blocks (home-made, pharmacy-made). The key-code was then sealed in a closed envelope by a pharmacist who was not involved in the remainder of the study. The randomized bottles were then distributed to the volunteers for the actual inhalation simulation study (n=40, 20 of each group) and to the laboratory for electrolyte analysis (n=10, 5 of each group) [Figure 1].

A sample (4 ml) collected from each bottle was aseptically cultured on chocolate agar, blood agar, and liquid broth media

Figure 1. Inhalation simulation study—a double blind control study



for bacteria and Sabouraud's dextrose agar for fungi. The approximate identification of the recovered organisms was accomplished using standard microbial identification techniques.

In the second double-blind controlled study a separate batch of 25 bottles of B-HM-HS were compared to 25 PM-HS 3% bottles. The bottles were further divided into two groups. The first group of 10 bottles (5 for each block: B-HM-HS and PM-HS) had their salt concentration indirectly measured at the lab by an Olympus AU 2700 analyzer (Hamburg Germany), using ISE module.

The second group consisted of 40 bottles (20 B-HM-HS and 20 PM-HS) all distributed to the volunteers' homes for simulation of realistic treatment by drawing 4 ml HS three times a day for up to 5 days. The bottles were cultured 1, 5, and 7 days after preparation [Figure 1].

All bottles were immediately transferred, after the indicated day of use, to the medical microbiology laboratory and processed in less than 3 hours to minimize contamination during the testing process. The majority of volunteers were parents (13/17) and 4/17 were grandparents to babies and preschool children. Thus, they were comparable to the target population that typically would administer HS treatment.

FINAL STUDY: STERILIZATION BY MICROWAVE RADIATION

The possible proof of the sterilization potency of microwave radiation consisted of radiating the five most infected bottles from the first study in a microwave oven for 2 minutes for sterilization.

In the final study, volunteers prepared 108 baby bottles containing 6 grams of sea salt in 200 ml water and placed each in a home microwave oven (1100–1850 W) for 2 minutes [Figure 2]. These bottles were not manipulated after the preparation. Culture samples were collected 24 hours, 48 hours and 1 month, after preparation.

Instructions to the volunteers, microbiologic methods, and volunteer population were identical to the previously described first study [Supplementary material 2].

Figure 2. Procedure for concocting homemade hypertonic saline using microwave radiation

1. Fill a microwave-proof baby bottle with 200 cc of tap water.
2. Add 5.6 ml sea salt measuring spoon filled to the brim with sea salt.
3. Close the lid with the baby nipple and place it in the microwave oven for 2 minutes.
4. Wait until the bottle is cool



In all parts of the study, both the supervising investigators and the laboratory team were blinded to the origin of the solutions.

RAW PURE SALT STERILITY CONFIRMATION

To confirm that raw pure saline, which is usually presumed to be sterile, was indeed sterile, we cultured 40 samples of pure salt without water (20 samples of Merck NaCl salt [Merck KGaA, Darmstadt, Germany] and 20 of cooking salt, which is sea salt [Salt of the Earth Ltd. Atlit, Israel]).

The study was approved by the ethics committee of the Wolfson Medical Center, research number 0060-12-WOMC.

STATISTICAL ANALYSIS

Categorical variables, such as culture results were compared by using chi-square test or by Fisher’s exact test when appropriate. Continuous variable with approximately normal distribution are reported as mean ± standard deviation. Continuous variables, such as electrolyte concentrations, were compared using unpaired two-tailed *t*-tests.

The calculated chance for another MICRO-HS bottle to be contaminated was calculated using Hanley’s simple formula: maximum risk = 3/n (for n > 30) [13].

Results were considered statistically significant when a two-tailed *P* value was < 0.05.

RESULTS

FIRST STUDIES: STERILIZATION BY BOILING WATER

B-HM-HS were significantly more contaminated than PM-HS (56% vs. 14%, respectively, *P* = 0.008) 48 hours after preparation [Table 1]. NaCl concentration was similar for HM-HS and PM-HS: 3.7% ± 0.4 and 3.5% ± 0.3, respectively (*P* = NS). In one day of simulating inhalations at home,

Table 1. Contamination rates with no inhalation simulation

Positive cultures 48 hours after preparation	MICRO-HS n=108	B-HM-HS n=50	PM-HS n=50	<i>P</i> value
n (%)	0 (0)	28 (56)	7 (14)	< 0.001

