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Inside this Issue:

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An Evaluation of Common Cleaning Methods for the Removal of a Clinical Isolate of *Escherichia coli* in Personal Hydration System Water Reservoirs

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ABSTRACT

Waterborne infection is an important cause of morbidity and mortality throughout the world. Personal hydration packs have been used by military personnel since the Gulf War and are now a common issue item. Since military personnel tend to operate under austere conditions and may use a variety of water sources, preventing the acquisition of waterborne infections is extremely important. Further, since hydration pack water reservoir replacements may not be available during combat operations, the development of a reliable cleaning protocol for use in the field is essential. Several methods for cleaning have been described. In the current study, three common cleaning methodologies—bleach treatment, baking soda treatment, and proprietary CAMELBAK Cleaning Tabs™—were evaluated for the ability to remove *Escherichia coli* contamination from hydration pack water reservoirs. The study results suggest that the use of bleach and proprietary CAMELBAK tablets should be encouraged since they both operate by releasing bactericidal chlorine compounds into solution, which is more effective at reducing post-treatment bacterial burden. It should be noted that no method was 100% effective at completely eliminating bacteria from the reservoirs and that mechanical cleaning was not attempted.

KEYWORDS: *CAMELBAK Cleaning Tab™; infection, waterborne; hydration packs, personal; cleaning methodologies; Escherichia coli contamination*

Introduction

Waterborne infection is an important cause of morbidity and mortality throughout the world.[1] Proper hydration is an essential part of any physical activity.[2] This is especially true for military personnel, who often perform strenuous activities under stressful and austere conditions, where a clean and reliable water source may not be readily available. Indeed, the degradation of infrastructure, population displacement, and loss of public services that occur during war have often been associated with increases in waterborne diseases.[3–5] This has been demonstrated in conflict and natural disaster zones in locations that are both geographically and culturally distinct.[4,5] Personal hydration packs have been used by military personnel since the Gulf War and are now a common issue item.[6] The typical hydration pack (commonly known as a CamelBak) consists of a backpack-type sack and a removable 2L to 3L water reservoir to which a drinking tube can be attached. Since military personnel tend to operate under austere conditions and may use a variety of water sources, preventing the acquisition of waterborne infections is extremely important. Further, since hydration pack water reservoir replacements may not be available during combat operations, the development of a reliable cleaning protocol for use in the field is essential.

Interestingly, although the US military has studied the integrity of particular hydration packs in a CBRN (chemical, biological, radiological, nuclear) environment, the ability of the current cleaning methods to eliminate pathogenic microorganisms from the interior of the water reservoir has not been evaluated.[7] Several methods for cleaning have been described. These methods include draining the reservoir and rinsing with bleach, draining the reservoir and cleaning with a baking soda solution, and cleaning with a variety of commercially available, water-soluble cleaning tablets. The evaluation of these methods using clinical bacteria is essential given that clinical isolates are

often more robust than their environmental counterparts and given that there can be great variability in the stability of individual isolates.[8] In the current study, three common cleaning methods—bleach treatment, baking soda treatment, and proprietary CAMELBAK Cleaning Tabs (CamelBak Products LLC, Petaluma, CA)—were evaluated for the ability to remove *E. coli* contamination from hydration pack water reservoirs.

Materials and Methods

Bacterial Isolate Used in This Study

A clinical isolate of *E. coli* was obtained from the Tripler Army Medical Center (TAMC) Department of Pathology. This isolate was maintained on tryptone soy agar supplemented with 5% sheep blood (TSA/blood) at 37°C without CO_2, and fresh plates were streaked prior to inoculation. All *E. coli* growth experiments were performed on TSA/blood agar.

Hydration Pack Water Reservoirs

A total of 66 new and unused surplus hydration pack water reservoirs were obtained from the military supply detachment at Schofield Barracks, HI. Each water reservoir had a capacity of 3L (100oz) and was composed of polyether-thermoplastic polyurethane (polyether-TPU).

