EVALUATION OF THE OCCURRENCE AND RISK OF MICROBES IN LAUNDRY AND LAUNDRY-ASSOCIATED SURFACES

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jeanne McDonald Nordstrom entitled Evaluation of the Occurrence and Risk of Microbes in Laundry and Laundry-associated Surfaces and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

Viable bacteria have been found on environmental surfaces, including washed and unwashed clothing, and places that come into contact with laundry. Under certain conditions, clothing contaminated with pathogenic organisms may present a health risk to the laundry handler. This research project focused on i) evaluating *Staphylococcus aureus* and MRSA survival using front-load and top-load washing machines; ii) determining relative microbial levels on new, disposable, laundered and unlaundered hospital scrubs; iii) characterizing the relative hygiene of public and apartment laundromat surfaces; and iv) developing a quantitative risk assessment for laundry handlers.

Standard microbial evaluation techniques were used to identify and quantify a variety of microorganisms on fabrics and environmental surfaces, including HPC bacteria, *S. aureus*, MRSA, total coliforms and *Escherichia coli*. *S. aureus* and MRSA were exclusively used during evaluation of bacteria reduction levels achieved by front- and top-load washers.

Results from this research indicate:

i) Washing in either a top- or front-load washer affords a 5 – 6 $\log_{10}$ reduction of *S. aureus* and MRSA when detergent is used. If complete drying and/or bleach are also employed, a 6 – 7 $\log_{10}$ reduction is achieved and few organisms remain.

ii) Bacteria cross-contamination of other fabrics within a laundry load is common for both types of washers and between loads on the interior of top-load washers.

iii) Significantly fewer bacteria ($p=0.044$) were detected on hospital-laundered scrubs than on home-laundered scrubs.

iv) Laundromat surfaces can be contaminated with substantial numbers of bacteria and the potential exists for transfer of bacteria from a past user to the next laundromat patron.

v) The risk of acquiring a *S. aureus* infection after handling unwashed laundry contaminated with an initial *S. aureus* level of 10^6 CFU/cm^2 was estimated to be 0.59 infections per person per year. The estimated risk became negligible if handling washed laundry.

INTRODUCTION

Problem Definition

Statistical information from the World Health Organization (2007) suggests that infectious diseases are the leading cause of death in the world. Significant changes in the normal patterns of home situations and hygienic practices have contributed to the spread of infectious diseases. Infectious disease transmission has been shown to occur in 6-60% of households in which one member is ill (Kagan *et al*. 2002).

Pathogens are brought into homes via people, food, water, insects, pets, and the air (Bloomfield *et al*. 2003) and can contaminate a variety of surfaces, including fabrics. To date there have only been a few documented reports of nosocomial infections transmitted via a fabric route (Standaert *et al*. 1994), however, increased frequency of homecare for the elderly, immunocompromised, and/or known and unknown carriers of methicillin resistant *Staphylococcus aureus* (MRSA) raise concerns regarding human health risks of contaminated laundry. Numerous variables such as new fabric types, increasing numbers of healthcare workers washing scrubs at home, and changing laundering practices (i.e. lower wash temperatures, energy and water efficient appliances, and reduction in bleach use) may impact the effectiveness of current laundry practices (Scott 1999).

Larson *et al*. (2001) performed a correlation prevalence survey to describe the relationship between home hygiene practices and prevalence of infectious disease symptoms among household members and was the first to suggest a potential link between using self-service laundromats and disease transmission within the home. Three

hundred and ninety eight inner-city households (96.4% Hispanic) were surveyed and the only hygienic practices in a home setting that were associated with transmission of infectious disease symptoms among household members were use of communal laundries or a failure to use bleach when washing laundry. Other home hygiene practices that were not identified as being significant ($p<0.05$) to the prevalence of infection included personal hygiene habits such as bathing, frequency of general cleaning, duration of kitchen sponge use, wearing gloves when cleaning the toilet, and use of an automatic dishwasher.

Literature review

Changes in home-laundering practices

The primary objectives of the laundering process are removal of soil, stains, creases, and pathogens, and haven't changed over time; however, the equipment available to consumers has. In a household washing process the main hygienic effect is probably due to removal of dirt itself. Microorganisms, affixed to soil or fibers of fabric, are dislodged by mechanical and chemical action, suspended in the suds, and then removed along with the dirt during one of the rinse cycles (Terpstra 1998). Incomplete soil removal can allow microorganisms to attach to soil remaining on the fabric, making them less sensitive to the influence of thermal and chemical disinfection and thereby leading to insufficient pathogen removal as well. Microorganisms do not reproduce well on clean fabrics (Terpstra 1998).

Laundering practices have changed dramatically over the past several decades. There are several reasons behind these alterations. Environmental concerns and cost

impact how both home and industrial laundering processes are evolving and have resulted in procedural changes that include a general trend toward lower wash temperatures, the utilization of less water, and the reduction in bleach use (Scott 1999).

Eighty-five to 90 percent of the energy used by a washing machine goes to heating water (Bluejay 2007). Besides rising energy costs, consumers have switched to cold water wash cycles because it extends the life and minimizes shrinkage of fabrics. Only five percent of households currently use hot water wash cycles (Gerba 2001). Currently, the majority of domestic laundering is done at 40˚C or 60˚C which is followed by tumble drying or ironing and does not use many of the chemicals routinely added in industrial machines (Patel *et al.* 2006). At washing temperatures below 60˚C, soil removal becomes more difficult and the effectiveness of bleach systems decrease, so not only are clothes less clean, they are also less hygienic. Washing clothes at reduced temperatures is encouraged by The Department of Energy (DOE), who has developed lower energy usage goals for new washing machines to support Federal sustainability initiatives (U.S. Department of Energy 2008).

DOE's Energy Star target values also include reductions in water usage per cycle for all newly constructed washing machines. To meet DOE Energy Star rating goals, washing machines must have a maximum ratio of 8 gallons of water used per cubic foot of washer capacity. Lower water use in new washing machines is being achieved through design modifications that reduce the initial water fill level and eliminate one or two rinse cycles per wash load. Subsequently, rinsing efficiency has decreased, resulting in greater amounts of soil and microorganisms left behind in washed laundry (Terpstra

1998). Rinsing programs are again being modified somewhat to reverse this trend, but it is unknown whether these changes will be sufficient to reduce transmission of microorganisms (Terpstra 1998).

Steps to minimize the environmental impacts associated with the laundering process have also influenced detergent composition and availability. Household detergent alkalinity has been lowered by 1 to 2 pH units making them less effective as disinfectants in the wash process, particularly when no bleach products are used (Terpstra 1998). In addition, low phosphate, biodegradable detergents containing less effective builders are mandated to comply with environmental regulations, and concentrated soap products have been introduced to not only lessen the amount of product in effluent, but also reduce the amount of product packaging needed. Similarly, by 1993 at least 35 states had issued guidebooks recommending the use of alternative products to sodium hypochlorite bleach additives (Parnes 1997), citing among other reasons, it's potential to produce an adverse environmental impact. Worry about the effect detergents and bleaching agents have on the environment has been driven in part by the fact that washing machine effluent is increasingly used as "gray" water by homeowners to eliminate water waste.

New types of fabrics and dyes also have affected both the type of laundry products used and wash cycle temperatures. New fabrics have significantly reduced the amount of time consumers spend doing laundry. The introduction of wrinkle-resistant fabrics has made hanging clothing and linens outside where sunlight can aid in denaturing many of the microbes, and ironing which allows steam to penetrate and reduce

the microbial load in the fabric, much less common (Kagan *et al*. 2002). The current generation of fabrics has new fibers, construction, quality of dyes, and special finishes that cannot withstand traditional bleach, and therefore laundering of these items requires use of non-chlorine products (Belkin 1998). The germicidal effectiveness of these nonchlorine type bleach substitutes has not been well documented. The changing composition of fabrics ultimately may impact the retention and release of viable microorganisms (Sattar *et al*. 1999).

Over the years, laundry "soap" has been replaced with laundry "detergent". Soap is made of materials found in nature, while detergents are composed of synthetic cleaners. Detergents remove soil better over a wider range of water hardness levels and do not require hot water to catalyze their action (Galt Technologies Incorporated 2008). Laundry detergents are composed of several primary ingredients, each designed to perform a different role in the cleaning process. There exists a great diversity among laundry detergent formulas, but nearly all detergents contain a mixture of surfactants, builders, whitening agents, colorants, and fragrance.

The type of home washing machine used is also changing. Approximately 30% of new washers purchased in the United States are the front-load variety (Consumer Reports 2001). Currently, this type of machine accounts for 90% of the European market (Wikipedia 2008). In the U.S., front-load washing machines are gaining in popularity because they have several unique benefits over top-load models. They are significantly more energy efficient, using 30-50% less energy or approximately 400-560 kwh/year versus 800 kwh/year for top-load models (Bluejay 2007). Front-loaders also use 40-60%

less water than their top-load counterparts. In addition, they help extend the life of clothing washed in them, are quieter, and have larger capacities than conventional washing machines (Morrissette 2008). Front-load washing machines also have faster spin cycles resulting in shorter time periods needed for drying. Finally, front-load washers use less detergent which saves the consumer money and is less stressful on the environment.

Front-load washing machines use entirely different mechanisms for washing clothes than top-load models. Conventional washing machines wash clothes by using a large agitator to force clothes back and forth through soapy water, and then again through clean water to rinse out the detergent. Top-load machines are also known as vertical-axis washers, because the tub spins vertically. Front-load washers spin horizontally and are often referred to as horizontal-axis washers. There is not an agitator to move the clothes through the water, rather the tub itself moves, causing the clothes to be repeatedly lifted out of the water and dunked back in (Morrissette 2008). While top-load washers fill their tub up with a standard volume of water at the beginning of the wash cycle, front-loaders control water usage through the surface tension of water and capillary wicking action of the fabric weave. Front-load washers always fill to a same low water level, but the pile of dry clothes soaks up the moisture, causing the water level to drop. The washer then refills to maintain the original water level. Because it takes time for water absorption to occur in a motionless pile of laundry, nearly all front-load machines begin the wash process by slowly tumbling the clothing under the stream of water entering and filling the drum. This facilitates a more rapid saturation of the dry laundry with water.

Hospital- versus home-laundering practices

The steps in an industrial laundry are essentially the same as those performed in a home setting, only the magnitude is different. The process begins with collecting and sorting, and then proceeds to washing, drying and whatever subsequent actions may be required for the particular type of item being washed. Ironing and folding, either by hand or machine, are performed for most items. Packaging and distribution are the final steps in a commercial setting.

The two main differences between industrial and home-laundering are 1) the water to fabric ratio in an industrial laundering operation is about 5:1 (w/w), whereas in a home or coin-operated washing machine, the ratio is about 10:1 (U.S. EPA 2007), and 2) most commercial hospital laundries follow CDC guidelines (2003) to provide thermal disinfection in the wash cycle by washing for $\geq$ 25 minutes, holding the temperature for 3 minutes at 71˚C, and by using chemicals suitable for low-temperature washing if $\leq$ 70˚C laundry cycles are used (Smith *et al.* 1987).

Previous laundry research

In 1938, Lloyd Arnold performed the first laundry studies, enumerating bacteria counts in wash water and on textiles. Arnold's work showed that exposure to water temperature above 71.1˚C for 25 minutes was sufficient to kill nearly all bacteria forms except spores. Laundering at lower temperatures allowed bacteria contamination to accumulate inside the commercial washing machines of the era, a condition attributed to the tallow-based soaps used which required hot water for proper emulsification. He also identified seasonal variation in bacteria counts on clothes coming into the laundry that ranged from

325,000 bacteria counts/cm^3 of wash water during the coldest months to well over 18 million/cm^3 in the warm weather months of the year. His results formed the basis of hospital laundry policies (Blaser *et al*. 1984).

Since that initial study, a variety of other wash conditions have been evaluated to determine microbial survival during and after the laundering process. Besides varying temperature as Arnold (1938) did, the effectiveness of chlorine bleach use, detergent type, duration of wash cycle, and drying have all been assessed with sometimes disparate results.

By far the most frequently studied parameter is that of wash water temperature. Wiksell *et al*. (1973) tested *S. aureus* survival on polyester cotton blend fabric. He found that when wash temperatures were increased from 35˚C to 46˚C and from 46˚C to 57˚C, a significant reduction in the number of viable *S. aureus* cells recovered occurred. However, survival was substantial even at 68˚C, with 3.5 organisms per square centimeter of fabric. His data also demonstrated that Gram positive bacteria could be transferred to uncontaminated fabrics at all tested wash water temperatures which ranged from 24˚C to 68˚C, and that they were much more resistant to the laundering process than Gram negative bacteria and viruses. His findings were similar to those published earlier by McNeil (1964) and Sidwell *et al*. (1971). Christian *et al*. (1983) testing bacteria concentrations in hospital laundered fabrics, concluded that low temperature washing (47.8˚C to 60˚C) eliminated bacteria groups, including *S. aureus* at least as effectively as did wash temperatures above 70˚C. Although, they qualified their findings by admitting that the effectiveness may have resulted in part from increased concentrations of bleach

used at lower temperatures. Battles and Vesley (1981) reviewed nearly a dozen laundry studies published after 1937. They concluded that most vegetative organisms are killed by laundering at 60˚C, 66˚C is effective for more resistant species such as *Streptococcus*, and chlorine bleach greatly enhances the effectiveness of heat.

Numerous investigators have established the importance of longer wash cycles or the need for chemical disinfection when water temperatures were reduced below 60˚C: Walter and Schillinger (1975) in hospital and hotel linen, Lemaire *et al*. (1996) in an industrial laundering process, and Terpstra (1991) and Ainsworth *et al*. (1993) in household laundry. Wiksell *et al*. (1973) found that the longer "regular" wash cycle was significantly more effective in removing microorganisms than a shorter, gentler "permanent press" cycle. Ainsworth and Fletcher (1993) showed that a liquid detergent and a laundry powder containing activated bleach (nonanoyloxybenzene sulfonate added) each vastly improved the antimicrobial action of a wash cycle, both at 30˚C and 50˚C. Bacteria transferred from the interior of a washer to wash water of a subsequent cycle was also reduced when bleach was used (Wiksell *et al*. 1973).

Blaser *et al.*'s 1984 research was one of the few studies to evaluate the importance of each individual stage of disinfection during the laundry process. He found that a standard low temperature (22.2˚C) wash cycle without laundry additives removed 3 $\log_{10}$ of bacteria in hospital sheets and towels by agitation, dilution and drainage alone. Addition of soap and bleach further reduced post-wash colony counts by one to two logs, and drying removed yet another one to two logs. They concluded that bacteria counts were comparable whether low temperature or high temperature washing was employed,

and that the addition of a bleaching agent was the most important bacteria reduction step when washing at low temperatures. Later studies by Smith *et al*. (1987) mirrored these results. Contrarily, work by Jaska and Fredell (1980) determined that water temperature was more significant in determining bacteria survival than other wash system parameters such as detergent amount or type, soil load, water hardness or wash time.