MICRO-HS = homemade preparation using microwave radiation, B-HM-HS = home preparation using boiled water, PM-HS = pharmacy-made hypertonic saline using distilled sterile water
P < 0.001 for MICRP-HS vs. B-HM-HS and vs. PM-HS

Table 2. Contamination rate during inhalation simulation

Positive Cultures	B-HM-HS, n (%)	PM-HD, n (%)	<i>P</i> value
After 24 hours	15 (75%)	4 (20%)	0.01>
After 5 days	15 (75%)	11 (55%)	0.3
After 7 days	20 (100%)	17 (85%)	0.23

B-HM-HS = home preparation using boiled water, PM-HS = pharmacy made hypertonic saline using distilled sterile water
 In one day of simulating inhalations at home, B-HM-HS bottles became significantly more contaminated than PM-HS. However, by day 5, most of bottles in both groups were contaminated

B-HM-HS bottles became significantly more contaminated than PM-HS (75% vs. 20%).

By the end of the simulation (day 7), 85% of PM bottles and 100% of HM bottles were contaminated [Table 2]. The main microorganisms that were isolated were Coagulase-negative staphylococci, *Acinetobacter* and *Enterobacteriaceae* species.

FINAL STUDY: STERILIZATION BY MICROWAVE RADIATION

All 108 MICRO-HS cultures remained sterile 24 hours, 48 hours, and 1 month after preparation.

These results were significantly better compared to HM-HS and even to PM-HS cultures (*P* < 0.001) [Table 1]. As all 108 MICRO-HS cultures remained sterile, the statistical calculated risk by Hanley’s simple formula for a consecutive MICRO-HS to be contaminated is negligible: maximum risk (upper limit of 95% confidence interval) < 0.028 [13].

RAW PURE SALT STERILITY CONFIRMATION

All the 40 raw pure salt cultures were sterile after 24 hours, 48 hours, and 7 days.

DISCUSSION

Our study demonstrates that both methods of HS preparation, home-made (HM) and pharmacy-made (PM), are prone to baseline contamination, as well as contamination when administered in a realistic setting. Even previously sterile PM-HS bottles had a contamination rate of 20% within 1 day of simulating inhalations. Our findings are comparable to previous studies that demonstrated contamination of hospital inhalation equipment [14,15]. These observations led us to design our final study using a microwave oven as an effective, available, and daily applicable method for sterilization of the solutions, regardless of the preparation method.

The main reason for conducting the study was to simulate the conditions in daily life. The majority of volunteers were parents of children at ages similar to those who typically require such treatment for acute viral bronchiolitis. The HM preparations were made in their home kitchens in the midst of their daily routine, as is typical. We chose tools and ingredients that are available or are found in most homes with small children, including baby bottles, a microwave oven, boiling water, and sea salt.

Electrolyte concentrations were shown to be accurate in HM as compared to PM preparations. This further strengthens the assumption that HS can be prepared at home.

Our final study demonstrated the utility of microwave radiation in the final study, and found that boiled water was not as effective a sterilization method in the first trial. This result is similar to findings from other studies [16,17,18]. Microwave radiation leads to sterilization, not only by heating and boiling the water. Research suggests that the rapid movement of water molecules in the microwave oven produces an additive sterilization effect. One study on *Candida albicans* demonstrated microwave radiation destruction of cell membranes, which did not occur when only boiling water was applied [16]. In a different study, substantial damage to *Staphylococcus aureus* was noted using sub-lethal temperature microwave radiation, which was not achieved with regular heating [17]. Moreover, it was demonstrated that exposure to microwave radiation at boiling temperature for 60 seconds damaged all bacteria membranes [18]. This additional effect occurs only in the presence of water.

We chose natural sea salt over table salt as it is made by steaming sea water, thus it consists of only NaCl. By using natural sea salt we avoided the consequences of inhaled additives contained in table salt (i.e., iodine, flour, and anti-crystallizing agents).

The vast majority of contamination in our study stems from handling the HS preparations in real life. Thus, we tell the parents of our patients to first prepare the final HS solution and only then to sterilize it in a microwave oven. Subsequently, we advise minimal handling by just pouring the HS from the sterile bottle directly to the inhalation cup and not using any syringes or needles.