Water Source

The goal of this study was to evaluate the capacity of three common cleaning methodologies with respect to the removal of *E. coli* contamination from a hydration pack water reservoir. A preliminary study was conducted to compare bacterial growth and survival in a nutrient-limited, closed environment using water obtained from the Honolulu County Municipal Water System and distilled-deionized water obtained from the Department of Clinical Investigation at TAMC (Lab Pure Clinical Laboratory Reagent Water System by Water Solutions Inc., Honolulu HI). Water from the Honolulu County Municipal Water System had a total dissolved solute level of 202ppm (HM Digital TDS-EEZ Handheld Meter, HM Digital Inc., Culver City, CA), and the distilled-deionized water had a total dissolved solute reading of 0ppm, indicative of an efficient purification process.

Two water reservoirs were filled with 500mL of the municipal water and distilled/deionized water, respectively. Both reservoirs were inoculated with 1mL of *E. coli* suspended in phosphate-buffered saline at an optical density (OD) of 1 at 600 nm (Cary 60 UV-Vis spectrophotometer; Agilent Technologies, Santa Clara, CA). A 1OD solution of *E. coli* is approximately equal to a concentration of 500 million bacterial cells/mL, also known as colony-forming units (CFUs). The reservoirs were incubated in a closed room at TAMC Department of Clinical Investigation with a constant temperature of 25°C and limited sunlight for 2 weeks. It was found that only the distilled water reservoir maintained viable organisms. No organisms could be recovered from the reservoir containing the Honolulu municipal water. Given the results of this preliminary study, distilled water was chosen for all further inoculation and cleaning experiments.

Reservoir Inoculation and Storage

Sixty-one hydration water reservoirs were filled with 500mL of distilled water and inoculated with 1mL of phosphate-buffered saline containing an *E. coli* clinical isolate at an optical density of 1. All reservoirs were capped and stored in a covered, open-air shed for 4 weeks at TAMC to simulate the temperature and humidity of field conditions. The average high temperature during the 4-week period was 29.4°C/84.92°F during the day. After 4 weeks, reservoirs were gently mixed and 100µL of water was collected from each reservoir. Collected water samples were immediately plated on TSA/blood agar and incubated overnight at 37°C.

Water Reservoir Cleaning

A total of three cleaning methods were evaluated. Twenty reservoirs were used for each treatment method. For the bleach cleaning method, the reservoirs were first emptied and then refilled with 1L of hot tap water (43°C/110°F) and 30mL of bleach (Clorox Regular-Bleach®). The reservoirs containing bleach solutions were vigorously agitated for 30 seconds and allowed to lay flat for 30 minutes. The bleach solutions were emptied out and then refilled with 500mL of distilled water. Reservoirs were agitated for 30 seconds and a 100µL sample was collected. The water samples were immediately plated on TSA/blood agars.

The same procedure was used for the baking soda (Arm and Hammer Pure Baking Soda®) treatment, with the exception of substituting 30mL of baking soda and 1L of hot tap water for bleach. The proprietary CAMELBAK Cleaning Tablet™ treatment was performed as described for the bleach and the baking soda with the exception that a single tablet was used instead of the bleach/baking soda and the incubation time was 5 minutes instead of 30 minutes as suggested by the manufacturer.

Data Analysis

E. coli was identified by colony morphology. Colony counts before and after treatment were tabulated in Microsoft® Excel. Fisher's exact test was used to determine the statistical significance of the tabulated results.

Results

A total of 60 water reservoirs were used for experimental cleaning treatments. Each was filled with 500mL of

distilled water and inoculated with approximately 500 million bacterial cells, or CFUs. Two reservoirs were used as controls: a positive control that was inoculated but not cleaned and a negative control that was filled with distilled water but not inoculated. All were incubated for a total of 4 weeks at ambient temperature in an open-air outdoor shed. Water samples from the reservoirs were collected and plated for bacterial growth before and after cleaning with the bleach, baking soda, and a proprietary CAMELBAK Cleaning Tab.