While some studies have shown that wash-water temperatures and the addition of laundry disinfectants affect the initial success of viable microbial elimination (Blaser *et al*. 1984, Wiksell *et al*. 1973), there is virtually no difference seen in resulting microbial levels once fabrics are tumble dried or ironed (Patel 2006). Gerba (2001) found that washing and drying together reduced bacteria levels in inoculated laundry loads by at least 99.99 percent.

Wiksell *et al*. (1973) reported that in most studies, the type of laundry detergent used made little difference on microbial reduction. Several subsequent reports have refuted these findings. Jaska *et al*. (1980) found that the detergent action of a variety of nonphosphate detergent products and soaps were consistently less efficient than that of typical phosphate formulations even though the pH was similar in all products. Kagan *et al*. (2002) quantified the effectiveness of both liquid and powdered detergents containing either oxygen bleach or non-sanitizing bleach alternatives and concluded that products containing sanitizing components demonstrated superior performance. New powder and liquid sanitizing laundry detergents were shown to reduce *S. aureus* and *Klebsiella pneumoniae* (a Gram negative bacteria) in laundry fabric more effectively than other laundry detergents (Bloomfield 2002). Gerba *et al*. (1999) found that *S. aureus* survival

was greatly reduced when laundered with a Tide (Proctor and Gamble, Cincinnati, OH) detergent formula that contained enzymes. The Gram positive bacteria were resistant when washed with Clout (Pharmacal, Naugatuck, CT), a detergent without enzymes.

Some studies have shown that Gram positive bacteria appear to survive the laundry process better than Gram negative bacteria. This increase in survival is thought to be because of differences in susceptibility of the two types of bacteria to drugs and other chemicals (Belkin 2001). The outer membrane of Gram positive bacteria may protect them from antibiotics, dyes, detergents, influencing survival. Bauer *et al*. (1994) discovered that high pH levels caused by bleaching altered hydrogen ion concentrations around bacteria cells which may have inactivated surface elements of Gram negative *E. coli* more readily than Gram positive *S. aureus*. Neely *et al*. (2000) surmised that the antibiotic sensitivity of Gram positive bacteria had no consistent effect on survival, Takashima (2004) showed similar results.

Numerous studies have shown that regular washing at or below 40˚C without the use of a bleach product does not completely destroy the microflora in fabric, and the result may be a buildup of biofilms where cells have become embedded within the clothing matrix. The resistance of resident and transient biofilms to detergents and bleach will most likely be much different than where fabric surfaces have been prepared using laboratory grown inocula (Bloomfield *et al*. 2002), so this must be taken into account when evaluating bacteria survival rates. Washing machines may also provide sites for adhesion and development of biofilms over time if microorganisms are not completely eradicated, then allowed to re-grow (Sheane 2000).

Studies over the past four decades have demonstrated that cloth may become contaminated with high levels of microorganisms which can survive for long periods of time in the fabric (Larson 2001). Distribution of bacteria on soiled fabrics is not uniform and four to six orders of magnitude variations in density have been recorded (Christian *et al.* 1983). Wilkoff *et al*. (1969) found that cloth type and construction, mode of bacteria exposure, exposure to light, and relative humidity can affect the survivability of *S. aureus* on fabric prior to washing. These investigators theorized that the physical characteristics of the cloth fibers themselves such as the scales of wool fibers, twisted cylindrical cotton fibers, and tightness of the textile weave, as well as the charge on the surfaces of both the fiber and the bacteria may influence attachment capability of *S. aureus*. *S. aureus* survived for one week on cotton and two weeks on terry cloth. This may have occurred because the bacteria became imbedded within deep woven terry cloth used in towels. While most initial studies examined survival of *S. aureus* using cotton as the test fabric, as cotton-polyester blends became more common, so did testing of them. Neely *et al*. (2000) found that all bacteria survived for at least a day on cotton-polyester blend fabrics, and concluded that *S. aureus* survived longer on polyester than on cotton, indicating that fabric type may also influence survival. These results were similar to those of Wilkoff *et al*. (1969). Takashimi *et al*. (2004) measured binding of bacteria to fibers. They similarly determined that polyester or acrylic fibers bound *S. aureus* at higher levels (>80%) than wool (63.2%), cotton (10%), or nylon (0.9%). Takashima was not able however, to demonstrate this same result when using entire cloth materials instead of cloth fibers. They theorized that fibers had more binding sites available to

microorganisms and that cloth interiors might not be as accessible to bacteria. They noted that the amount of moisture absorbed by the various types of fiber bundles was not uniform and therefore it was hard to get reproducible results. A study performed by Bloomfield and Scott (1997) showed opposing results and concluded that *S. aureus* survived longer on 100% cotton fabrics. Neely *et al*. (2000) theorized that the difference in persistence between the two studies was due to a much lower initial inoculum level (10^2 CFU versus 10^8 CFU) used by Bloomfield and Scott (1997).

Besides looking at bacteria attachment, Wilkoff *et al*. (1969) also investigated the effects of humidity and mode of contamination as they pertain to *S. aureus* survival on fabric. They found that in general, fabrics contaminated by aerosolized *S. aureus* cultures and dust containing bacteria survived longer than those exposed by direct contact. Relative humidity also affected bacteria persistence in their studies. They determined that at a higher relative humidity (78%), bacteria populations on fabrics survived a substantially shorter period of time than at a lower humidity level (35%).

Lidwell and Lowbury (1950) noted that bacteria death rate in dust was approximately five times higher when it was exposed to daylight, low intensity ultra-violet light and fluorescent lights than when in the dark. This phenomenon may also apply to bacteria dusts attached to fabrics, however no studies have been conducted to date verifying this.

There are equally as many factors that can influence the level of transfer of bacteria, or cross-contamination, among fabrics (Montville *et al*. 2003). Type of bacteria, initial inoculum level, type of source and destination fabric, as well as moisture level may

all affect a fabric's ability to transfer bacteria. The degree to which a garment is hydrophobic or hydrophilic has also been shown to impact bacteria transfer. Sattar *et al*. (2001) found that bacteria transfer from cotton blends was consistently higher than that seen from all-cotton materials, implying that bacteria were less able to penetrate deeper into the fabric because of the hydrophobic nature of the polyester component. As in previous studies, he also noted that bacteria transfer levels were always greater when coming from moist donor fabric. However, Rusin *et al*. (2002) reported lower transfer rates from 50:50 cotton/polyester swatches than 100% cotton swatches. The discrepancy in these results may be due to Sattar's significantly shorter contact time and much smaller contact surface area as compared to the Rusin study.

Residual quaternary ammonium compounds on laundry fabrics is another factor which has been hypothesized as affecting both bacteria attachment and transfer ability. Cody *et al*. (1983) concluded that Gram positive organisms are more susceptible than Gram negative ones to the bacteriostatic properties of this class of compounds, which are present in nearly all new as well as previously washed fabrics as an antimicrobial finish. As the use of synthetic fibers and blends continue to increase in manufacture of shirts, hosiery, blouses, and underwear, so does the use of bacteriostatic finishes. These types of fabrics display drastically different moisture-transport characteristics than those of natural fibers, resulting in a greater degree of perspiration wetness for wearers (Aegisasia 2005). The antimicrobial finishes are added to clothing made from synthetic fabrics and blends to combat increased odors and bacteria counts seen. Durability of these finishes varies among articles of clothing, but is purported to last through at least 10 laundering

cycles. While still active, a bacteriostatic finish has the side benefit of reducing transfer of microorganisms from other fabrics to the clothing where it has been applied.

Microbial contamination of uniforms

Speers *et al*. (1969) found that approximately one third of microorganisms recovered from a nurse's uniform originate from the flora of the wearer with uniforms most frequently becoming contaminated below the waist during procedures such as dressing wounds. Sixty-two percent of the microorganisms recovered were attributed to patients.

Loh *et al*. (2000) tested the cuffs, side pockets and backs of white coats of one hundred medical students using contact plates. Every coat was contaminated on all three sites to some degree. As with similar studies (Babb *et al*. 1983, Wong *et al*. 1991), *Staphylococcus* spp. was most frequently seen (all 100 students), and *Acinetobacter* spp. (7 students) and dystheroids (12 students) were also isolated from the white coats. No MRSA was found and only three instances of a Gram negative organism were detected. None of the Gram negative organisms identified were considered normally pathogenic. A study by Wong *et al*. (1991) found that the maximum level of *S. aureus* contamination on doctor's white coats is reached within a week of use and doesn't change significantly until the coat is laundered.

Callaghan (1998) examined the effect of laundering frequency on bacteria levels for nurses' uniforms, with or without use of an additional cover apron. Wide variations in bacteria contamination levels were seen. Unwashed uniforms were found to be equally and heavily contaminated at all sites sampled. End of shift samples produced no statistically higher contamination levels than beginning or middle of a shift samples.

Nurse participants who did not additionally use an apron (59.4%, 116/196) reported that they wore a clean uniform each day. Fewer nurses who wore aprons (7.3%, 14/196) felt it necessary to wear a clean uniform each day and 30.6% (60/196) of the survey participants did not always wear clean uniforms at the start of a shift. More than half of the nurses used the hospital's laundry, so further research was conducted to determine initial bacteria counts on clean uniforms. From the dozen hospital-laundered uniforms tested, no bacteria were recovered.

Perry *et al*. (2001) used a vacuum method to analyze 57 nurse's home-laundered uniforms at the beginning and end of a shift for MRSA, VRE, and *C. difficile*. Thirty-nine percent (22/57) of uniforms tested were positive for one or more of the organisms prior to the start of the shift. VRE was detected on 21% (12/57) of uniforms, while MRSA and *C. difficile* were each found on 12% (7/57). Contamination levels varied from one to greater than 100 colonies. Scrubs at the end of a shift showed that 54% (31/57) of uniforms were positive for at least one of the test organisms. VRE was found on 31% (22/57) of uniforms, *C. difficile* on 19% (11/57) of uniforms and MRSA on 15% (8/57) of uniforms. Perry *et al*. (2001) noted that some uniforms had fewer organisms after being worn for a shift, and that the levels of post-duty contamination varied based on the type of ward.

Conversely, Babb *et al*. (1983) did not detect an increase in *S. aureus* or Gram negative bacilli when gowns and plastic cover aprons were used for periods up to 11 days in a main isolation unit. This study employed contact plates and detected *S. aureus* in 12.6% (26/207) of the fronts/shoulders of cotton gowns and 9.2% (22/239) of the plastic

aprons tested. Gram negative bacilli were only recovered from one gown (1/207). While 47% (42/89) of strains identified could not be associated with either the patients or staff, 35% (31/89) were linked to patients and 18% (16/89) were matched to the nurse's own nasal strain. Two percent (11/707) of garments evaluated produced *S. aureus* counts greater than one per square centimeter, however little difference was seen in the numbers of bacteria recovered between the two different areas tested.

Pilonetto *et al*. (2004) analyzed specific types of microbiota from uniforms using RODAC contact plates at both the beginning and end of a work shift. Samples were analyzed from the cuffs of long-sleeved gowns and the abdominal region from short-sleeved gowns for total viable and Gram negative bacteria counts. Researchers found a significant ($p=0.027$) increase in total bacteria from the beginning to the end of a work shift, with average total viable counts increasing from 2.2 to 4.9 CFU/cm^2. Converse to findings in Loh *et al*. (2000), bacteria levels were higher in the abdomen region than at the cuff. Pilonetto speculated this was because the earlier work by Loh only evaluated contamination in physicians clothing, while his work involved gowns from staff that generally had a much closer patient contact. Pathogens were isolated from 48% (15/31) of the gowns. Of the isolated pathogens, 61% (11/18) were *S. aureus* none of which were MRSA. Gram negative isolates found included *Acinetobacter baumanni* (2/18), *Klebsiella pneumonia* (2/18), *Stenotrophomonas maltophilia* (2/18), and *Serratia rubidate* (1/18). No *E. coli* or *Pseudomonas* spp. were detected, however, it was felt that the fewer number of Gram negative organisms was due in part to their poor ability to attach to fabrics

Fijan *et al*. (2005) also attempted to identify specific organisms on fabrics from a hospital setting. RODAC plates were used to evaluate the number and types of microorganisms on surfaces from a hospital laundry's clean area as well as ironed and folded textiles processed at the laundry. The most common microorganisms found were normal skin bacteria from the *Micrococcus* and *Staphylococcus* genera. Specimens from the genus *Bacillus* and the genus *Corynebacterium* were also frequently detected, even after surface disinfection measures had been implemented by the hospital laundry.

Hospital- versus home-laundered scrubs

Few studies compare the microbial flora of hospital- versus home-laundered attire. Previous research focused mainly on enumeration rather than identification of specific biota.

After recovering no bacteria from 12 randomly selected hospital-laundered uniforms, Callaghan (1998) inoculated uniforms previously laundered by the hospital with *Serratia marcescens* and washed them using a home washing machine. A variety of temperature settings, wash cycles, and laundry load contents were employed. All loads were tumble dried. She concluded that uniforms could be laundered at home provided they were washed with no other items of clothing, at no less than 50˚C, and ironed dry with a hot iron.

Jurkovich (2004) swabbed the left front shoulder of 50 operating room personnel, 60% (30/50) of whom had laundered their scrubs at home. No pathogenic microorganisms were found on either the home- or hospital-laundered scrubs. Also, no significant differences were found when comparing the normal skin flora on the two

different types of scrubs. Most staff who had home-laundered their scrubs (70%, 35/50) used warm water cycles, 73% (37/50) had washed their scrubs separate from other clothing, and all had dried their scrubs completely in the drier.

Outbreaks attributed to contaminated laundry

Only a few isolated studies have explored the possible transfer of organisms from nursing scrubs and uniforms to patients during identification of an outbreak, and those have taken place mainly in specialized wards such as burn or cardiothoracic units.

After two patients developed *Bacillus cereus* meningitis following neurosurgery at a London hospital, extensive environmental testing was performed by Barrie *et al*. (1992) to determine the source of the organism. Operating room linen was found to be the most probable origin of the infections. Hospital administrators made alternate laundering arrangements until the outbreak investigation, including analysis of the laundry facility, was complete. No further cases were reported after these changes were implemented. It was eventually determined that a contaminated continuous batch tunnel washer harbored large numbers of the organism and was not effectively disinfecting the linen (Wilcox *et al*. 1995).