The common bacteria that were cultured during our trial were consistent with infections originating from water sources and human skin. The potential clinical impact of contaminated inhalations is inconclusive due to lack of data from controlled trials. Moreover, it was recently shown using advanced molecular methods, that the lower human respiratory tract is not entirely sterile as was previously determined, thus questioning the need for strict sterile inhalation material [19]. However, the current dogma that contaminated inhalations should be avoided is based on a logical assumption. The inhalation apparatus produces specifically sized droplets designed to reach the lower respiratory tract and remain there [20]. Introducing a mix of microorganisms in this manner is unsettling. Taking into

consideration these findings, together with our data, suggests that microwave radiation is adequate sterilization for inhaled materials, and questions the need to go through unreasonable additional efforts to reach further a reduction in microorganism concentration beyond the sensitivity of standard culture.

The significance of the specific microorganisms that were cultured in this trial and the threshold for the bacterial load is yet to be determined. Considering the overwhelmingly fast rate of contamination in actual settings, we suggest pouring the solution straight from the microwaved bottle into the inhalation cap and avoiding the use of additional tools (i.e., syringe, needles, spoons, hands), which are all prone to contaminations, through a course of inhalation.

Another recommendation for minimizing contaminations is using disposable solutions and tools if available, although these might be too expensive, especially for the average global population [7].

A few recent publications raised some doubt regarding the short term efficacy of treating acute viral bronchiolitis with HS in the emergency department (ER) [21]. However, in most of these studies the majority of infants were not hospitalized and had short follow-up, only after one or two doses of inhaled HS. Moreover, a recent multi-center double-blind controlled study from California, the largest study performed to date (n=480) in which a substantial number of infants were hospitalized, showed that HS combined with bronchodilators decreased hospitalization rate by almost half [22]. Further analysis of the data shows significant benefit for the length of stay (LOS) in the hospital if considering the intention to treat all children (not only those who were hospitalized) who arrived at the ER. Accordingly, the consensus of the Israeli Pulmonary Pediatric Society concluded in December 2014 that HS 3% combined with bronchodilators could be used in ambulatory patients, in the ER, and in the pediatric ward for children with acute viral bronchiolitis. In a recent reanalysis of previous meta-analyses, Brooks et al. [24] compared hospital LOS in U.S. populations as opposed to other populations. They manipulated results regarding populations and statistics and excluded, based on a geographic location, some Cochrane eligible studies. They concluded that, specifically for a 'typical' U.S. population, there is no benefit for HS treatment, regarding LOS, in contrast to the previous 2014 Cochrane review [3] and the 2015 systematic meta-analyses [4], which included all the data. Nonetheless, the Brooks selective analysis misinterpreted and manipulated the results to conclude that there was no benefit for HS in treating a typical U.S. population. Moreover, the biggest randomized double-blind control trial to examine LOS in patients receiving HS for bronchiolitis in the United States found that the decrease in LOS from 3.92 to 3.2 days did not reach significance. However, this result should be explained by a significant pre-hospitalization effect of HS treatment decreasing substantially the hospitalization rate from 42.6% in the normal

saline (NS) group to 28.9% in the HS group, thus precluding for further potential statistically significant effects [22]. This phenomenon is known as the ill surviving effect: In this study, 197 and 211 infants were intended to be treated with NS and HS, respectively. However, a large majority (71.1%) of the HS treated patients were discharged from the hospital (and from the study analysis regarding LOS). As by definition infants who are discharged are more mildly affected than hospitalized infants. Furthermore, a much higher number of infants from the HS group than from the NS group were discharged. Consequently, the hospitalized infants remaining in the HS group were presumably more severely affected than infants in the NS group. As a result, the LOS of the HS group decreased only by 0.76 day (as compared to 1 day in the randomized control studies included in the Cochrane review). Thus, this is by no way a negative study regarding the effect of HS treatment of infants presenting to the ER with acute viral bronchiolitis. If the hospitalization criteria were less strict and more infants were hospitalized, which is probably the case in some counties outside the U.S., this trend of LOS would have reached significance, and the calculations of Brooks analysis might be different regarding the LOS outcome, even for the U.S. populations. Accordingly, Zhang et al. [4], in their recent systematic meta-analysis, including data published in 2016, concluded that HS treatment decreases, both hospitalization rate and LOS, and is also an effective treatment in ambulatory pediatric patients with acute respiratory syncytial virus bronchiolitis.

