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Acceptability of Household Practices to Prevent Boils in Rural Alaska

Supplement 1

Effect of adding chlorine bleach to contaminated laundry in households without piped water

Rationale

In many communities in rural Alaska, households do not have piped water. Instead, water may be pumped to local collection points where it may be hauled into homes for storage and use (in 'self-haul' communities) (Thomas, Hickel, & Heavener, 2016). Typically, household laundry is washed by adding water stored at room temperature. To conserve water, the same water is re-used frequently to perform multiple laundry loads. Many households use a 'Danby' washer that is connected to electricity but is not hooked up to a water supply (Razniak et al., 2015). Laundry is added to water with detergent in the tub, and a wash cycle is run to agitate the laundry with the detergent. Depending on the household, a rinse cycle to wash off the soap may be run, or the water may be conserved by re-use for a subsequent laundry load.

Studies elsewhere have demonstrated that contamination of household surfaces, objects and textiles are important sources of *S. aureus* transmission in the home, and that contamination of textiles (such as clothing or bedding) can persist despite household laundry practices (Fritz et al., 2014; Honisch, Stamminger, & Bockmühl, 2014). The probability of decontamination of laundry is increased by higher wash temperatures and longer cycle durations (Honisch et al., 2014). However, households without piped water may not be able to use higher temperatures or longer cycle durations, and frequently re-use the same water for multiple loads (Razniak et al., 2015). We established a simple model of contamination of laundry with *S. aureus* and then evaluated the effect of adding household chlorine bleach.

Inoculation with *S. aureus*

Following the approach taken as part of a previous study of sterilization by household laundry (Patel, Murray-Leonard, & Wilson, 2006), we modeled contamination of clothing by inoculating an autoclaved

5cm^2 cloth swatch (50% cotton, 50% polyester). We added 1 colony of a strain of *S. aureus* (ATCC 25923, MSSA) to 5mL tryptic soy broth (TSB), vortexed to distribute the organisms evenly, and pipetted 400µL of the mixture evenly on the swatch, allowing to air-dry for an hour.

Measurement of staphylococcal growth

To assess contamination we also adapted methods from an earlier study (Patel et al., 2006). To prepare a 5cm^2 cloth swatch for evaluation, we placed the swatch in a tube containing 5mL of TSB using sterile forceps. For samples from the washer tub, we swabbed the interior of the washer using a sterile swab and placed in 5mL TSB. To sample effluent water from the washer, we added 1mL of the water to 5mL TSB. For each method, we vortexed the tube mixture and left it to sit, to distribute organisms evenly. We then pipetted 100 µL of the TSB mixture from each sample type, which we plated onto blood agar plates at the following concentrations: neat, 1:10, 1:100, 1:1000, 1:10000. We recorded growth on each plate at 24 hours and estimated the amount of *S. aureus* by the number of colonies present at the lowest concentration of the mixture.

Simulation of household laundry

To establish a model of household laundry in the absence of running water, a Danby® washer was set up in a microbiology laboratory. The model included a wash tub which could be filled via an external water hose and drained through an effluent pipe, and an adjacent spin tub. For each laundry run, we used 50L tap water kept at 20–22 ºC in the washer tub, and added the recommended volume of a commercially available liquid detergent designed for laundry at cold temperatures (Tide Cold Wash, approximately 60mL per load). To simulate a laundry load we added standardized clothing was added to the wash tub that was 60% cotton and 40% polyester (pants, a sweater, and a hooded top) using a 30-minute autoclave gravity cycle to re-sterilize the clothing between experiments. To allow assessment of contamination, four autoclaved 5cm^2 cloth swatches were added to each laundry load (Figure).

A wash cycle was run for 30 minutes, during which the wash tub contents were mechanically agitated. No rinse step was used, since during a preliminary visit community members reported that the rinse step was usually omitted to conserve water. To simulate re-use of water for an additional load, we repeated the standard laundry cycle, except that the water in the tub was used for a further laundry load after the initial laundry load was removed. After running a wash cycle, the clothing and 4 swatches were moved to the spin dryer compartment, and spun for 4 minutes, according to manufacturer recommendations. To sterilize the washer between experiments, we repeated the standard laundry process with detergent and water only, without added bleach or clothing.

Evaluation of contamination

To assess the effect of adding an inoculated cloth swatch, we compared staphylococcal growth after adding an inoculated swatch to a standard laundry run with a control experiment in which only sterile swatches were added. To assess the effect of adding chlorine bleach we then compared growth following the laundry run with an inoculated swatch with the same experiment but with additional chlorine bleach (1 capful, approximately 10mL). We repeated this comparison in a second load of laundry by re-using the same water with 4 new sterilized swatches and a new laundry load.