Laundry contaminated with *Streptococcus pyrogenes* was identified as the cause of infection in a maternity ward outbreak involving several babies. The organism was traced to a heavily contaminated dryer used during laundering of the babies' clothes. The outbreak ended once the clothes were autoclaved (Fijan *et al.* 2005)

Microbes on laundry-associated surfaces

Studies show that pathogenic microorganisms survive on environmental surfaces for extended periods of time providing an opportunity for the transmission of infectious diseases. (Kramer *et al.* 2006, Manangan *et al.* 2001, Reynolds *et al.* 2005, Rusin *et al.* 1998, Scott *et al.* 1982, Weber *et al.* 2001). Little is known about bacteria levels in public laundries, as few studies have been performed. Buford *et al.* (1977) found bacteria ranging from geometric mean counts of 5 to 73,960 CFU/cm^2 in the interior tub surfaces of automatic washers in self-service laundry facilities, with a count of 490,000 CFU/cm^2 obtained on one occasion. The researchers hypothesized that even greater numbers would be found if samples had been taken from less accessible areas that receive little abrasion, or wet surfaces. Legnani and Leoni (1997) also tested interior washing machine surfaces and wash water in commercial launderettes. They concluded that bacteria contamination was highest in the most heavily used machines and those where customers, trying to reduce costs, overloaded them or used lower temperature programs to wash various kinds of clothing together (underwear, pants, shirts, shoes, etc.). Higher wash water temperature or using an oxygen-based bleach with a low temperature cycle provided a significant bacteria reduction ($p<0.001$) both in terms of percentages of positive samples and mean concentrations. However, both bleach and hot water were necessary to ensure a nearly complete elimination of bacteria from fabric, wash water and washer interiors, including the less accessible parts of the washing machine, such as the drum. No published data were found for bacteria numbers on home washing machine interiors.

Viable bacteria have also been found on the interior tub surfaces of automatic washers in self-service laundry facilities. Buford *et al*. (1977) found the $\log_{10}$ of the geometric mean counts ranged from 1.260 to 2.489 in 160 swab samples taken at each of four locations. Microorganisms can be disseminated within loads, between loads, and between families who use communal laundries (Wiksell 1973). As would be expected, bacteria contamination of communal washing machines was higher in the most heavily used units and levels found were relatable to bleach use as well as wash cycle temperatures. Washing at 55˚C or adding an oxygen-based bleach to a lower temperature cycle did provide a significant reduction in bacteria recovery, but did not prevent all bacteria from surviving inside the washer. Adding bleach to a hot water cycle ensured almost complete elimination of bacteria from protected parts of the washing machine drum (Legnani *et al*. 1998). Along similar lines, Larson and Duarte (2001) also identified lack of bleach use in a communal laundry as a significant predictor of increased disease transmission among family members. This was the first study in a home setting to demonstrate a potential link between laundry practices and disease transmission in the household.

Risk assessment for handling laundry

A quantitative risk assessment is the process of estimating and describing the probability that an event will occur. Originally developed for chemical hazards, it is now being applied to disease-causing microorganisms. In 1990, the Environmental Protection Agency first used a quantitative risk assessment to establish regulations for drinking water treatment. They set a goal to reduce the risk of waterborne diseases like Giardia

and Cryptosporidium to 1 per 10,000 people per year (Gerba 2001). There are several reasons to use a risk assessment instead of a typical epidemiological study when attempting to quantify hazards. Quantifying the probability that an infection will occur from handling contaminated laundry cannot be done using typical epidemiological studies because of the low rate expected (Gerba 2001, Gibson *et al*. 1999). Risks less than 1 per 10,000 require a very large study population and epidemiological studies are not able to evaluate risks over time. Also, risks associated with unwashed laundry are difficult to document by using standard disease surveillance and epidemiologic tools since most cases of disease in the home would not be immediately reported and the route of exposure would not be clear. Alternatively, a four step risk assessment involving hazard identification, exposure assessment, dose-response, and risk characterization can be employed to increase sensitivity of the endpoint analysis.

The two instances where laundry may be most likely to act to disseminate infection are during handling before laundering, and after laundering in the event the laundry process fails to fully remove microorganisms, the laundry remains damp for a period of time before being handled, and residual bacteria are allowed the opportunity to re-grow. Gibson *et al.* (1999) developed a quantitative risk assessment for the fecal-oral transfer of *Shigella* from handling contaminated laundry. No risk estimate has been done for handling laundry contaminated with *S. aureus*.

Information needed to estimate the likelihood of *S. aureus* infection transmission includes: 1) concentration of pathogens in soiled laundry; 2) the effectiveness of laundering practices used, taking into account wash water temperature, additives, type of

washing machine, and drying technique; 3) potential for cross-contamination of other laundry items in the same load or to the inside of the washing machine for transference to subsequent loads (communal laundries have been found to be particularly problematic, Larson *et al*. 2001); 4) potential for contamination of nose or abraded skin surface; and 5) immune status of laundry handler. The ultimate purpose of the risk assessment analysis is to determine the number of cases of *S. aureus* disease that can be prevented by implementing certain control strategies. By evaluating microbial counts in laundry and on laundry-associated surfaces, a more accurate risk assessment can be developed for persons handling laundry.

Dissertation Format

The research reported in the appendices of this dissertation consists of four related experiments designed and undertaken by the candidate: 1) comparison of *Staphylococcus aureus* and MRSA survival in laundry between top-loading and front-loading washing machines; 2) assessment of bacteria on new, disposable, laundered and unlaundered scrubs; 3) bacteria contamination at community-use laundry facilities; 4) quantitative risk assessment for home-laundering fabrics contaminated with *Staphylococcus aureus*.

Each experiment has been primarily designed, implemented and interpreted by the candidate. Drs. Reynolds and Gerba are co-authors on the papers and have served to advise, but not design, the candidate's research.

Each candidate for the advanced degree in the Department of Soil, Water and Environmental Science is expected to submit their original research to peer reviewed scientific journals for publication. By using this alternative format, these papers will essentially be ready for publication. Preparation and submission of the manuscripts were undertaken by the candidate. Drs. Reynolds and Gerba served as co-editors of the manuscripts.

PRESENT STUDY

The methods, results and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the most important findings.

Colonization rates of *S. aureus* decreased in recent years, however, MRSA rates nearly doubled during the same time period causing more than 94,000 infections and 19,000 deaths in 2005 alone. Infections transmitted via a fabric route are rare but do occur. The following studies examine *S. aureus* and MRSA prevalence on laundry and laundry-related surfaces. Information obtained was then used to estimate the risk of infection transmission to laundry handlers.

The first article discusses the decontamination efficiency of top-load and front-load washing machines using loads consisting of swatches inoculated with either *S. aureus* or MRSA, sterile ballast and an artificially soiled pillowcase designed to simulate conditions found in a load of naturally soiled clothing. Parameters of temperature, laundry additives, and subsequent drying of laundry loads were varied. Front-load washing machines reduced *S. aureus* and MRSA more effectively in loads only when no laundry additives (detergent or detergent and liquid bleach) were used. This most likely was a result of the front-load washer's longer rinse cycles. Bacteria were transferred to other clothing within loads, particularly before drying or if bleach was not used, as well as to washing machine interiors, with top-load washers becoming cross-contaminated much more frequently.

The second article quantifies the numbers and types of microorganisms found on unwashed operating room, hospital-laundered, home-laundered, new cloth and new

disposable scrubs. Scrubs were tested for the presence of HPC bacteria, *Clostridium difficile*, fungi, *S. aureus*, total coliforms and *Escherichia coli*. Bacteria isolates were identified to species. Opportunistic bacteria pathogens and those capable of causing nosocomial infections were identified on every type of tested scrub except for hospital-laundered scrubs. Home-laundered scrubs had significantly greater HPC bacteria numbers than did hospital-laundered scrubs.

The third article discusses bacteria contamination at community-use laundry facilities. Public and apartment laundromat surfaces were sampled for the presence of HPC and total coliform bacteria, *E. coli*, *S. aureus* and MRSA. HPC bacteria was detected on all sampled sites with levels exceeding 100,000 CFU/cm^2 in some cases. Washing machine interiors generally had the highest HPC numbers. Thirty-five percent of sites tested positive for coliform bacteria, but no *E. coli* was found. One interior washer lid tested positive for MRSA.

The fourth article applies information obtained in the other studies to calculate a quantitative risk assessment for determining the risk of acquiring an infection when handling washed and unwashed laundry initially contaminated with *S. aureus* levels of 10^6 CFU/cm^2 fabric. The worst case risk was estimated to be 0.59 infections per person per year if handling unwashed laundry, but negligible if handling contaminated laundry after it has been washed with detergent that reduces *S. aureus* and MRSA levels by 5 – 6 $\log_{10}$.

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APPENDIX A:

COMPARISON OF *STAPHYLOCOCCUS AUREUS* AND MRSA SURVIVAL IN LAUNDRY BETWEEN TOP-LOAD AND FRONT-LOAD WASHING MACHINES

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ABSTRACT

Approximately 30% of healthy people in the United States are colonized with *Staphylococcus aureus*, usually on the skin or in the nose. If *S. aureus* enters the body it may produce no symptoms, cause self-limiting infections such as boils or pimples, or occasionally produce serious infections such as pneumonia or septicemia. Changes in laundering practices (i.e. lower wash temperatures, energy and water efficient appliances, and reduction in bleach use) may affect the survival rate of pathogens on fabrics. This study was conducted to determine whether *S. aureus* and its methicillin resistant form (MRSA) added to cotton cloth swatches survive typical household laundry practices. Decontamination efficiency of top- and front- load washing machines were compared using an artificially soiled pillowcase designed to simulate the conditions (pH, organic load, etc.) encountered in naturally soiled laundry. Use of plain water, detergent, detergent and liquid bleach (5.25% sodium hypochlorite) under various wash temperatures were evaluated. Initial *S. aureus* and MRSA inoculum ranged from 6 to 7 $\log_{10}$ colony forming units per swatch. Front-load washers were significantly ($p<0.05$) more effective than top-load washers in reducing bacteria numbers on inoculated swatches within laundry loads when no additives (plain water) were used, probably due to longer rinse cycles. This trend was shown regardless of the type of test organism (*S. aureus* or MRSA), temperature used (17˚C or 27˚C) or whether the laundry load was subsequently dried. However, once detergent or detergent and liquid bleach were added to laundry loads, no significant differences were seen between bacteria reductions on swatches washed using the two different washing machine types.

No statistical differences ($p<0.05$) were found when comparing the number of bacteria remaining on inoculated swatches within laundry loads at 17˚C versus 27˚C, regardless of the type of washer, the laundry treatment, or whether the loads were dried.

S. aureus and MRSA reductions in plain water washing ranged from 2.37 – 3.37 $\log_{10}$ for top-load washers and from 2.70 – 3.92 $\log_{10}$ for front-load washers before drying. If detergent was used, average reductions before drying ranged from 5.72 – 6.46 $\log_{10}$ and from 6.04 – 6.78 $\log_{10}$ when detergent and liquid bleach were used. After loads were dried, average reductions in loads using plain water ranged from 3.69 – 6.17 $\log_{10}$, detergent increased average reductions to 6.21 – 6.95 $\log_{10}$, and when detergent and liquid bleach were added average reductions exceeded 6.12 $\log_{10}$.

Bacteria were transferred to other clothing within loads, particularly before drying or if bleach was not used. Cross-contamination of washing machine interiors occurred more frequently (36% versus 4%) between loads when top-load machines were used than when front-load machines were used. Laundering practices in the United States may not completely eliminate *S. aureus* or MRSA from clothes if contamination levels exceed 10^6 CFU/cm^2, however the use of detergent or detergent with liquid bleach is adequate to minimize exposure potential to *S. aureus* and MRSA while handling washed laundry.

INTRODUCTION

Numerous studies implicate the home environment as a primary source for exposure to disease causing microbes (Sattar *et al*. 1999, Larson *et al*. 2001, Bloomfield 2006, Terpestra 1998). Shopsin *et al*. (1999) evaluated the potential for a colonized family member to transmit *S. aureus* within the family. When both child and parent/guardian tested positive for MRSA, they generally had the same strain, indicating that transmission between household members probably occurred. Beggs (2003) verified the contact transmission transfer of MRSA from healthcare provider to family members.

During a six-year study by Kniehl *et al*. (2005), the infection status of 87 healthcare workers identified as being MRSA-positive was tracked. Although 73 (84%) workers were able to eradicate their MRSA status by treatment with topical antibiotics and removal from the workplace, this methodology failed in 14 cases with recolonization suspected in 11 cases. Of the 11 recolonized cases, MRSA was detected among other residents in eight cases and at seven of the home environments. Even with appropriate medical treatment of the other residents, complete disinfection of household surfaces took as long as two years in heavily contaminated home environments. The researchers concluded that failure to eradicate MRSA among compliant healthcare workers resulted from recolonization within the home rather than from ineffective medical treatments.

Laundry has been overlooked as a potential route of pathogenic agent transmission in the home. Exposure to pathogens on contaminated clothing, bed linen and other types of fabrics may occur either by direct contact or indirectly through airborne particles (Sattar *et al*. 2001). MRSA was isolated from 40% of household

surfaces, including laundered items (Reynolds *et al*. 2006). To date there have only been a few documented reports of nosocomial infections transmitted via a fabric route (Standaert *et al*. 1994), however, increased frequency of homecare for the elderly, immunocompromised, and/or known and unknown carriers of MRSA raise new questions regarding human health risks of contaminated laundry. Numerous variables such as new fabric types, increasing numbers of healthcare workers washing scrubs at home and changing laundering practices (i.e. lower wash temperatures, energy and water efficient appliances, and reduction in bleach use) affect the survival rate of pathogens (Scott 1999) calling for additional research.

Previous laundry studies attempted to quantify bacteria reductions under varying conditions such as temperature, sodium hypochlorite use, detergent type and wash cycle duration. The specific factors most effective at eliminating pathogens during the washing process remain unidentified, but most likely all exert some influence. These studies were performed using top-load washing machines (Walter *et al*. 1975, Jaska 1980, Christian 1983, Tompkins *et al*. 1988, Belkin 1998). Currently, thirty percent of new washers purchased in the United States are front-load models (Consumer Reports 2001). This type of washing machine uses significantly less detergent and less water (Bluejay 2007), but has longer rinse cycles.

Previous research indicates one item of heavily contaminated clothing can cross-contaminate an entire load, transfer bacteria to the inside of washers, and contaminate a subsequent laundry load (Wiksell 1973). The interior surfaces of a front-load washing machine must be dried or disinfected routinely, to prevent water from remaining inside

the drum and around the front window seal, facilitating the growth of bacteria and fungi which results in adverse odors often reported by consumers (appliancejournal.com 2007).

