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ORIGINAL RESEARCH

10% Povidone-Iodine May Be a Practical Field Water Disinfectant

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Objective.—A paucity of data exists regarding the use of iodophores such as povidone-iodine (PVI) to disinfect water. We sought to determine a practical minimal disinfecting concentration of 10% PVI over different contact times and temperatures when added to water inoculated with *E. coli*.

Methods.—1:100, 1:1,000, and 1:10,000 dilutions of 10% PVI were created. *Escherichia coli* was exposed to these dilutions for 5, 15, and 30 minutes at 10, 20, and 30°C. Bactericidal activity was neutralized with 0.5% sodium thiosulfate. Mean viable colony forming units (CFUs) was determined after triplicate plating on Luria-bertani agar and 24 hours of incubation at 37°C. Effective bactericidal activity was defined as a 5-log reduction.

Results.—Of the 200,000 *E. coli* plated, no CFUs were observed after exposure to the 1:100 dilution. After 5 minutes of contact time with the 1:1,000 dilution, at 10°C CFUs were too numerous to count (TNTC), at 20°C the mean CFU count was 92 (standard error ±11), and at 30°C the mean CFU count was 25 (standard error ±8). No CFUs were observed after 15 minutes of exposure to the 1:1,000 dilution across experimental temperatures. The 1:10,000 dilution always yielded CFU growth that was TNTC.

Conclusions.—The lowest disinfecting concentration of 10% PVI was the 1:1,000 dilution at 15 minutes of contact time. This supports the use of PVI for water disinfection against *E. coli*, the organism most commonly responsible for traveler's diarrhea. Further studies may assess its effectiveness against more virulent water borne pathogens.

Key words: water disinfection, povidone-iodine, *Escherichia coli*, traveler's diarrhea

Introduction

Even the most pristine appearing surface water may contain harmful infectious contaminants, and, without treatment, almost no surface water should be considered safe to drink.[1–4] The main benefit to treating drinking water is to prevent gastrointestinal illness by enteric pathogens such as the bacterium *Escherichia coli, E. coli.* is the most common causative agent of traveler's diarrhea and is recognized as an important waterborne pathogen and as an indicator organism for monitoring water quality.[5–7] Enteric pathogens such as *E. coli* are found naturally in tropical, temperate, and cold waters. The diarrheagenic *E. coli* include such members as enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, and others.[8]

Iodophores such as povidone-iodine (PVI) are compounds consisting of iodine and inert polymers such as polyvinylpyrrolidone that have several advantages over elemental iodine preparations. As a topical disinfectant, iodophores tend to be less irritating to the skin, are more soluble in water, and are less staining, and yet maintain the antibacterial activity of iodine.[9] Organic iodine and iodine containing filter products have been well established as effective, safe, and simple methods of water disinfection. These iodinated products have been used extensively by the United States Army, aboard the US Space Shuttle, and in situations necessitating the emergency disinfection of potable water supplies such as in austere environments and during times of natural disasters.[10–15] The efficacy of iodophores to disinfect drinking water, however, has not been well established, and to our knowledge there are no previous studies that have

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effectively evaluated the potential for utilizing 10% povidone-iodine (PVI) as a practical field water disinfectant. We sought to experimentally determine a practical minimal disinfecting concentration of PVI when added to different temperatures of water inoculated with a known concentration of *E. coli*, and across water temperatures similar to those at which this organism is commonly found.

Methods

POVIDONE-IODINE SOLUTION

Betadine 10% Povidone-Iodine Topical Solution (1% available iodine, Purdue Frederick Company, Norwalk, CT, Lot #087-0215) was used in our experiment. Full-strength 10% PVI stock solution was added to sterile deionized water to create 1-L volumes of 1:100, 1:1,000, and 1:10,000 dilutions of 10% PVI. The PVI stock solutions and subsequent dilutions were used on the day that they were opened. The dilutions were kept at room temperature until placed in the temperature-controlled water bath as described below. The pH of each dilution was measured.

BACTERIA AND CULTURE MEDIUM

The clinical strain of *E. coli* used was ATCC 25922 and is our institution's laboratory quality control strain. Significant effective bactericidal activity was defined per convention as a 5-log reduction (99.999% bacterial kill).[9,16] To detect this 10^5 colony-forming unit (CFU) reduction, an initial stock inoculum of 10^8 CFU/mL of bacteria was created, then washed, and suspended in phosphate-buffered water. Final viable bacteria CFU count was determined after incubation on Luria-bertani (LB) agar plates. All final plating was performed in triplicate, and final results were expressed as the mean CFU count of the 3 plates with calculated standard error.

NEUTRALIZATION

Neutralization is essential to ensure that, after timed exposure to PVI, the bactericidal action of PVI does not carry over into the final survivor culture medium. Sterile 0.5% sodium thiosulfate (STS) was used in our experiment (Sigma-Aldrich Company, St. Louis, MO, Product #S7026, Lot #106K0178). STS is well established as an appropriate neutralizing agent for PVI in experiments employing many different species of bacteria.[17–20] Experimental controls as described in the "experimental procedure" section were performed to ensure that the neutralizing agent itself did not inhibit bacterial growth.

EXPERIMENTAL PROCEDURE

Temperature was a controlled variable in this experiment. The same protocol was performed with the PVI dilutions maintained in baths at 3 different temperatures: 10, 20, and 30°C. Sterile test tubes containing 9 mL of a given PVI dilution were placed in the water baths, and temperature equilibration was achieved in 30 to 60 minutes. One milliliter of our stock *E. coli* inoculum (at 10^8 CFU/mL) was then added to each of these test tubes with the PVI dilutions. One milliliter of this mixture (PVI + 10^7 CFU/mL of *E. coli*) was then withdrawn at set intervals of 5, 15, and 30 minutes, and added to 4 mL of the 0.5% sodium thiosulfate (STS) to neutralize the bactericidal activity of the PVI. This final solution (PVI + STS + 2×10^6 CFU/mL of *E. coli*) was gently agitated for 30 seconds, and the number of viable organisms present was determined by plating 0.1-mL samples containing 2×10^5 CFU of *E. coli* on LB plates in triplicate using standard surface plating techniques. Surviving CFUs from these 2×10^5 plated *E. coli* were counted after incubation at 37°C for 24 hours.

Experimental controls to assess for measurable antibacterial activity of the STS neutralizing agent were performed at each of the above temperatures and time intervals by substituting 9 mL of sterile deionized water for the 9 mL of PVI dilution. All other steps for the experimental control, including the details regarding the stock *E. coli* inoculums, addition of the STS, plating methods, incubation temperature, and incubation time, were otherwise identical to the steps that utilized PVI dilutions.

Results

Differences in disinfection ability were observed between the 3 concentrations of 10% PVI (Table). Of the 200,000 *E. coli* plated, no CFUs were observed at any sampling times after exposure to the 1:100 dilution of 10% PVI. After 5 minutes of contact time with the 1:1,000 dilution, at 10°C CFUs were too numerous to count (TNTC), at 20°C the mean CFU count was 92 (standard error ±11), and at 30°C the mean CFU count was 25 (standard error ±8). However, at 15 minutes of contact time, no CFUs were observed after exposure to the 1:1,000 dilution across experimental temperatures. The 1:10,000 dilution always yielded CFU growth that was TNTC. A trend toward faster disinfection at warmer experimental temperatures was observed with the 1:1,000 concentration. However, no significant difference in the time to effective disinfection (5-log reduction) relative to different temperatures for a given concentration of PVI was observed at any of our sampling times. The