



Comparison of two plain soap types for removal of bacteria and viruses from hands with specific focus on food service environments



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ABSTRACT

Handwashing (HW) is a long established and widely accepted method to prevent disease transmission. Ensuring effectiveness of current HW methods is essential for the optimization of HW and enhanced disease prevention. The objective of this research was to determine the difference in microbial reduction between plain foaming and liquid handsoap. The hands of 24 participants were inoculated by the palmar surface method with an average of 1.25×10^8 CFU *Escherichia coli* C3000 or 1.36×10^8 PFU MS2 bacteriophage. Participants washed their hands following a standard protocol with a standardized soap volume and a 10 s HW time. A glove juice method was used to recover microorganisms from hands. Remaining microorganisms were quantified by standard spread plate and plaque assays for *E. coli* and MS2, respectively. Hands inoculated with *E. coli* had an average log reduction of 2.76 ± 0.70 and 2.52 ± 0.58 log CFU for foaming and liquid handsoap, respectively. The mean log reduction for hands inoculated with MS2 was 2.10 ± 0.57 and 2.23 ± 0.51 log PFU for foaming and liquid handsoap, respectively. Data indicate no significant difference in overall microbial removal when comparing the efficacy of plain foaming and liquid handsoap. However, regardless of soap type, the type of microorganism impacted overall log reduction with a greater reduction for *E. coli* when compared to MS2 with a significant difference ($p = 0.0008$) in reduction for foaming handsoap. This study is the first comparison of the efficacy of plain liquid and foaming handsoap for microbial reduction on hands during HW.

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1. Introduction

It is estimated that foodborne pathogens, both major known pathogens as well as unspecified agents, cause 47.8 million illnesses, 127,830 hospitalizations, and 3037 deaths in the U.S. each year with the leading causes of illness including noroviruses (58%), nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%) (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). Pathogenic strains of *Escherichia coli* and *Salmonella* are more commonly associated with raw meat (i.e. beef and poultry, respectively) as animals are often hosts for these pathogens (Forsythe, 2010). However, cross contamination of pathogens between raw meat and ready-to-eat food products via food handlers' hands is a potential risk; therefore, proper handwashing (HW) is an essential control measure for risk reduction (USFDA, 2013). With respect to foodborne viruses, an epidemiologic investigation of foodborne norovirus outbreaks in the U.S. from 2001 to

2008 found that 53% (473) of the 886 outbreaks were caused by food handler contamination (Hall et al., 2012). Additional analysis of foodborne norovirus outbreaks from 2009 to 2012 confirmed these findings with food workers implicated in 70% of 520 outbreaks, and bare hand contact was identified in 54% of the outbreaks (Hall, Wikswo, Pringle, Gould, & Parashar, 2014). The recommended interventions for preventing norovirus in a food service environment primarily include following US Food and Drug Administration (FDA) Food Code 2013 guidelines for HW and glove use (Hall et al., 2012; USFDA, 2013).

The general population uses HW as an important step in disease prevention, and this is especially critical within a food service environment (Miller, James-Davis, & Milaneis, 1994). The hands of food service employees may become contaminated with foodborne pathogens during critical stages in food service including after using the restroom, handling raw materials (e.g., meats, vegetables, eggs, etc.) and after touching contaminated surfaces (Miller et al., 1994). Therefore, studies on the efficacy of HW agents are essential to ensure that HW procedures are optimized for removal of pathogenic microorganisms from hands during food service.

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Numerous soaps (i.e. brands, types, formulations, etc.) are available on the market today, and food service staff and the general population use these soaps daily. Plain (non-antimicrobial) hand-soap reduces soil, dirt, and in the case of food service, various physical and biological materials on hands through physical removal with detergents. Meanwhile, antimicrobial handsoap combines physical removal with the inactivation of microorganisms by antimicrobial compounds in the soap that differentially affect viruses and bacteria (Fuls et al., 2008; Sickbert-Bennet et al. 2005). While there have been numerous studies comparing the efficacy of antimicrobial and plain handsoap (Edmonds, McCormack, Zhou, Macinga, & Fricker, 2012; Fuls et al., 2008; Montville & Schaffner, 2011), the soaps used in these studies are typically liquid handsoap. In a recent review by Conover and Gibson (2016), the methodologies and results of 24 HW studies published since 1985 are discussed and despite the vast range of HW agents tested in these studies, only one study evaluated foaming handsoaps (Fuls et al., 2008) and none compare the efficacy of foaming and liquid handsoap. For this reason, the authors of the present study selected to compare plain foaming and liquid handsoaps. One of the primary differences between foaming and liquid handsoap is the level of surfactant. Foaming soaps generally have a lower level of surfactants, and as a result, these soaps do not form micelles as readily as liquid handsoap. Meanwhile liquid handsoaps typically have increased surfactant levels as well as additional salts that allow for the formation of micelles (personal communication provided by M. Caetta, VCI Formulation Specialist at GOJO Industries, Inc.) that aid in the removal of dirt and oils as well as microorganisms.