The objectives of this research were to: 1) compare the reduction of *S. aureus* and MRSA after washing at various water temperatures, with and without drying, under varying laundering conditions of no additives (plain water), detergent, detergent and bleach, 2) evaluate whether front-load washing machines, designed to be more environmentally-friendly than top-load washing machines, impact the ability of washing to effectively reduce bacteria loads, and 3) measure the potential for bacteria cross-contamination of other fabrics within a laundry load and of the inside of the washer between laundry loads for the two different types of washers.

MATERIALS AND METHODS

Maintenance and preparation of bacteria isolates

Staphylococcus aureus ATCC 25923 and *S. aureus* ATCC 700698, a methicillin-resistant strain isolated from the sputum of a patient with pneumonia after surgery who had failed vancomycin therapy, were used as seed organisms. The bacteria were maintained on Tryptic Soy Agar (TSA; Difco, Sparks, MD). Erlenmeyer flasks containing 300 ml of Tryptic Soy Broth (TSB; Difco, Sparks, MD) were inoculated and incubated in a shaking water bath (250 rpm) at 37˚C overnight prior to testing.

After incubation, the bacteria suspension was poured into six 50 ml conical tubes and centrifuged at 2,900 x g for 9 minutes in a Beckman J2-21 centrifuge (Beckman Coulter, Inc., Fullerton, CA) to remove gross cellular debris. The supernate was poured off each of the pellets and discarded. Pellets were resuspended in 15 ml of sterile saline (0.85% NaCl; Difco, Sparks, MD) using a vortex then combined with one of the other pellets to make a total of three concentrated suspensions. Sterile saline was added to these suspensions to make 50 ml and they were again centrifuged at 2,900 x g for 9 minutes. Finally, the supernate was again poured off the new pellets, 10 ml of sterile saline was added to each, and they were vortexed once more to resuspend the bacteria. Bacteria concentration of the titer solution was determined using a standard dilution/plating method.

Soiled pillowcases

One pillowcase pretreated with a known quantity of organic dirt mixture was added to each laundry load in the study. The organic dirt mixture (soil) was applied to new,

washed pillowcases in two phases as detailed in a protocol used in previous laundry studies (Strazdas 2007). First, 50 ml of a salt solution was applied to each pillowcase using a pipette to distribute the liquid over the entire surface. The salt solution was made by adding 336.6 g urea (Fisher, Pittsburgh, PA), 336.6 g sodium chloride (Sigma, St. Louis, MO), 118.8 g calcium chloride (Sigma), and 72.6 g magnesium chloride (Sigma) to 3,760.4 ml of distilled water. The mixture was blended thoroughly using a stir plate before being applied to the pillowcases, and the pillowcases were dried together for 30 minutes in a standard dryer. Next, 100 ml of a second solution was applied to each salted pillowcase, again using a pipette. The second solution was made by adding 260.8 g sebum base (Textile Innovators, Rock Hill, SC), 20.2 g triethanolamine (Sigma), 157.5 g black charm clay (Textile Innovators), and 31.5 g gelatin (Sigma) into 1850 ml of 60˚C distilled water. This mixture was stirred continuously using a hot/stir plate to keep the particles in solution. The pillowcases were dried together once more.

With a naturally soiled load of laundry, soil is released gradually throughout the wash cycle. A synthetically soiled pillowcase added to sterile laundry ballast is necessary to simulate conditions of pH, oxidant degradation, undissolved solids, turbidity, whitening, and stain and soil removal found in a typically soiled wash load (Watson, 1999).

Preparation of cloth swatches

Swatches made from 100 percent light blue cotton sheets (purchased from Target, Roseville, MN) were laundered and cut into 232 cm^2 inch pieces. The swatches were wrapped in paper and autoclaved before use. A set of six swatches were each inoculated with 5 ml of bacteria suspension containing approximately $10^6 – 10^7$ colony forming

units per ml and allowed to air dry completely. Cloth swatches to remain sterile for use when evaluating bacteria transfer were marked in one corner with a small "X" prior to autoclaving.

Ballast material

Ballast material consisted of men's white cotton t-shirts. Ballast clothing was autoclaved prior to each experiment. Two large pieces of autoclave tape were placed in the center of each ballast bundle prior to autoclaving to ensure complete steam penetration of the fabric.

Summary of laundry load contents

Each laundry load contained one soiled pillowcase, four inoculated swatches, and 3.2 kg of sterile ballast. Two sterile swatches were included in at least three experiments for each laundry treatment to evaluate bacteria transfer potential among the fabrics and to the washing machine interior. One swatch was analyzed for bacteria concentrations after washing and one after drying.

Washing treatments

A diagram summarizing experimental procedures is presented in Figure 1.1. Loads were washed in either a top-load type (Frigidaire, model number FLEB8200DS0, Martinez, GA) and a front-load type (Whirlpool duet, model number WFW9200S, Benton Harbor, MI) washing machine. All experiments with top-load machines were performed using the highest water level setting (69 liters). A 12-minute wash cycle was followed by a 3-minute rinse. During some loads, the water temperature was adjusted from the ambient cold water value of 25˚C-27˚C to 17˚C-23˚C by adding ice to the water in the machine.

This is the average temperature of a cold water wash in the United States (E. Shaheen, personal communication, Clorox Company). Test conditions included: 1) water only, no detergent nor bleach, 2) 3/4 cup of powdered Tide powdered detergent, original scent, without bleach (Procter and Gamble, Cincinnati, OH), and 3) 3/4 cup of Tide detergent and 2/3 cup of Clorox bleach (5.25% sodium hypochlorite, Oakland, CA). The amount of both detergent and bleach added were determined using recommendations listed on the product packages. The detergent was added first to the washing machine followed by the ballast clothing and pillowcase. Next, the inoculated and sterile swatches were placed in locations within the load so that none of them were touching each other, and finally the water was added. After the wash and rinse cycles were complete, the clothes were immediately transferred to a tumble dryer (Electrolux Home Products, Martinez, GA) and completely dried (50 minutes). The temperature of the ballast clothing after drying was approximately 54˚C. All treatments were repeated at least 3 times.

Treatments using a front-load washer were performed using a normal setting for wash cycle type, size load, and spin speed. Because wash cycle times vary based on water pressure and temperature, detergent, and clothes load, the wash cycle duration was timed at 13 $\pm$ 1 minutes, while the rinse cycles ran 20 $\pm$ 1minutes. The entire load took approximately 48 minutes, 16 minutes longer than for the top-load washer. Ice was added during some loads to lower the temperature from ambient 25˚C - 29˚C to approximately 17˚C - 20˚C before adding the ballast, pillowcase and swatches. Test conditions for this type of washer included: 1) water only, no detergent or bleach, 2) 1/2 cup of powdered Tide HE (high efficiency) detergent without bleach (Procter and

Gamble, Cincinnati, OH), and 3) 1/2 cup of Tide HE detergent and 2/3 cup of Clorox bleach (5.25% sodium hypochlorite, Oakland, CA). Amounts of both detergent and bleach added were again based on recommendations listed on the product packages. While the amount of bleach recommended was identical for both machines, the amount of detergent was ¼ cup less for the front-load model. In this machine, detergent and/or bleach were both added once water started filling the drum, via a dispensing drawer. After the wash and rinse cycles were complete, the clothes were immediately transferred to a tumble dryer (Kenmore 70 Series, Heavy Duty Super Capacity, Hoffman Estates, Illinois) and dried. Because the front-load washer had a significantly longer and faster spin cycle, complete drying took only 28 minutes. The temperature of the ballast clothing after drying was 55˚C. All treatments were repeated at least 3 times.

After each wash cycle, a bleach-only load was run to disinfect the washer between treatments, then a sodium thiosulfate-only load was run to neutralize any residual chlorine left in the washer after the disinfection process. Finally, surface swabs were collected on washing machine interiors to ensure no residual bacteria remained.

Similarly, empty dryers were run on the highest setting for 30-minutes after each use. Again, interior surface swabs were collected to ensure no residual bacteria remained.

Recovery of bacteria from the swatches

Bacteria was recovered from the swatches by placing each one into a plastic stomacher bag (Model 3500, Seward, London, UK) with 5 ml of phosphate buffered saline and 0.1 ml of 0.1% sodium thiosulfate. Each bag was placed in a Seward (Seward, London, UK)

stomacher and pummeled for 120 seconds at high speed. As much liquid as could be removed was pipetted out of the bags and bacteria concentration was determined using a standard dilution/plating method. Mannitol salt agar (MSA; Difco, Sparks, MD), selective for *S. aureus*, was used for plating the bacteria. The plates were incubated at 37˚C for 48 hours before being counted.

For each experiment, bacteria were recovered from two swatches before either washing or drying to quantify baseline inoculum levels, after washing only, and after washing with drying to determine bacteria reduction at each laundry phase. Bacteria levels from the sterile swatches were also determined as controls.

Recovery of bacteria from inside the washing machine

Swab samples were collected immediately after at least three loads per laundry treatment to determine if bacteria were transferred from fabric to interior washing machine surfaces. Following seeded trials but prior to disinfection between laundry loads, several locations inside of the washing machine, including the lid, on the drum and under the tub rim (top-load) as well as on the drum and around the door seal (front-load), were swabbed using a spongesicle (Biotrace International, Bridgend, UK). Liquid was eluted from the spongesicles via hand manipulation for 60 seconds, then samples were serially diluted and assayed on MSA using the spread plate technique. Plates were incubated at 37°C for 2 days to determine presence of *S. aureus*.

Calculations

All data represents the arithmetic mean of at least three experiments for each condition. In every experiment, two swatches were analyzed after each laundry step (washing or washing with drying). Student t-tests were employed to determine if there was a significant difference ($p < 0.05$) between bacteria reductions in 1) washing at two different water temperatures, with and without drying, under varying laundering conditions of no additives (plain water), detergent, detergent and bleach, and 2) front-load washing machines and top-load washing machines, with and without drying, under varying laundering conditions of no additives (plain water), detergent, detergent and bleach.

T-tests were calculated by comparing bacteria numbers remaining per swatch for the different treatments. Because initial bacteria concentrations varied between tests, each data point was normalized before the t-test calculations using the following formula:

$$\frac{N}{N_o} = \frac{\text{bacteria number on swatch after treatment}}{\text{bacteria number on swatch before treatment}}$$

Data points for each condition were then averaged and the final value log transformed.

Student t-test comparisons, as well as, average and standard deviation values were performed using Microsoft Office Excel software (2003). Due to small sample sizes, differences shown are judged merely as trends. Using a statistical power calculation program (DSS research 2006) to determine necessary sample size, as many as 22 repetitions of each experiment would be needed to achieve a 95% confidence interval with an Alpha error level of 5% and a Beta error level of 50%.

RESULTS

Front-load washers reduced both *S. aureus* and MRSA on inoculated swatches within laundry loads (each consisting of 3.2 kg sterile t-shirt ballast and a standardized dirt load) significantly better ($p<0.05$) versus top-load washers at 17˚C and 27˚C when no additives (plain water) were used. This also held true after the swatches washed in loads without additives subsequently went through a drying cycle. However, once detergent or detergent and liquid bleach were added to laundry loads, no significant differences were seen between bacteria reductions on swatches washed using the two different washing machine types, regardless of whether the loads had been dried (Table 1.1).

No statistical differences ($p<0.05$) were found when comparing the number of bacteria remaining on inoculated swatches within laundry loads at 17˚C versus 27˚C, regardless of the type of washer, the laundry treatment, or whether the loads were dried.

No consistent trends were seen when comparing *S. aureus* versus MRSA reductions on inoculated swatches within laundry loads between like treatments. If no laundry additives (plain water) were used, S. *aureus* reductions on inoculated swatches were statistically greater than MRSA reductions in two instances (front-load after washing at 27˚C and top-load after washing with drying at 17˚C). MRSA reductions on inoculated swatches within laundry loads were statistically greater than *S. aureus* in four instances (front-load after washing with drying at 27˚C, top load after washing and washing with drying at 17˚C, and front-load after washing at 17˚C). Once detergent or detergent and liquid bleach were added to laundry loads, no significant differences were

shown between bacteria reductions on swatches within laundry loads for the two different organisms after washing or washing with drying.

Table 1.2 shows the log reductions of *S. aureus* and MRSA numbers on inoculated swatches within laundry loads using a 17˚C or a 27˚C wash cycle, under different laundry treatments: with no additives (plain water), with detergent, and with detergent and liquid bleach. After washing with no additives (plain water) average reductions within the eight different laundry categories (top-load *S. aureus* 27˚C, front-load *S. aureus* 27˚C, top-load MRSA 27˚C, front-load MRSA 27˚C, top-load *S. aureus* 17˚C, front-load *S. aureus* 17˚C, top-load MRSA 17˚C, front-load MRSA 17˚C) varied from 2.37 – 3.92 $\log_{10}$. Adding detergent to the loads increased the average reduction from 5.72 – 6.46 $\log_{10}$ and when detergent and liquid bleach were used, average reductions exceeded 6.04 $\log_{10}$ within these same categories. Similarly, after washing with no additives (plain water) followed by drying, average reductions ranged from 3.69 – 6.17 $\log_{10}$, adding detergent to the loads then drying increased the average reduction to 6.21 – 6.95 $\log_{10}$, and once detergent and liquid bleach were added to loads then dried, average reductions exceeded 6.12 $\log_{10}$ within all eight categories.

As noted in earlier studies (McNeil 1964, Wiksell *et al*. 1973), cross-contamination between items within a laundry load was common, most frequently occurring before drying or if bleach was not used along with detergent in a load. Table 1.3 lists the percentage of sterile swatches contaminated during the various types of laundry treatments performed. Percentages ranged from 0 to 100 percent. Numerous instances were noted where after treatment, bacteria numbers on the laundered sterile

swatches exceeded those that remained on the laundered inoculated swatches by one to three logs indicating that *S. aureus* was easily disseminated during the wash cycle.

During several trials, the inside of both types of washing machines were tested to determine whether cross-contamination might also occur between laundry loads (Table 1.4). In the top-load washer, contamination was found 37% (10/27) of the time on the inside of the machine lid and 36% (10/28) of the time on either the tub rim or side of the drum. In only one instance (out of 24 swab sample tests) microbial contamination was detected on the window seal/door of the front-load washer. Contamination was never found on the inside drum of the front-load washer (0/24).

DISCUSSION

Historically, *S. aureus* has been the microorganism of choice to study reduction of bacteria by laundering because it is known for its ability to survive washing and drying better than other bacteria commonly found in laundry (Neely *et al*. 2000). All previously published studies were performed using top-load washers. This study is novel due to the inclusion of front-load washing machines that operate using less water and detergent but have longer rinse cycle times.