Evaluation of sustained effect of chlorine bleach

To assess the effect of adding 1 capful of chlorine bleach on repeated wash loads using the same water, we performed an additional experiment in which the same tap water was re-used three times for four separate wash loads (each with new detergent, swatches and clothing added). We assessed for the presence free chlorine using Hach Aquachek High Range Chlorine Test Strips (detection threshold 0-600 parts per million [PPM] in mg/mL). Hach Aquachek Total Chlorine and Free Chlorine Test Strips were used to assess the presence of total and free chlorine with a detectable range of 0-10 ppm in mg/mL. We assessed chlorine content in tap water and after addition of one capful of chlorine bleach to 50L tap water. We then assessed concentration of total and free chlorine and of bacterial contamination of the swatches and effluent water after four successive laundry loads reusing the same water.

Effect of inoculation

After inoculation with *S. aureus*, a previously sterilized cloth swatch was found to have 100,000 CFU/mL. Using the same inoculation method, after a standard laundry cycle including detergent, clothing, and other cloth swatches, 5,000 CFU/mL was detectable on an inoculated swatch. In addition, colony counts of 10–20 CFU /mL were detectable on 3 other cotton swatches in the laundry that were not inoculated, and 10 CFU/mL was detectable in samples of effluent water. When water was re-used with new swatches and clothing added, 4,000 CFU/mL was detectable on the inoculated swatch, 10–30 CFU /mL was detectable on other swatches, no colonies were detectable on the washer tub, and 20 CFU/mL was detectable in effluent water. A control experiment of a single laundry load using only autoclaved swatches yielded no *S. aureus* from any swatches or the washer tub, but also included detection of 10 CFU/mL in effluent water (Table 1).

Effect of adding chlorine bleach

After adding chlorine bleach there was no detectable contamination with *S. aureus*, whether or not the same water was re-used (Table 1). Results of a follow up experiment to assess the persistent effects of bleach during 4 wash cycles re-using the same water are summarized in Table 2. The addition of 1 capful of bleach to 50mL tap water resulted in detectable total chlorine concentration of 4–10 ppm and a free chlorine concentration of <25 ppm that was not detectable using 0–10 ppm test strip. Total chlorine of ~4ppm was detectable through subsequent wash loads with the same water whereas free chlorine was no longer detectable during subsequent wash loads. *S. aureus* was not detectable on any swatches exposed to the water or in the effluent water (Table 2). A swab of the washer tub at the end of the 4th wash cycle was also negative.

Figure: Simulated laundry load including water, detergent, clothing, and four cotton swatches

Table 1: Contamination of four cloth swatches, washer, and effluent water under different conditions in simulated household laundry without piped water

Additions to simulated laundry	**Swatch 1** (*S. aureus* CFU/mL*)	**Swatch 2** (*S. aureus* CFU/mL*)	**Swatch 3** (*S. aureus* CFU/mL*)	**Swatch 4** (*S. aureus* CFU/mL*)	**Washer tub** (*S. aureus* CFU/mL*)	**Effluent water** (*S. aureus* CFU/mL*)
Control (no inoculated swatch)	0	0	0	0	0	10
Inoculated swatch †	5,000	10	10	20	‡	10
Inoculated swatch, reused water	4,000	10	20	30	0	20
Inoculated swatch, chlorine bleach	0	0	0	0	‡	0
Inoculated swatch, reused water, chlorine bleach	0	0	0	0	0	0

* Colony forming units per mL, measured on blood salt agar after 24 hours.

† Swatch inoculated with *S. aureus* (ATCC 25923, MSSA). Using the same inoculation method, a test swatch was found to have 100,000 CFU/mL on repeat testing.

‡ The washer tub was not tested in laundry cycles for which the water was re-used in a subsequent cycle.