After 4 weeks of incubation, it was found that all samples had significant contamination before any cleaning treatment. Organisms other than *E. coli* were noticed and determined by visual inspection. All the plates had too many colonies to count after 24 hours, and there was no significant difference in bacterial growth among the three treatment groups.

After treatment, it is significant to note that the proprietary cleaning tablet was most effective at removing all *E. coli* with no organisms remaining in the posttreatment water samples (Figure 1). Bleach was also effective at removing *E. coli*. Seventeen of the 20 reservoirs (85%) had no *E. coli* after the bleach treatment. In addition, both of these treatment methods were also effective at reducing total bacterial levels (*E. coli* and other contaminating organisms) (Figure 2).

However, baking soda was the least effective in cleaning, with at least 70% of the samples having at least one organism posttreatment. The proprietary tablet and bleach did significantly better than baking soda ($P < .001$ based on a Fisher's exact test). There were no significant differences between the cleaning tablet and bleach ($P = .231$ based on Fisher's exact test).

Figure 1 *Number of water reservoirs containing* E. coli *after cleaning treatments. Both bleach and the CamelBak cleaning tablet are effective at reducing the numbers of* E. coli *(expressed as colony forming units [CFUs]) after treatment. The blue bar represents reservoirs that had at least one organism post-treatment and the maroon bar represents reservoirs that had more than one organism post-treatment.*

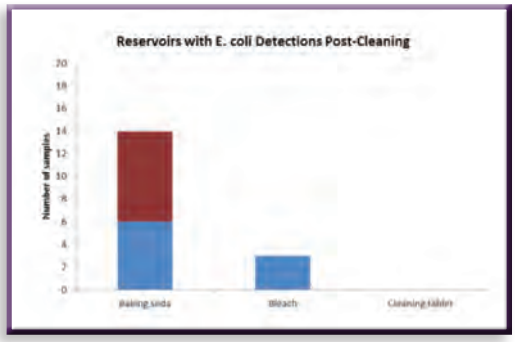
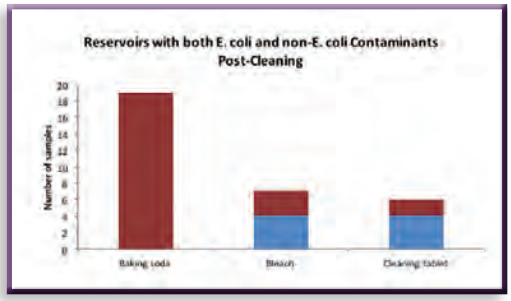


Figure 2 *Number of water reservoirs containing* E. coli *and other biological contaminants following cleaning treatment. Both bleach and the CamelBak cleaning tablet are effective at reducing the numbers of total bacteria (expressed as CFUs) after treatment. The blue bar indicates reservoirs from which 1 CFU was detected. The maroon bar indicates reservoirs from which more than 1 CFU was detected.*



Discussion

One interesting result of this study was the fact that sterile distilled water allowed *E. coli* to survive for at least 4 weeks in the water reservoir while Honolulu County municipal city water did not. Our initial hypothesis was that the bacteria would survive longer in municipal city water due to the greater abundance of dissolved solutes and micronutrients. This result may be explained by the fact that city water is often treated with chlorine, which is known to inhibit bacterial growth.[9] A subsequent search of the literature revealed that certain bacterial species may be able to survive for up to 16 years in pure distilled water.[10,11] Based on these results, it is not advisable to clean a water hydration system by merely washing it with distilled water, as distilled water is not antibacterial and may favor the survival of certain bacteria. It is also not advisable to fill a hydration system reservoir with distilled water and let it sit for any period of time as this may allow the accumulation of environmental bacteria. Chlorinated water appears to be the best choice for use with these systems for its antibacterial property.