The effectiveness of bacteria reduction was compared for a number of different laundry treatments. The major trend identified was that front-load washers were significantly more effective than top-load washers in reducing bacteria numbers on inoculated swatches within laundry loads when no additives (plain water) were used. This trend was shown regardless of the type of test organism (*S. aureus* or MRSA), temperature used (17˚C or 27˚C) or whether the laundry load was subsequently dried. Bacteria reduction achieved without detergent or detergent and liquid bleach is primarily due to agitation, dilution and drainage (Blaser *et al.* 1984), implying that front-load washing machines are better designed to accomplish these functions.

Front-loading washing machines use entirely different mechanisms for washing clothes than top-load models. Top-load washing machines use a large agitator to force clothes back and forth through soapy water, and then again through clean water to rinse out the detergent. Front-load washers spin horizontally, and have no agitator to move clothes through the water. Rather the tub itself moves, causing clothes to be repeatedly lifted out of water and dunked back in (Morrissette 2008). While top-load washers fill

their tub up with a standard volume of water at the beginning of the wash cycle, front-loaders control water usage through the surface tension of water and capillary wicking action of the fabric weave. Front-load washers always fill to a same low water level, but the pile of dry clothes soak up the moisture, causing the water level to drop. The washer then refills to maintain the original water level.

The difference in cycle time for the two types of washers used in these experiments further explains the results shown. The top-load washer used had a 3-minute rinse cycle with a load taking 32-minutes to complete. The front-load washer used had a 20 ± 1 minute rinse cycle and it took 48 ± 1 minutes to finish a complete load.

Blaser *et al.* (1984) and Smith *et al.* (1987) also performed studies to determine bacteria load reduction in plain wash water cycles. Both used large capacity hospital washer-extractors and respectively found 3 $\log_{10}$ and 1.5 – 1.8 $\log_{10}$ total bacteria reductions. Average reductions shown in the current study ranged from 2.37 – 3.37 $\log_{10}$ for top-load washers and from 2.70 – 3.92 $\log_{10}$ for front-load washers before drying.

Once either detergent or detergent and liquid bleach were added to laundry loads, no significant difference in *S. aureus* or MRSA reduction was shown between the two types of washing machines. Average reductions before drying ranged from 5.72 – 6.46 $\log_{10}$ for loads where detergent was used and from 6.04 – 6.78 $\log_{10}$ when detergent and liquid bleach were used. Similar studies show bacteria reductions ranging from 5 – 6 $\log_{10}$ (Watson 1999, Blaser *et al*. 1984) if laundry chemicals were used. Blaser noted that drying removed an additional 1 – 2 $\log_{10}$ organisms. Average reductions after drying in

this study were comparable to Blaser's, ranging from 6.21 – 6.95 $\log_{10}$ if detergent was used and from 6.12 – 6.85 $\log_{10}$ when both bleach and detergent were used.

Washing machines normally offer several wash temperature settings. However, altering the setting only changes the temperature of wash water. Rinse water remains at whatever the "cold" (ambient) wash cycle temperature is regardless of the setting chosen. Ambient water temperatures remained relatively constant throughout the study period at 27˚C, varying only by +/- 2˚C even though outside temperatures fluctuated from below 0˚C to above 38˚C. Ice was added to lower wash water temperature to a level more representative for much of the United States (17˚C – 23˚C). There was no effect of lower wash water temperature (17˚C versus 27˚C) on *S. aureus* or MRSA reductions. Blaser *et al.* (1984) found a comparable outcome for total bacteria (CFU/cm^2), varying wash water temperatures by a much larger range (22.2˚C to 71˚C), but noted that some organisms (B*acillus* spp. and *S. aureus*) survived the wash process better than others. Later studies by Smith *et al.* (1987) confirmed these findings. For the current study, the highest wash water achievable only reached 40.2˚C in the top-load washer and 44.6˚C in the front-load washer. Bacteria reductions at higher temperatures were beyond the scope of this study.

The type of washing machine used did not consistently affect *S. aureus* versus MRSA reduction efficiencies. No other laundry studies compare this parameter, however environmental persistence of MRSA versus *S. aureus* has been investigated. Most studies show only minor differences exist (Kramer *et al*. 2006).

The differences in the number of surviving bacteria varied by as much as a factor of 10,000 within replicate experiments. The highest variability was seen in loads where

no additives (plain water) were used. Numerous factors could be responsible for the variability. Location of a swatch relative to other items within a load (detergent, ballast, etc.), the inherent variability when isolating and growing bacteria, as well as in recovery efficiency can all affect resulting bacteria numbers.

During the experimental design stages, shorter drying times were trialed when using the top-load washer, since the average drying time per load documented in previous studies (Gerba *et al.* 2007) was 28 minutes. Fifty minute drying cycles had to be used in this study to ensure complete drying of loads washed in the top-load washer. It was evident that the spin cycle of the top-loading machine used was not as efficient at removing water as the spin cycle of the front-loading washer used during the experiments. In all cases where swatches were removed and analyzed from loads dried for 28 minutes instead of 50 minutes (n=6), bacteria reductions were lower (3.50 ± 0.28 $\log_{10}$ versus 2.37 ± 0.60 $\log_{10}$) by approximately one log ($p=0.025$) . The longer drying time necessary for use with the top-load washer most likely influenced survival rates and lessened the differences in reduction efficiencies shown between the two types of washers after loads were dried.

In the current study, a Seward stomacher was employed to recover bacteria from fabric. An estimated 8.2% of organisms were recovered using this method, higher than the 1.4% efficiency found during our earlier lab studies using the Waring blender recovery method. Christian *et al* (1983) showed that the stomacher method provided a better recovery rate than nondestructive methods (using sweep plates or swabs), but

determined it was not as good as the blender method which recovered 85% of *S. aureus* and *E. coli*. It is unknown why our studies yielded dissimilar results.

Studies have shown that bacteria contamination of laundry varies considerably and is not uniform (Bloomfield *et al*. 2002, Christian *et al.* 1983). Reports have dealt primarily with hospital-based laundry (Walter *et al*. 1975, Battles *et al*. 1981, Christian *et al*. 1983, Blaser *et al*. 1984, Tompkins *et al*. 1988), and no data are available regarding *S. aureus* specific concentrations in home laundry. Items in the home, including laundry, can become contaminated especially when a family member is ill (Bloomfield *et al*. 2003). Saltzman *et al*. (1967) determined total bacteria levels to be approximately 10^5 CFU/cm^2 in used articles of clothing. Similar levels ($10^5 – 10^6$ CFU/cm^2) were found in hospital laundry by Blaser *et al*. (1984). Christian *et al*. (1983) analyzed hospital laundry specifically for *S. aureus* and reported concentrations of <0.1 to 4 x 10^3 CFU/cm^2.

In conclusion, we found that use of detergent effectively removes *S. aureus* levels previously reported in unwashed laundry regardless of whether a front-load washer or a top-load washer is used, 5 – 6 $\log_{10}$ reductions were shown and if bleach was also used 6 – 7 $\log_{10}$ reductions occurred. If only plain water is used, front-load washers reduce *S. aureus* and MRSA significantly better than top-load washers most likely due to inherently longer rinse cycles which improve bacteria removal through agitation, dilution and drainage. However if detergent is not used, even when using a front-load washer, *S. aureus* and MRSA reductions achievable are 3 $\log_{10}$ lower than the 6 – 7 $\log_{10}$ level recommended for minimizing risk of nosocomial infection (Walter 1975, Christian *et al.* 1983). Washing at 27˚C instead of 17˚C had no significant effect on bacteria reduction.

S. aureus and MRSA are readily transferred to the interiors of washing machines as well as to other clothing within loads, particularly before drying or if bleach was not used. Top-load washer interiors became contaminated more frequently than front-load washer interiors. A data gap exists for the potential of these cross-contamination organisms to cause infection in laundry handlers.

The level of MRSA in laundry has not yet been adequately evaluated, therefore more research is needed to quantify current exposure risks. This research focused specifically on reduction of *S. aureus* and MRSA during laundering. Because bacteria numbers and pathogenicity varies from situation to situation, further research is needed on the effectiveness of laundering for other potentially pathogenic organisms.

Figure 1.1 Flowchart of laundry experiments.

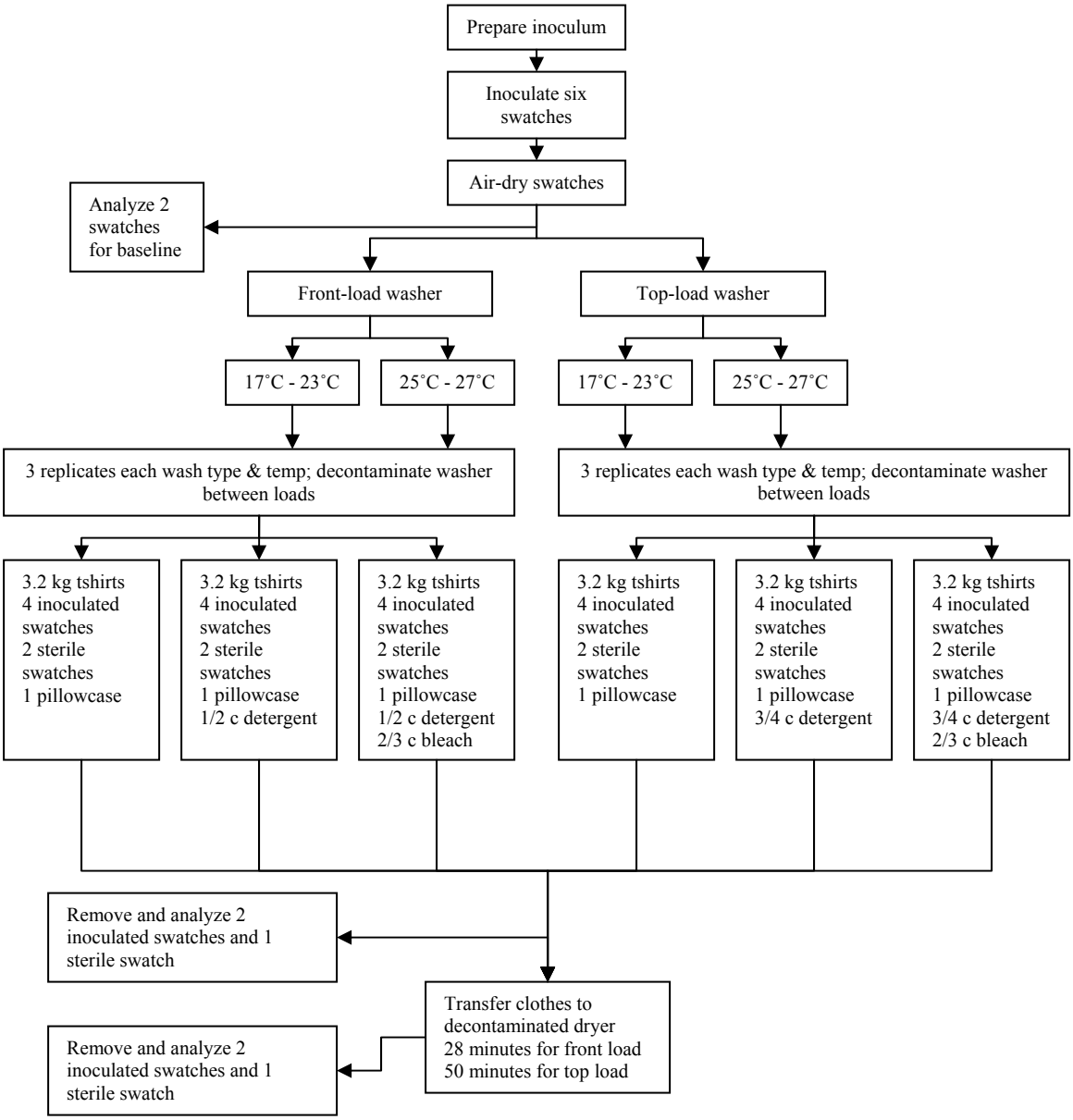

Table 1.1 T-test results for comparisons of N/N_o* after washing and after washing with drying^

Comparison	Organism	Laundry treatment	"p" value after washing	"p" value after washing and drying
Top- vs front-load @ 27˚C	*S. aureus*	No additives, water only	**p<0.001**	**p=0.05**
		Detergent	p>0.05	p>0.05
		Detergent and bleach	p>0.05	p>0.05
Top- vs front-load @ 27˚C	MRSA	No additives, water only	**p=0.03**	**p=0.04**
		Detergent	p>0.05	p>0.05
		Detergent and bleach	p>0.05	p>0.05
Top- vs front-load @ 17˚C	*S. aureus*	No additives, water only	**p=0.03**	**p=0.05**
		Detergent	p>0.05	p>0.05
		Detergent and bleach	p>0.05	p>0.05
Top- vs front-load @ 17˚C	MRSA	No additives, water only	**p<0.001**	**p<0.001**
		Detergent	p>0.05	p>0.05
		Detergent and bleach	p>0.05	p>0.05

*N/N_o = (number of bacteria remaining)/(bacteria concentration before treatment)

^Loads each included 3.2 kg sterile cotton t-shirts, standardized dirt load on 1 pillowcase, and four seeded swatches with initial bacteria inoculum ranging from 6 to 7 logs per swatch.

Table 1.2 Log_{10} reduction after washing at 17˚C and 27˚C wash water with and without drying*

Organism & Drying Status	Laundry conditions	Log_{10} reduction after washing at 17˚C	Log_{10} reduction after washing at 27˚C
***S. aureus,* no drying**	Top load - no additives, water only	2.96 ± 0.29	2.88 ± 0.59
	Top load - detergent	5.92 ± 1.27	6.13 ± 1.24
	Top load - detergent and bleach	6.44 ± 0.76	6.78 ± 0.36
	Front load - no additives, water only	3.92 ± 0.78	4.66 ± 0.35
	Front load - detergent	6.46 ± 0.28	6.21 ± 0.45
	Front load - detergent and bleach	6.04 ± 0.29	>6.58
MRSA, no drying	Top load - no additives, water only	2.37 ± 0.60	3.37 ± 0.96
	Top load - detergent	5.72 ± 1.08	6.02 ± 0.62
	Top load - detergent and bleach	6.04 ± 1.33	>6.85
	Front load - no additives, water only	3.77 ± 0.36	2.70 ± 0.52
	Front load - detergent	6.41 ± 0.45	6.22 ± 0.94
	Front load - detergent and bleach	>6.53	6.43 ± 0.00
***S. aureus,* with drying**	Top load - no additives, water only	5.45 ± 0.90	4.45 ± 0.67
	Top load - detergent	6.75 ± 0.03	6.95 ± 0.55
	Top load - detergent and bleach	>6.73	6.43 ± 0.90
	Front load - no additives, water only	4.68 ± 0.75	4.66 ± 0.35
	Front load - detergent	6.46 ± 0.14	6.21 ± 0.45
	Front load - detergent and bleach	6.12 ± 0.02	>6.58
MRSA, with drying	Top load - no additives, water only	3.69 ± 1.13	6.17 ± 0.71
	Top load - detergent	6.21 ± 1.23	6.39 ± 0.50
	Top load - detergent and bleach	6.67 ± 0.06	>6.85
	Front load - no additives, water only	5.69 ± 0.58	5.73 ± 0.40
	Front load - detergent	6.29 ± 0.57	6.49 ± 0.53
	Front load - detergent and bleach	>6.53	6.43 ± 0.00

*Loads each included 3.2 kg sterile cotton t-shirts, standardized dirt load on 1 pillowcase, and four seeded swatches with initial bacteria inoculum ranging from 6 to 7 logs per swatch.