In general, a huge number of patients around the world will continue to be treated with HS 3% in acute viral bronchiolitis, and with a higher concentration of NaCl in many other lung diseases. This strengthens the relevancy and generalizability of our study, which is especially important for many patients who cannot afford or have no access to PM HS. To this end, we used a surrogate population of small children with acute viral bronchiolitis to demonstrate the availability and affordability of HS to treat all other airway disease. Obviously, the same methods we used for HS preparations can be easily modified for all useful 3–7% HS concentrations and can be sterilized using a microwave oven in a similar way.

LIMITATIONS

One potential drawback to the assumption of mimicking a real-life situation is that we explained to the volunteers that the purpose of this study was to examine sterility outcomes while using home inhalations. Thus, we assume their awareness to sterility issues affected their preparations. However, even in these conditions we observed high rates of microbial contaminations in the actual inhalation simulations. It thus seems the only solution is daily sterilization of the HS, which as demonstrated can be easily done using microwave radiation.

Electrolyte concentrations were shown to be accurate in both HM and PM preparations. However, electrolyte con-

centrations were tested in the boiling-water method and only briefly in microwave radiation. This issue should be examined more thoroughly. However, NaCl concentration has large safety margins, and even much higher concentrations of electrolytes are considered safe and effective due to the wide safety and therapeutic range of HS [2-4].

Using a microwave oven as a sterilization method raises some possible safety issues, such as exposing the children to inhalation of phthalates and other plastic products, burns on hands when engaging with the hot solution, and burns in the respiratory tract of children inhaling hot solution. These concerns emphasize the importance of proper cooling and using microwave-safe plastic products. However, parents today are already dealing with these issues when they sterilize their baby bottles using a common microwave sterilizer. Fortunately, during our trials there were no such incidences.

The bottles that were distributed to volunteers (HM-HS and PM-HS) were not cultured before distribution right after preparation. The bottles that were cultured after preparation were different bottles from a different preparation batch. The baseline frequency of contamination was assumed to be similar, since the solutions were prepared using the same protocol, by the same individuals, and in the same conditions. However, this assumption may not necessarily be accurate.

The volunteers boiled the water before adding the salt while the microwave sterilization was done to the bottles after adding the salt. We did not culture boiled water before adding salt compared with cultures of boiled salty water (when the hypertonic saline preparation is prepared before boiling). Thus, we do not know how many of the water bottles, before the boiling procedure, were not sterile. However, the pure raw salt was proved to be sterile and we did not presume that the water used by the volunteers was sterile.

CONCLUSIONS AND IMPLICATIONS

Homemade preparations provide HS with adequate electrolyte concentrations. HM-HS preparation using microwave radiation provides sterile HS. Even sterile inhalation solutions are contaminated at an alarmingly fast rate in real settings, regardless of preparation method. Microwave ovens are a cheap, fast, and widely available method to prepare HS treatment with minimal handling.

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Appendix 1. Supplementary material provided to the volunteers**Supplementary material 1**

Boiling water procedure:

- Wash hands with soap and running water.
- Boil water in a clean dried pot.
- Wait until the water is cold.
- Pour 250 cc of water into the bottle.
- Fill the syringe with 7.5 grams (7 ml) of sea salt. You can do this with a clean dry spoon. You need to compress the salt using the piston of the syringe.
- Empty the syringe into the bottle with the water.

*If the syringe gets dirty or falls, please throw it away and use the spare one.

Supplementary material 2

Microwave radiation procedure: [Figure 2]

- Fill a microwave-proof baby bottle with 200 cc of tap water.
- Add a 5.6 ml of sea salt.
- Close the lid with the baby nipple and place in the microwave oven for 2 minutes.
- Wait until bottle is cool.

(The volunteers used their own home microwave ovens, which ranged in wattage from 1100W to 1850W. Boiling was reached after 1 minute of applying microwave radiation.)

Inhalation retraction:

- Wash your hands with soap in running water.
- Open the bottle.
- Open a new syringe.
- Use the syringe to extract 4 ml of solution and empty the syringe into the sink/trash.
- Close the bottle.

“Medicine is not only a science; it is also an art. It does not consist of compounding pills and plasters; it deals with the very processes of life, which must be understood before they may be guided”

Paracelsus, (1493–1541), born Theophrastus von Hohenheim (full name Philippus Aureolus Theophrastus Bombastus von Hohenheim), was a Swiss physician, alchemist and astrologer of the German Renaissance