It was also interesting to note that bleach and the proprietary cleaning tablet manufactured by CamelBak were the most effective cleaning agents. Both compounds tend to kill bacteria by the release of chlorine into solution, which acts to block cellular metabolism.[12,13] As there was no statistically significant difference between the two, it can be assumed that they were both capable of delivering bactericidal chlorinated compounds into the bacterial cell. Although baking soda has been reported to have some antibacterial activity, it appears to be more effective when combined with sodium dodecyl sulfate. The lack of that compound in the baking soda used in this study may have contributed to its failure in adequately cleaning the water reservoirs.[14]

Conclusion

This study suggests that the use of bleach and proprietary CamelBak tablets should be encouraged since they both operate by releasing bactericidal chlorine compounds into solution, which is more effective at reducing posttreatment bacterial burden. It should be noted that no method was 100% effective at completely eliminating bacteria from the reservoirs and that mechanical cleaning was not attempted. Further, it should be noted that the bleach solution left a strong odor in the reservoirs, which may negatively impact perceptions of palatability and may require multiple risings to remove. The proprietary tablets, on the other hand, left a faint but somewhat clean and pleasant smell in the reservoirs. When time and/or water resources are limited, the tablets may be a more convenient method for cleaning. Future studies will evaluate the addition of mechanical cleaning methodologies, such as scrubbing with brushes or washcloths, as a means of eliminating biofilms and bacterial buildup that may accumulate on the interior surface of the reservoir.

Disclaimers

The views expressed in this abstract/manuscript are those of the author(s) and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US government.

Disclosures

The authors have nothing to disclose.

References

1. **Ramírez-Castillo FY, Loera-Muro A, Jacques M, et al.** Waterborne pathogens: Detection methods and challenges. *Pathogens*. 2015;4:307–334.
2. **Casa DJ, Armstrong LE, Hillman SK, Montain SJ.** National Athletic Trainers' Association position statement: fluid replacement for athletes. *Jf Athl Train.* 2000;35:212–224.
3. **Swerdlow DL, Woodruff BA, Brady RC, et al.** A waterborne outbreak in Missouri of Escherichia coli O157: H7 associated with bloody diarrhea and death. *Ann Intern Med*. 1992;117: 812–819.
4. **Walton DA, Ivers LC** (2011). Responding to cholera in post-earthquake Haiti. *N Engl J Med*. 2011;364:3–5.
5. **Zolnikov TR**. The maladies of water and war: addressing poor water quality in Iraq. *Am J Public Health*. 2013;103:980–987.
6. **Hart S.** CamelBak expands into new markets. *The Press Democrat* (Santa Rosa, CA), 8 April 2012.
7. US Army approves Camelbak CBR X for use in the worst environments. http://www.militarytimes.com/story/military/gearscout/2010/11/16/us-army-approves-camelbak-cbr-x-for-use-in-the-worst-environments/32168047/. Accessed 14 April 2016.
8. **van Elsas JD, Semenov AV, Costa R, Trevors JT.** Survival of Escherichia coli in the environment: fundamental and public health aspects. *ISME J*. 2011;5:173–183.
9. **Camper AK, McFeters GA.** Chlorine injury and the enumeration of waterborne coliform bacteria. *Appl Environ Microbiol.* 1979;37:633-–641.
10. **Iacobellis NS, DeVay JE**. Long-term storage of plant-pathogenic bacteria in sterile distilled water. *Appl Environ Microbiol.* 1986;52(2), 388–389.
11. **Liao CH, Shollenberger LM.** Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline. *Lett Appl Microbial.* 2003;37:45–50.
12. **WHO.** Sodium dichloroisocyanurate in drinking water, World Health Organization report. http://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/sodium_dichloroisocyanurate_2add_feb2008.pdf. Accessed 14 April 2016.
13. **Estrela C, Estrela CR, Barbin EL, et al.** Mechanism of action of sodium hypochlorite. *Brazil Dent J*. 2002;13:113–117.
14. **Drake D.** Antibacterial activity of baking soda. *Compend Contin Educ (Jamesburg, NJ: 1995)*. 1995;17(Suppl):S17–S21.

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