">" values indicate log reduction exceeded limit of detection for experiment.

Table 1.3 Percent (number contaminated/number tested) of sterile swatches cross contaminated during laundering

	Top Load		Front Load	
Laundering Conditions	**MRSA**	***S. aureus***	**MRSA**	***S. aureus***
27˚C, Wash, water only	50 (10/20)	100 (4/4)	67 (4/6)	75 (3/4)
27˚C, Wash, soap only	29 (2/7)	38 (3/8)	33 (2/6)	33 (2/6)
27˚C, Wash, soap and bleach	50 (2/4)	25 (1/4)	0 (0/6)	0 (0/3)
27˚C, Wash and Dry, water only	0 (0/12)	100 (4/4)	17 (1/6)	50 (2/4)
27˚C, Wash and Dry, soap only	0 (0/5)	0 (0/8)	0 (0/6)	25 (1/4)
27˚C, wash and Dry, soap and bleach	0 (0/4)	25 (1/4)	0 (0/6)	0 (0/3)
17˚C, Wash, water only	96 (22/23)	100 (4/4)	63 (5/8)	75 (3/4)
17˚C, Wash, soap only	45 (5/11)	50 (3/6)	0 (0/3)	0 (0/4)
17˚C, Wash, soap and bleach	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/4)
17˚C, Wash and Dry, water only	87 (13/15)	0 (0/4)	100 (6/6)	25 (1/4)
17˚C, Wash and Dry, soap only	100 (6/6)	17 (1/6)	0 (0/4)	0 (0/4)
17˚C, wash and Dry, soap and bleach	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/4)

Table 1.4 Number/percent of swabs taken from washing machine interiors testing positive for bacteria contamination

Top Load Machine Test Location	**Positive Samples**
Lid, after wash load	10/27 (37%)
Rim and sides, after wash load	10/28 (36%)
Lid, rim and sides, after bleach-only load	0/28 (0%)
Front Load Machine Test Location	
Window, after wash load	1/12 (8%)
Door, after wash load	0/12 (0%)
Sides, after wash load	0/24 (0%)
Window and door, after bleach only load	0/24 (0%)

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APPENDIX B:

COMPARISON OF BACTERIA ON NEW, DISPOSABLE, LAUNDERED AND UNLAUNDERED SCRUBS

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ABSTRACT

As a cost saving measure, an increasing number of hospitals allow personnel to launder their uniforms, lab coats, and even operating room scrubs at home. Recent studies indicate that lower wash temperatures are utilized and bleach is sometimes omitted to wash loads in the home setting. With rising nosocomial infection rates and increasing levels of multi-antibiotic resistant bacteria present in hospital settings, uniform contamination may be an environmental factor in the spread of infection. We quantified the number and identity of bacteria found on swatches cut from unwashed operating room (OR), hospital-laundered, home-laundered, new cloth and new disposable scrubs. Bacteria isolates were identified to species. Scrubs were tested for presence of HPC bacteria, *Clostridium difficile*, fungi, *Staphylococcus aureus*, total coliforms, and *Escherichia coli*. Seventy-nine percent (23/29) of unwashed hospital OR scrub swatches tested positive for some type of Gram positive cocci, with 10% (3/29) of those classified as *S. aureus*; and 69% (20/29) tested positive for coliform bacteria, three of which were *E. coli*. Home-laundered scrubs had significantly greater HPC bacteria than hospital-laundered scrubs ($p=0.044$). Forty-four percent (18/41) were positive for coliform bacteria, but none were *E. coli*. No *S. aureus* was isolated from laundered scrubs, however many other potentially pathogenic Gram positive rods and cocci were identified. Hospital-laundered scrubs had the lowest HPC bacteria numbers of any tested, including new cloth scrubs and new disposable scrubs. Fungi were frequently found on scrubs with 93% (28/30) positive among home-laundered scrub swatches, 36% (13/36) of new disposable scrub swatches, 27% (4/15) of new cloth scrub swatches, 22% (4/18) of

unwashed hospital OR scrub swatches, and 10% (2/21) of hospital-laundered scrub swatches testing positive. Opportunistic bacteria pathogens and those capable of causing nosocomial infections were identified on unwashed OR, home-laundered, and new cloth scrubs, but not on hospital-laundered scrubs. It is prudent to follow good hygienic practices, particularly when laundering items that may be worn during contact with persons vulnerable to infection. Hospital administrators, in conjunction with the infection control team staff should provide staff with written guidance to follow when home-laundering scrubs, to minimize the potential for microbial contamination of other laundry and the spread of disease.

INTRODUCTION

Hospital scrubs may harbor numerous types of pathogenic bacteria, fungi and viruses. How uniforms are handled, laundered, and stored appears to influence the level of bacteria contamination (Jurkovich 2004). Changes in laundry methods used both at home and in hospitals have occurred over the past several decades (Patel *et al*. 2006, Blaser *et al*. 1984, Smith *et al*. 1987). To reduce energy usage, some hospitals are washing at temperatures lower than the Centers for Disease Control recommendation of 71.1˚C (CDC 2003). Hospital laundry managers have adjusted chemical usage and increased wash cycle running time by 15-20 percent to compensate for this reduction in wash water temperature, resulting in higher water consumption and a chemical cost increase of 8-10 percent (Smith *et al*. 1987). A consequence of higher laundering costs is that an increasing number of hospitals are allowing personnel to launder their uniforms, lab coats, and even operating room scrubs at home. In the home setting, not only are lower wash temperatures being utilized, but because of increasing numbers of brightly-colored and permanent press fabrics in use, bleach is often not added to wash loads. Only a few studies have looked at the difference in decontamination effectiveness between domestic and industrial laundering of uniforms (Loveday, *et al*. 2007).

The frequency of home-laundering uniforms is unknown. As part of a United Kingdom study to determine the influence of current nursing practices on overall bacteria uniform contamination levels, Callaghan (1998) distributed a questionnaire to 224 nurses working in three hospitals where hospital laundry services were offered by the same contract company. Overall, hospital laundry use was 69%, but it varied significantly

between the three facilities. In July 2001, the Association of Perioperative Registered Nurses (AORN) in the United States conducted a website survey of its members to get an estimate of the percentage of nursing staff who send their uniforms to a hospital laundry instead of washing them at home. Twenty-six percent of responders said they home-laundered their scrubs (Jurkovich 2004).

The steps in an industrial laundry are essentially the same as those performed in a home setting, only the magnitude is different. The process begins with collecting and sorting, and then proceeds to washing, drying and whatever subsequent actions may be required for the particular type of item being washed. Ironing and folding, either by hand or machine, are performed for most items. Packaging and distribution are the final steps in the commercial setting.

Industrial laundries may use batch washers, which operate similarly to domestic washers by allowing laundry process modifications and using fresh water for each wash and rinse cycle (Barrie, 1994). The other common type of industrial machine, the continuous batch or tunnel washer, processes pre-weighed loads and automatically adds pre-determined amounts of chemicals at designated points during a complete cycle. After washing, items are unloaded directly onto the laundry's clean area. Tunnel washers provide a more continuously flowing process and are currently used in most higher-volume facilities (Barrie 1994). As a water saving measure, the clean water used during the rinsing process of a load in a tunnel washer is reused as relatively clean water in the washing cycle of a subsequent load.

The water to fabric ratio in an industrial laundering operation is about 5:1 (w/w), whereas in a home or coin-operated washing machine, the ratio is about 10:1 (U.S. EPA, 2007). This variation in water amounts can have a significant impact on the effectiveness of laundry additives, so formulations in the hospital and home differ (Barrie 1994). The other main dissimilarity between industrial and home-laundering is that most commercial hospital laundries follow CDC guidelines (2003) to provide thermal disinfection in the wash cycle by washing for $\leq$ 25 minutes and holding the temperature for 3 minutes at 71˚C, or by using chemicals suitable for low-temperature washing if $\geq$ 70˚C laundry cycles are used (Smith *et al*. 1987).

Microbial contamination of uniforms

Speers *et al*. (1969) found that approximately one third of microorganisms recovered from a nurse's uniform originate from the flora of the wearer with uniforms most frequently becoming contaminated below the waist during procedures such as dressing wounds. Sixty-two percent of the microorganisms recovered were attributed to patients.

Loh *et al*. (2000) tested the cuffs, side pockets and backs of white coats of one hundred medical students using contact plates. Every coat was contaminated on all three sites to varying degrees. As with similar studies (Babb *et al*. 1983, Wong *et al*. 1991), *Staphylococcus* spp. was most frequently seen (all 100 students tested), and *Acinetobacter* spp. (7 students) and dystheroids (12 students) were also isolated from the white coats. No MRSA was found and only three instances of a Gram negative organism were detected. None of the Gram negative organisms identified were considered normally pathogenic. A study by Wong *et al*. (1991), determined that the maximum level

of *S. aureus* contamination on doctor's white coats is reached within a week of use and doesn't change significantly until the coat is laundered.

Callaghan (1998) examined the effect of laundering frequency on bacteria levels for nurses' uniforms, with or without use of an additional cover apron. Wide variations in bacteria contamination levels were seen. Uniforms were found to be equally and heavily contaminated at all sites sampled, and the end of shift samples produced no statistically higher contamination levels than were observed at the beginning or middle of a shift. Of this study's participants, 59.4% (116/196) of nurses who did not additionally use an apron reported that they wore a clean uniform each day. Far fewer nurses who wore aprons (7.3%, 14/196) felt it necessary to wear a clean uniform each day and 30.6% (60/196) of the survey participants admitted to not always wearing clean uniforms at the start of a shift. More than half of the nurses used a hospital laundry, so further work was conducted to determine initial bacteria counts on clean uniforms. Of the dozen uniforms tested, no bacteria were recovered.

Perry *et al*. (2001) used a vacuum method to analyze 57 nurse's home-laundered uniforms at the beginning and end of a shift for MRSA, VRE, and *C. difficile*. Thirty-nine percent (22/57) of uniforms tested were positive for one or more of the organisms prior to the start of the shift. VRE was detected on 21% (12/57) of uniforms, whereas MRSA and *C. difficile* were each found on 12% (7/57). Contamination levels varied from one to greater than 100 colonies. Scrubs at the end of a shift showed that 54% (31/57) of uniforms were positive for at least one of the test organisms. VRE was found on 31% (22/57) of uniforms, *C. difficile* on 19% (11/57) of uniforms and MRSA on 15%

(8/57) of uniforms. Perry *et al*. (2001) noted that some uniforms had fewer organisms after being worn for a shift, and that the levels of post-duty contamination varied based on the type of ward.

However, Babb *et al*. (1983) did not detect an increase in *S. aureus* or Gram negative bacilli when gowns and protective clothing were used for periods up to 11 days in a main isolation unit. This study employed contact plates and detected *S. aureus* in 12.6% (26/207) of the fronts/shoulders of cotton gowns and 9.2% (22/239) of the plastic aprons tested. Gram negative bacilli were only recovered from one gown (1/207). While 47% (42/89) of strains identified could not be associated with either the patients or staff, 35% (31/89) were linked to patients and 18% (16/89) were matched to the nurse's own nasal strain. Little difference was seen in the numbers of bacteria recovered from the two different areas tested, and 11 of the 707 garments evaluated produced *S. aureus* counts greater than one per square centimeter.

One of the few studies to analyze specific types of microbiota from uniforms was performed by Pilonetto *et al*. (2004) using RODAC contact plates at both the beginning and end of a work shift. Samples were collected from the cuffs of long-sleeved gowns and the abdominal region from short-sleeved gowns and analyzed for total viable and Gram negative bacteria counts. Pathogens were isolated from 48% (15/31) of the gowns. Of the isolated pathogens, 61% (11/18) were *S. aureus* none of which were MRSA. Gram negative isolates found included *Acinetobacter baumanni* (2/18), *Klebsiella pneumonia* (2/18), *Stenotrophomonas maltophilia* (2/18), and *Serratia rubidate* (1/18). No *E. coli* or *Pseudomonas* spp. were detected, however, it was felt that the lower

number of Gram negative organisms was due in part to their poor ability to attach to fabrics. Researchers found a significant ($p=0.027$) increase in total bacteria from the beginning to the end of a work shift, with average counts increasing from 2.2 to 4.9 CFU/cm^2. Converse to findings in Loh *et al*. (2000), bacteria levels were higher in the abdomen region than at the cuff. Pilonetto speculated this was because the earlier work by Loh only evaluated contamination in physicians clothing, while his work involved gowns from staff that generally had a much closer patient contact.

Fijan *et al*. (2005) also identified specific organisms on fabrics from a hospital setting. RODAC plates were used to evaluate the number and types of microorganisms on surfaces from a hospital laundry clean area as well as on ironed and folded textiles processed at the laundry. Normal skin bacteria from the *Micrococcus* and *Staphylococcus* genera were most commonly found. Species from the genus *Bacillus* and the genus *Corynebacterium* were also frequently detected, even after surface disinfection measures had been implemented.

Hospital- versus home-laundered scrubs

Few studies compare the microbial flora of hospital- versus home-laundered attire. Previous research focused mainly on enumeration rather than identification of specific biota.

After recovering no bacteria from 12 randomly selected hospital-laundered uniforms, Callaghan (1998) inoculated uniforms previously laundered by the hospital with *Serratia marcescens* and washed them using a home washing machine employing a variety of temperature settings, wash cycles, and laundry load contents. All loads were

tumble dried. She concluded that uniforms could be laundered at home provided they were washed with no other items of clothing, at no less than 50˚C, and ironed dry with a hot iron.

Jurkovich (2004) swabbed the left front shoulder of operating room personnel, 60% (30/50) of whom had laundered their scrubs at home. No pathogenic microorganisms were found on either the home- or hospital-laundered scrubs. Also, no significant differences were found when comparing the normal skin flora on the two different types of scrubs. Seventy percent (35/50) of staff who had home-laundered their scrubs used warm water cycles, 73% (37/50) had washed their scrubs separate from other clothing, and all had completely dried their scrubs in a drier.