With the increasing prevalence of foaming handsoap on the market and within food service establishments, it is critical to determine if the associated microbial reductions are comparable to that of traditional, plain liquid handsoap. For this study, we hypothesized that there would be a significant difference in microbial reduction between foaming and liquid handsoap. More specifically, we hypothesized that reduction of bacteria and virus on hands would differ depending on soap type. Therefore, the overall goal of this study was to determine if a difference exists in the efficacy of plain foaming and liquid handsoap by measuring the reduction of microorganisms on hands inoculated with non-pathogenic *E. coli* and MS2 bacteriophage—a surrogate for the study of human enteric viruses such as norovirus.

2. Materials and methods

2.1. Study design

The study was based on a Latin square design. The treatment structure was a two by two factorial with microorganisms (*E. coli* C3000 and MS2) and soap type (foaming and liquid) as the two different factors. Each participant visited one time per week over a four week period and was randomly assigned to one of four sequences of treatment. Sequences were selected to alternate exposure of participants to microorganism type and soap type and to adjust for any possible confounding factors (e.g., learning by either the researchers or the experimental participants or any carryover effects that could potentially be present throughout the four weeks of the study).

2.2. Participant recruitment and training

Twenty-four participants (12 men and 12 women), 18 years and older, were recruited from the University of Arkansas (Fayetteville, Arkansas) community. Participants had healthy skin, with no presence of dermatitis, open wounds, cuts, burns, hangnails, or any

additional known disorders of the skin (ASTM, 2013a). Institutional Review Board and Institutional Biosafety Committee approval were obtained, and participants were informed about the safety of microorganisms used in the study. All participants signed an informed consent form to participate in the study. Sample size was determined based on a minimum power of 0.8 with the following parameters: $\alpha = 0.05$, standard deviation = 0.6, and a difference to detect of 0.5 log₁₀ CFU or PFU.

To employ a standardized HW procedure throughout the study, prior to participating, participants were trained on a standard HW protocol (Singapore Motherhood, 2012). Participants were given 30 s to complete the HW procedure during training as well as throughout the decontamination steps of the study. The actual experimental handwash was completed in 10 s which is considered more representative of actual HW time occurring in daily life (discussed in Section 2.7).

2.3. Preparation of inocula

2.3.1. Preparation of MS2 bacteriophage

A stock of MS2 bacteriophage (ATCC 15597-B1; American Type Culture Collection, Manassas, VA) was prepared through propagation in *E. coli* C3000 followed by chloroform extraction of the infected cell lysate as described previously by Gibson, Crandall, and Rieke (2012). The stock concentration of MS2 bacteriophage was determined to be approximately 10¹¹ PFU/mL by the double agar layer (DAL) method. One milliliter aliquots of MS2 were stored at −80 °C. The phage stock was diluted with 0.1% peptone (Becton Dickinson and Company, Sparks, Maryland) to approximately 6.78 × 10⁸ PFU/mL.

2.3.2. Preparation of *E. coli* C3000

Overnight stocks of *E. coli* C3000 (ATCC 15597; ATCC) was prepared in a culture flask containing 25 mL of tryptic soy broth (Acumedia, Lansing, Michigan) incubated at 37 °C with shaking at 110 rpm. Stock concentrations were determined by preparing a ten-fold dilution series and plating 1 mL of each dilution in duplicate on 3 M Petrifilm™ *E. coli*/coliform count plates (3 M, Maplewood, Minnesota). *E. coli* C3000 overnight culture (approximately 10⁹ CFU/mL) was diluted with 0.1% peptone (Becton Dickinson and Company) to approximately 6.26 × 10⁸ CFU/mL for inoculation on participants' hands.

2.4. Hand decontamination prior to inoculation

To eliminate resident microorganisms on the hands of participants prior to inoculation with test organisms, hands were treated with a conditioning wash as described by Fuls et al. (2008) with modifications. Modifications included using 1 pump of antibacterial handsoap (The Dial Corporation, Scottsdale, Arizona) with subjects scrubbing hands for 30 s and rinsing hands for 10 s. Hands were also twice soaked in 70% ethyl alcohol and dried thoroughly before inoculation with microorganisms.

2.5. Inoculation of hands

Hand inoculation of *E. coli* C3000 and MS2 was performed by the palmar surface method (PSM) as described in the ASTM Standard Test Method E2870-13 with modifications. One-hundred microliters of prepared *E. coli* or MS2 inoculum were pipetted onto the palm of each hand (200 µL total) for an average of 1.25 × 10⁸ CFU total (hands combined) or 1.36 × 10⁸ PFU total (hand combined), respectively. The participants were asked to rub the palms and fingers of each hand against each other for 10 ± 1 s in order to spread the inoculum on the palms and fingers of each hand.

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