Table 2: Chlorine content and contamination of four cloth swatches and effluent water after four simulated laundry loads reusing the same water

Timing of sample	**Total chlorine (range 0–10 ppm)**	**Free chlorine (range 0–10 ppm)**	**Free chlorine (range 0–600 ppm)**	**Swatch 1** (*S. aureus* CFU/mL) *	**Swatch 2** (*S. aureus* CFU/mL) *	**Swatch 3** (*S. aureus* CFU/mL) *	**Swatch 4** (*S. aureus* CFU/mL) *	**Effluent water** (*S. aureus* CFU/mL)
Tap water	2	2	0	-	-	-	-	-
1 capful of bleach in 50L tap water	4-10	0	<25	-	-	-	-	-
After laundry load 1	4-10	0	0	0	0	0	0	0
After laundry load 2	4-10	0	0	0	0	0	0	0
After laundry load 3	4	0	0	0	0	0	0	0
After laundry load 4	4	0	0	0	0	0	0	0

* One swatch was inoculated with *S. aureus* (ATCC 25923, MSSA). Using the same inoculation method, a test swatch was found to have 100,000 CFU/mL on repeat testing. Growth measured in colony forming units per mL, on blood salt agar after 24 hours

Conclusions

From a simulation of laundry in a household without piped water we found evidence that low temperature laundry may result in inadequate sterilization of clothing contaminated with *S. aureus* and therefore a potential source of transmission in the home. This is consistent with the finding that household textiles such as hand towels and bed sheets may be frequently contaminated with *S. aureus*, and that household surfaces may be contaminated with strains matching cases of community-associated infections with MRSA (Fritz et al., 2014). Although a study of home laundering of hospital uniforms reported adequate decontamination using similar methods of assessment to this study (Patel et al., 2006), cycles were run at 40°C in machines with a piped water supply. The lower temperature in the current study may have contributed to the persistence of *S. aureus* (Honisch et al., 2014). In addition, we found evidence of cross-contamination to other uninoculated swatches. This indicates that it may be possible for contamination to occur during laundry, although at low concentrations.

Adding even a capful of bleach (~10mL) in 50L of water was sufficient to neutralize the effects of introducing an inoculated cloth swatch, resulting in sterilization of the inoculated swatch as well as preventing transmission to other swatches. This is consistent with a study that found that adding ~100mL chlorine bleach (sodium hypochlorite) into a similar volume of water led to more than a 3-log-reduction in the concentration of *S. aureus* within 5 minutes (Sehulster, 2015). Similarly a study of recovery of *S. aureus* on cotton towels found that chlorine bleach was most effective at reducing bacterial contamination (Oller & Mitchell, 2009).

We also found evidence that a single capful of chlorine bleach could provide effective sterilization for multiple wash loads using the same water. This is important because re-use of the same water for laundry is frequent in villages without in-home water (Razniak et al., 2015). We found that a new inoculated swatch was effectively sterilized even after up to 4 uses of the same water with no detectable

S. aureus after the laundry cycle or transmission to other swatches. The persistent effect of bleach occurred despite no detectable free chlorine after laundry; the persistence of total chlorine may reflect conversion of free chlorine (such as sodium hypochlorite) into chloramines.

Although our study was designed to model laundry practices in a household using unpiped water, actual conditions may vary, and caution is needed in extrapolating the findings for several reasons. Firstly, we used the standard inoculation technique reported in an earlier study but the actual quantity of *S. aureus* contaminating clothing in the community is unknown, and is likely to vary depending on whether it is from a person who is asymptomatically colonized or with an open boil, among other circumstances. Secondly, we used a simplified laundry load for comparability but the actual wash load used may again vary. It is possible that soiled clothing or more clothing could result in greater agitation during the wash cycle but could also require higher concentrations of chlorine bleach for sterilization. Thirdly, we used tap water provided to the microbiology laboratory which may be chlorinated to a different level than water used in the community, which may affect the quantity of bleach required. Fourthly, it may not be possible to extrapolate to households using a different washer.

Our study also has some inherent limitations in inoculation and assessment of contamination by *S. aureus*. Although we quantified growth of *S. aureus* following a standard inoculation procedure, the level of *S. aureus* contamination may have varied for some test swatches. We found a concentration lower than reported from the study using similar methods (Patel et al., 2006), and we were not able to assess the level of *S. aureus* on these swtaches until after the laundry cycle. Our assessment of contamination was limited because the effluent fluid was found to have bacterial growth at baseline. A more general limitation is that we were not able to confirm that the growth was of *S. aureus*, and of the inoculated strain. However, these limitations do not directly limit comparison of experiments with or without the inoculated swatch, or with or without chlorine bleach.

Overall, this study indicates that adding a capful of chlorine bleach to the first wash load may be an effective strategy to reduce staphylococcal contamination that could lead to household transmission and skin and soft tissue infections in the community, even if the same water is re-used multiple times. A practical consideration is that the use of chlorine bleach may lead to bleaching of colours over time. One approach to this would be using a lower concentration of chlorine bleach—further experiments could help to clarify a minimum concentration of chlorine bleach to be recommended. The acceptability of adding a rinse step in laundry is also unknown—this may help reduce the risk of skin irritation from detergent or bleach.

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