Outbreaks attributed to contaminated laundry

Only a few isolated studies have explored the possible transfer of organisms from nursing scrubs and uniforms to patients during infection outbreaks, and those have taken place mainly in specialized wards such as burn or cardiothoracic units.

Barrie *et al*. (1992) collected environmental samples after two patients developed *Bacillus cereus* meningitis following neurosurgery at a London hospital to determine the source of the organism. Operating room linen was found to be the most probable origin of the infections. Alternate laundering arrangements until the outbreak investigation, including analysis of the laundry facility, was completed and no further cases were reported. It was eventually determined that a contaminated continuous batch tunnel washer harbored large numbers of the organism and was not effectively disinfecting the linen (Wilcox *et al*. 1995).

The other outbreak attributed to contaminated laundry involved a maternity ward where several babies contracted *Streptococcus pyrogenes*. The organism was traced to a heavily contaminated dryer used during laundering of the babies' clothes. The outbreak ended once the clothes were autoclaved (Fijan *et al.* 2005).

The CDC estimates that health-care associated infections in American hospitals accounts for 1.7 million infections and 99,000 associated deaths each year (CDC 2007) and by 2007, 2.4% of all hospital patients contracted a MRSA infection (Jarvis *et al.* 2006). These infection rates highlight the need to evaluate and develop new guidelines for laundering scrubs and other attire worn in the hospital. The purpose of this study was to quantify the number and identify types of heterotrophic (HPC) bacteria found on unwashed operating room, hospital-laundered, home-laundered, new cloth and new disposable scrubs. This information can be used by hospital administrators and infection control personnel who develop policies about laundering scrubs at their facilities and provide guidance to staff on home-laundering procedures.

MATERIALS AND METHODS

Preliminary testing using a standard dilution/plating method for heterotrophic count (HPC) bacteria was performed to determine scrub locations most likely to elicit highest numbers. Scrub tops, pants and jackets worn for one shift were taken from a local hospital operating room laundry collection bin at the end of a shift. Each clothing piece was placed separately into a clean plastic bag, sealed and refrigerated until analyzed. Fabric sections (swatches) with known areas were cut from necks, sleeves, pockets, front pants, front shirts, front jackets, crotches and under arms using sterile scissors and placed separately into sterile plastic bags with 50 ml of buffered peptone solution. Each sample was pummeled for 4 minutes on high speed in a Seward stomacher (Seward, London, UK) to recover the bacteria into the peptone liquid. The liquid was pipeted off and for each swatch, 0.1 ml and 0.01 ml of the liquid was plated on R2A (Difco, Sparks, MD) agar. The plates were incubated for 3-5 days at 37^{o}C and colonies counted. Bacteria identification was not performed during the preliminary study since only total bacteria numbers were of interest. Based on results from the preliminary study, subsequent sampling locations were narrowed down to the neck and front pocket area of scrub tops, and central front area of scrub pants. Further testing on operating room jackets was not conducted because of low HPC bacteria found. A diagram summarizing experimental procedures is presented in Figure 2.1.

Scrubs laundered at home or by the hospital came from a variety of manufactures and were constructed of either 100% cotton or a polyester/cotton blend. Hospital-laundered scrubs were all processed within the company-owned industrial laundry

facility. Ten steps were used during the 61-minute wash/mechanical action cycle for heavily soiled laundry items, such as operating room scrubs. Chemicals were added during five of the steps and consisted of a phosphate-free detergent, a product to restore water and soil repellency finish, a laundry rinse additive, a germicide, and a pH reducer (sour) to eliminate yellowing. Water temperature reached 71.1˚C for at least three minutes, as is recommended by the Centers for Disease Control (2003). No information on specific laundering practices or type of nursing assignment was sought from donors of the home-laundered scrubs, but all were obtained from nurses who had patient contact in a hospital setting. Two different styles of new disposable scrubs were purchased and tested (Molnlycke Healthcare, Anderson SC, and Lakeland Industries, Ronkonkoma NY) as well as two different colors of a single style of new cloth scrubs (Natural Uniform, 65% polyester, 35% cotton, model B101).

Heterotropic plate counts (HPC)

HPC testing was done using the dilution and plating method. Peptone collected after pummeling each swatch in the Seward Stomacher, as previously described, was serially diluted and assayed on R2A (Difco, Sparks, MD) agar. The plates were incubated for 3-5 days at 37^{o}C and counted. Unique-looking colonies that could be harvested without contamination from other colonies were plated on tryptic soy agar (TSA) (Difco, Sparks, MD) agar and MacConkey (MAC) (Difco, Sparks, MD) agar. Plates were incubated for 24 hours at 37ºC.

Further identification of isolated colonies was performed. Freshly-grown lactose forming colonies on MAC agar were Gram stained (BD Gram crystal violet stain, Difco,

Sparks, MD), then observed under a microscope (Nikon HFX-II) to determine whether they were Gram negative cocci or rods. Further characterization of Gram negative rods and cocci was done using API-20 (BioMerieux, St. Louis, MO) test strips. Freshly-grown non-lactose forming colonies on MAC agar were Gram stained (BD Gram crystal violet stain, Difco, Sparks, MD), then observed under a microscope (Nikon HFX-II) to determine whether they were Gram positive cocci or rods. Further characterization was done using the Biolog GP2 microplate (Biolog, Hayward, CA) identification system.

Presence/absence of *Clostridium difficile*

For each swatch, 10 ml of peptone was placed in a test tube and capped. The sample was then heat shocked for 10 minutes at 70^{o}C and subsequently filtered through a Millipore 0.45 μm, 47-mm membrane. The intact membrane was placed on cycloserine-cefoxifin fructose agar (CCFA) (Hardy Diagnostics, Santa Maria, CA), placed in an anaerobic chamber, and incubated for 24 hours at 37^{o}C. After incubation, colonies were counted. Because no *C. difficile* was found during the first 30 sets of scrubs tested, including several sets of unwashed scrubs, it was not performed in later testing.

Presence/absence of *Staphylococcus aureus*/MRSA

Peptone collected after pummeling each swatch in the Seward Stomacher, as previously described, was serially diluted and plated on tryptic soy agar (TSA) amended with 5% sheep blood (Hardy Diagnostics, Santa Maria, CA), 0.015 g/L of nalidixic acid (Sigma, St. Louis, MO) and 0.01 g/L of colistin (Sigma, St. Louis, MO), then incubated at 37°C for 48 hours. Creamy-white and yellow colored colonies demonstrating β-hemolysis were further subjected to a series of biochemical tests to confirm *S. aureus*. Biochemical

tests included slide coagulase (Becton Dickinson, Sparks, MD), tube coagulase, catalase, and polymixin-B (Becton Dickinson, Sparks, MD) sensitivity. Confirmed *S. aureus* isolates were plated on two different types of MRSA agar, a differential CHROMagar media (Becton Dickinson, Sparks, MD) containing cefoxitin, and a selective MRSA Screening Plate (Hardy Diagnostics, Santa Maria, CA) containing oxacillin. If full growth occurred on both types of media, isolates were confirmed as MRSA. If no growth or only residual growth occurred, the strains were considered methicillin susceptible *S. aureus*.

Presence/absence of total coliforms and *Escherichia coli*

For each swatch, 1 ml of peptone was added to tubes containing Colilert (IDEXX, Westbrook, ME). Tubes were then incubated for 24 hours at 37ºC. A yellow tube indicated presence of coliforms and yellow tubes that fluoresced under UV light were preliminarily deemed positive for *E. coli*. Liquid (0.01 ml) from tubes that had fluoresced were plated onto MAC for further confirmation testing using API-20E test strips. In addition to using the Colilert test, entire 6 inch square sections of scrubs were added to 100 ml bottles containing Colilert media (IDEXX, Westbrook, ME). Contents were shaken for 60 seconds and then incubated for 24 hours at 37ºC. This was done to determine if the two methods would achieve identical results.

Presence/absence of fungi

Peptone was collected after pummeling each swatch in the Seward Stomacher, as previously described, then serially diluted and plated on sabouraud dextrose agar with chloramphenicol (Hardy Diagnostics, Santa Maria, CA) at 37˚C for 48 hours. Over the

following 21 days, plates were checked for growth every two days, any growth was noted, and positive plates were immediately discarded.

Statistical analysis

Student t-tests were employed to determine if there was a significant difference ($p< 0.05$) between numbers of HPC bacteria found per square centimeter for the different categories of scrubs. Student t-test comparisons, as well as, average, geometric mean, and standard deviation values were performed using Microsoft Office Excel software (2003).

RESULTS

Heterotrophic plate count (HPC)

A summary of HPC bacteria results for the different classifications of scrubs is shown in Table 2.1. Unwashed hospital OR scrubs had the highest HPC numbers (geometric mean 85 CFU/cm^2, range 5 – 473 CFU/cm^2), followed by home-laundered scrubs (geometric mean 16 CFU/cm^2, range 1 - 848 CFU/cm^2), new cloth scrubs (geometric mean 5 CFU/cm^2, range 1 - 145 CFU/cm^2), new disposable scrubs (geometric mean 5 CFU/cm^2, range 1 – 118 CFU/cm^2), and hospital-laundered scrubs (geometric mean 2 CFU/cm^2, range 1 – 27 CFU/cm^2). Hospital-laundered scrubs had significantly fewer ($p=0.044$) HPC bacteria than home-laundered scrubs.

Presence/absence of *C. difficile*

No *C. difficile* was isolated from any of the scrubs tested, although positive controls for *C. difficile* were obtained.

Presence/absence of *S. aureus*/MRSA

Seventy-nine percent (23/29) of swatches tested on unwashed hospital scrubs were positive for some type of Gram positive cocci (Table 2.2), with 10% (3/29) of those identified as *S. aureus* (Table 2.2). None were confirmed as MRSA. No *S. aureus* was isolated from any of the home-laundered (0/41), hospital-laundered (0/36), new disposable (0/48), or new cloth (0/15) scrubs, however many other potentially pathogenic Gram positive rods and cocci were identified using a Biolog GP2 Microplate identification system (Table 2.3).

Presence/absence of total coliforms and *E. coli*

Sixty-nine percent (20/29) of swatches on unwashed hospital OR scrubs tested positive for coliforms with three of those confirmed as *E. coli*. Eighteen swatches (18/41, 44%) on home-laundered scrubs tested positive for coliforms. None of these were identified as being *E. coli*. No coliforms or *E.coli* were found on hospital-laundered, new disposable or new cloth scrubs. Identical results were achieved whether the samples were run using the colilert tubes or the 100 ml bottles containing colilert media.

Presence/absence of fungi

Fungi were frequently found on scrubs with 10% (2/21) of hospital-laundered swatches, 93% (28/30) of home-laundered swatches, 36% (13/36) of new disposable swatches, 22% (4/18) of unwashed hospital swatches and 27% (4/15) of new cloth swatches testing positive.

Identify Gram positive bacteria using Biolog GP2 microplate

Gram positive cocci were identified from unwashed hospital OR scrubs (*S. aureus*, *Micrococcus luteus*, *S. saprophyticus*, *Aerococcus viridans*, *S. caprae*, *S. delphini*, *S. kloosii*, *S. pasteuri*, *S. cohnii*, *Enterococcus flavescens*, and *S. epidermis*) and home-laundered scrubs (*Micrococcus luteus*, *Micrococcus lylae*, *Lacoccux lactiss lactis*, *S. lugdunensis*) using the Biolog MicroLog system, version 4.20. In all cases, similarity rates exceeded 0.5, indicating an acceptable species identification result for each isolate (BIOLOG, MicroLog System 4.20, User Guide). Results are shown in Table 3.

No Gram positive rods were identified from unwashed hospital OR scrubs, however several different Gram positive rods were isolated from home-laundered scrubs

(*Bronchothnix thermosphacta*, *Microbacterium esteraromaticum*, *Brevibacterium liquefaciens*, *Tsukamurella inchonesis*, *Curtobacterium luteum*, and *Sanguibacter inulinus*) and new cloth scrubs (*Tsukamurella inchonensis*, *Microbacterium* spp.) using the Biolog MicroLog identification system (Table 2.3).

Identify Gram negative bacteria using API 20E test strip

Enteric Gram negative rods were isolated from unwashed hospital OR scrubs using the API 20E test strip (*E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), all exceeding 90% significant taxa identification. Similarly, Gram negative rods were isolated from home-laundered scrub swatches using the API 20E test strip system. Taxa identified were *Klebsiella pneumoniae*, *Pantoea* spp 3, *Pantoea* spp 4, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Raoultella terrigena*, and *Serratia rubidaea.* Test results were compared to the apiweb system database and identification was carried out using Biomerieux apiweb version 4.1 (2009). Percent significant taxa identification was above 90%, indicating "good identification" except for *Pantoea* spp 4 at 63% (acceptable identification to the genus) and *Serratia rubidaea* at 87.7% (very good identification to the genus). These results are listed in Table 2.4.

DISCUSSION

HPC bacteria were chosen to show the relative cleanliness of scrubs, based on similar studies performed by Pilonetto *et al.* 2004, Perry *et al.* 2001, and Callaghan 1998. Significantly fewer bacteria ($p=0.044$) were detected on hospital-laundered scrubs than on home-laundered scrubs (average HPC bacteria 4 CFU/cm^2, range 1 – 27 CFU/cm^2 versus 143 CFU/cm^2, range 1 – 848 CFU/cm^2). Most of this difference is attributable to two sets of home-laundered scrubs with average HPC bacteria of 848 CFU/cm^2 and 627 CFU/cm^2 and appears less pronounced when geometric mean values are compared (geometric mean HPC bacteria 2 CFU/cm^2 versus 16 CFU/cm^2). Both average and geometric mean HPC values were calculated because average values take into account worst-case scenarios and can be used in the development of a quantitative risk assessment, while geometric means were used for comparison of this data with data from similar studies. The highest home-laundered HPC values were greater than any seen in unwashed hospital OR scrubs (highest HPC bacteria 436 CFU/cm^2 and 473 CFU/cm^2). For this study, detailed laundering practices used by the hospital-owned and operated laundry were known but home-laundering treatments were not. Each set of home-laundered scrub tested ($n=10$) came from a different owner. A Seward stomacher was employed to recover bacteria from scrub fabric. In our earlier lab studies using this method, an estimated 8.2% of organisms were recovered, so actual HPC numbers present on the scrubs will likely be higher.

Many variables will affect the level of contamination detected in washed scrubs, including initial level of contamination, laundry procedures used, and storage conditions.

The average HPC levels on home-laundered scrubs were 80% of the bacteria counts recovered from unwashed hospital OR scrubs (average of 143 CFU/cm^2 versus 180 CFU/cm^2). In previous studies, Callaghan (1998) and Perry *et al.* (2001) found bacteria counts varied little between scrubs worn at the beginning of a shift compared to those tested in the middle and at the end of a shift.

The level of post-duty contamination of hospital uniforms also varies between ward areas (Perry et al. 2001). In Perry's study, only 7.7% (4/57) of surgical staff uniforms tested positive for MRSA, *C. difficile*, and/or VRE , while 92% (52/57) of general medicine ward area staff uniforms, 83% (47/57) of renal medicine staff uniforms and 62% (35/57) of renal transplant staff uniforms tested positive for at least one of these pathogens. Ward staff have direct contact with many more patients during a single shift than surgical staff which may account for the contamination differences seen between ward and operating room apparel.

C. difficile was not isolated from any scrub tested, washed or unwashed. Positive controls were achieved, and while few organisms were expected on washed or new fabrics, because *C. difficile* is a common causes of hospital infections and had been previously isolated from worn hospital clothing (Perry *et al.* 2001) it was expected that *C. difficile* would have been found on the worn hospital scrubs tested in this study.

On the other hand, some type of Gram positive cocci were isolated from seventy-nine percent (23/29) of unwashed hospital OR scrub swatches, with 10% (3/29) of those classified as *S. aureus*. This is not surprising since nearly 32% of people carry *S. aureus* on their skin and/or nares at any one time (CDC 2007).

Of the coliform bacteria isolated from worn hospital scrubs and home-laundered scrubs, ten percent (3/29) tested positive for *E. coli*, however many of the other Gram negative bacteria identified (*Enterobacter cloacace*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Serratia rubidaea*) are capable of causing serious nosocomial infections including pneumonia, meningitis and septicemia (Emori *et al*. 1993). A number of Gram positive organisms identified (*S. aureus*, *Aerococcus viridans*, *Enterococcus flavscens*) also are capable of causing infections in high-risk individuals, such as those with underlying diseases.

The high frequency of fungi found on scrubs was unexpected since samples were collected from areas known to have low relative humidity (Tucson and Phoenix, Arizona) during a typically dry part of the year (December). Even hospital-laundered scrubs showed a 10% (2/21) positive rate for fungi and yeasts. Storage conditions (humidity and temperature) for the home-laundered scrubs, as well as time since last washing was not known for the home-laundered scrubs, however neither visible fungi nor noticeable moldy smell was detected on any of the scrubs prior to testing. Still, 93% (28/30) of swatches from home-laundered scrubs tested positive for fungi. Fungi have the ability to thrive almost anywhere as long as food, moisture and humidity are present.

Sabouraud Dextrose Agar with chloramphenicol was the selective media chosen for fungi growth because it inhibits many species of bacteria via an acidic pH (5.6) and added chloramphenicol supplement. Identification of specific fungal species was beyond the scope of this project and not determined, so the health hazards from the fungi found are uncertain. Some species of *Aspergillus*, known to grow well on clothing, are

opportunistic pathogens (Bio-medicine 2001). Other fungi may not cause infections, but can cause strong allergic reactions in sensitive individuals. Recommendations to reduce fungal growth in clothing include drying all items completely or hanging them out to dry in the sun and storing clothes in a cool, dry environment with adequate ventilation.

Holton *et al*. (2006) evaluated the bioburden mass on nursing uniforms before and after low temperature laundering using the stomacher method. Holton found that pre-washed uniforms were contaminated with $10^5 – 10^7$ CFU, primarily *Staphylococcus* spp. Washing reduced contamination to $10^2 – 10^5$ CFU, and altered the flora to mostly Gram negative rods and *Bacillus* spp. Because characterization of biota in the present study was not performed for each organism found, it is impossible to determine if results are similar in the two studies. However in the present study, both Gram positive and Gram negative bacteria were isolated from home-laundered scrubs. Although similar analytical methods were used to enumerate HPC bacteria levels in these two studies, results are not comparable since in Holton reported bacteria counts obtained for an entire scrub. This study enumerated CFU per square centimeter of fabric.

Whether this contamination is transferred to patients was not assessed during this study, however Hedin (1993) found three of five patients were colonized with a *S. epidermis* strain identical strain to one found on staff clothing. Hedin demonstrated that indirect contact with staff clothing was a route for cross-infection in a clinical setting. Even *S. epidermidis* and other normal skin organisms of usually low pathogenicity may cause infection, particularly during highly invasive procedures such as implant surgery and joint replacement operations (Bukhari *et al*. 1993). Boyce *et al.* (1997) found that

65% of nurses who performed care activities on patients with MRSA in urine or a wound contaminated their uniform or gown. Additionally, contact transfer from uniforms resulting in infections has also been noted by Hambraeus (1973) and Hambraeus and Ransjo (1977).

Conversely, after performing a literature review, Wilson *et al.* (2007) concluded that existing evidence does not support uniforms as a vehicle for the transmission of infections and; similarly, the CDC 2002 Guidelines for Laundry in Health Care Facilities states that "uniforms laundered at home have shown no link with an increase in infection rates and no pathogens have been recovered from either home- or hospital-laundered scrubs." A pilot study by Jurkovich (1999) showed no increase in nosocomial infection rate when OR staff laundered their scrubs in warm water, with household laundry soap and then dried them in a drier. In a longer duration pilot study, Kiehl *et al*. (1997) recruited 68 perinatal nurses at two Florida hospital campuses to purchase and then launder their scrub at home for approximately three years. Participants were provided guidance on home-laundering procedures, required to change home-laundered scrub clothing with hospital-laundered scrubs in the event clothing was penetrated by blood or other potentially infectious body fluids during work, and were encouraged to wear impervious gowns and protective eyewear as necessary. Over the course of the study, no outbreaks of infection occurred in the newborn nursery and the perinatal infection rate fell from 1.7% to 1.0% by the end of the pilot program.

There are conflicting opinions regarding whether to allow staff to home-launder hospital attire. Hospital administrators and infection control teams must weigh the risk of

potential infection transmission against the cost savings realized by the company if staff purchase and launder their own scrubs. Either way, health care providers should be made aware of the potential for uniforms to be come contaminated and steps they can take to minimize transmission of microorganisms within the hospital setting and when washing scrubs at home. By minimally providing written guidelines for staff to follow when home-laundering scrubs, the risk of nosocomial infection may be reduced. Laundering scrubs at 71˚C, as recommended by the CDC, is not feasible in a home setting. Washing at 60˚C or above using any laundry product or washing at 40˚C using a bleach-containing laundry product should be sufficient to kill most microorganisms, including *C. difficile* spores (Bloomfield, 2006). Bleach should be used when possible and always on grossly contaminated garments. All hospital attire should be dried completely in a drier, then stored in a manner to ensure continued cleanliness and minimize fungal growth.

As the frequency of people with impaired immunity rises and difficulty in treating antibiotic resistant disease strains magnifies, it is prudent to follow good hygienic practices, particularly when laundering items that may be worn during contact with people vulnerable to infection.

Most of the research to date focuses on bacteria types and quantities found on operating room personnel scrubs. It is recommended that further studies be performed to identify and quantify bacteria levels on uniforms from staff in alternate hospital areas where the risk of infection is also great, such as transplant and burn units.

Figure 2.1 Flowchart of scrub experiments

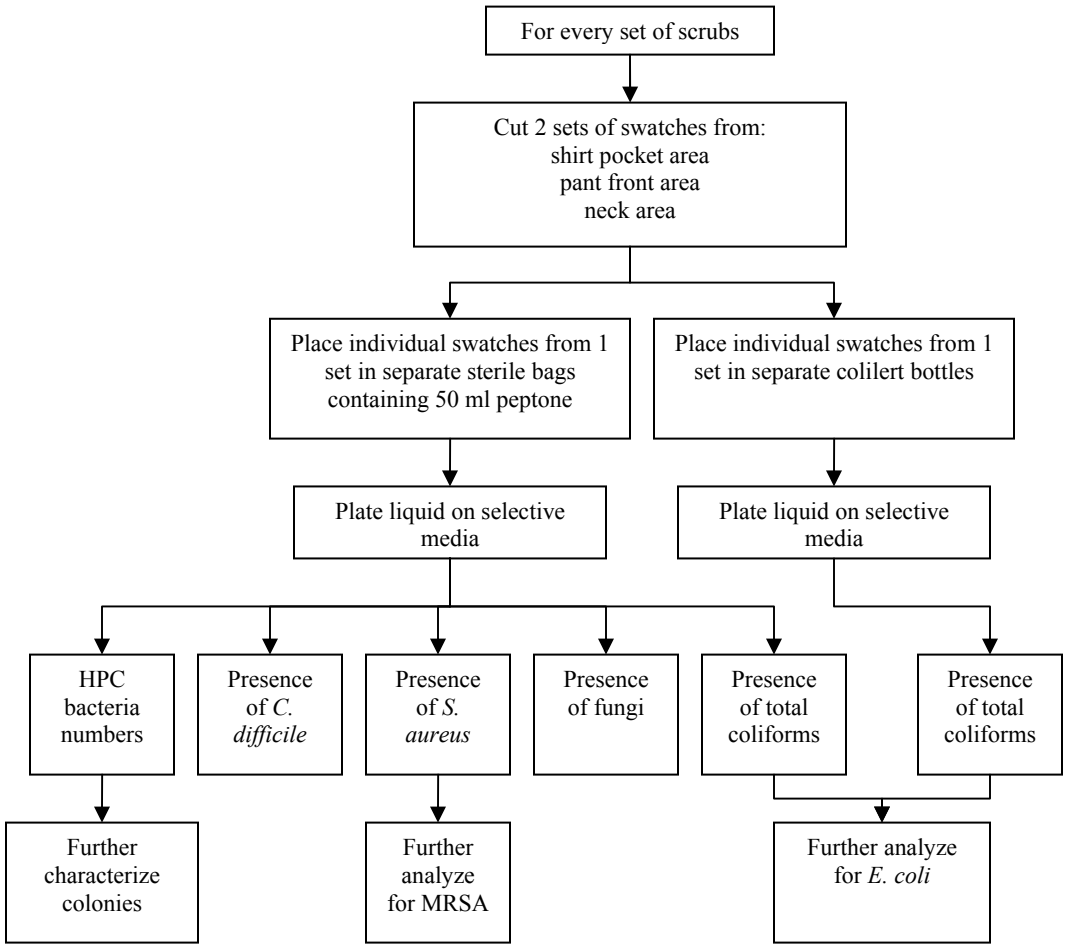

Table 2.1 Summary of Heterotropic Plate Counts for different classifications of scrubs

Type of scrub	Number of garments	Arith. mean CFU/sq cm	Geo. mean CFU/sq cm	Range CFU/sq cm	Standard Deviation
Unwashed hospital	18	180	85	5 - 473	178
Home-laundered	26	143	16	1 - 848	274
Hospital-laundered	20	4	2	1 - 27	8
New disposable	16	16	5	1 - 118	29
New cloth	10	35	5	1 - 145	62

Table 2.2 Percentage of swatches testing positive for selected microorganisms

Type of scrub	Fungi	Gram positive bacteria	*S. Aureus*	Coliforms	*E. coli*
Unwashed hospital	22 (4/18)	79 (23/29)	10 (3/29)	69 (20/29)	10 (3/29)
Home-laundered	93 (28/30)	12 (5/41)	0 (0/41)	44 (18/41)	0 (0/41)
Hospital-laundered	10 (2/21)	0 (0/36)	0 (0/36)	0 (0/36)	0 (0/36)
New disposable	36 (13/36)	4 (2/48)	0 (0/48)	4 (2/48)	0 (0/48)
New cloth	27 (4/15)	7 (1/15)	0 (0/15)	0 (0/15)	0 (0/15)

Table 2.3 Identification of Gram positive bacteria using Biolog GP2 Microplate system.

Type scrub tested	Organism name*	Illness; occurrence
unwashed hospital	*Staphylococcus aureus*	Common cause of nosocomial infections
unwashed hospital	*Micrococcus luteus* (ATCC 9341)	Environmental isolate
unwashed hospital	*Staphylococcus saprophyticus*	Bladder and urinary tract infections
unwashed hospital	*Aerococcus viridans*	Common cause of nosocomical infections; antiobiotic resistant infections; an enterococcus
unwashed hospital	*Staphylococcus caprae*	Bacterimia in neonates, meningitis
unwashed hospital	*Staphylococcus delphini*	Environmental isolate
unwashed hospital	*Staphyloccus kloosii*	Infections in colon cancer patients
unwashed hospital	*Staphylococcus pasteuri*	Environmental isolate
unwashed hospital	*Staphylococcus cohnii*	Water, air
unwashed hospital	*Enterococcus flavescens*	Antibiotic resistant nosocomial infections
unwashed hospital	*Staphylococcus epidermis*	Antibiotic resistant nosocomial infections
home-laundered	*Micrococcus luteus* (ATCC 9341)	Air and water contaminate
home-laundered	*Micrococcus lylae*	Found in water
home-laundered	*Lacoccux lactiss lactis*	Unknown
home-laundered	*Micrococcus luteus*	Found in water and air
home-laundered	*Staphylococcus lugdunensis*	Osteomyelitis infections; infections after cardiac surgery; 24% mortality
home-laundered	*Bronchothnix thermosphacta*	Food spoilage and seafood
home-laundered	*Microbacterium esteraromaticum*	Found in water and sewage; clinical isolates; no disease
home-laundered	*Brevibacterium liquefaciens*	Native soil bacterium
home-laundered	*Tsukamurella inchonesis*	Environmental isolate
home-laundered	*Curtobacterium luteum*	Native soil bacterium
home-laundered	*Sanguibacter inulinus*	Native soil bacterium
new cloth	*Tsukamurella inchonensis*	Lung infections
new cloth	*Microbacterium spp.* (CDC.A-4)	Infections in cancer and transplant patients

*The results were compared to the BIOLOG system database and identification was carried out using BIOLOG's MicroLog version 4.20. Similarity rates were all above 0.5.

Table 2.4 Identification of coliform bacteria using the API 20E system

Type scrub tested	Organism name*	Illness; occurrence
unwashed hospital	*Escherichia coli 1*	Indicator of fecal contamination. Virulent strains can cause diarrhea
unwashed hospital	*Enterobacter cloacae*	Antibiotic resistant nosocomial infections
unwashed hospital	*Klebsiella pneumoniae*	Antibiotic resistant nosocomial infections, particularly respiratory infections
unwashed hospital	*Pseudomonas aeruginosa*	Common cause of nosocomial infections
home-laundered	*Raoultella terrigena*	Environmental isolate, cause of waterborne diseases
home-laundered	*Enterobacter cloacae*	Antibiotic resistant nosocomial infections
home-laundered	*Klebsiella pneumoniae*	Common cause of nosocomial infections, particularly respiratory infections
home-laundered	*Klebsiella oxytoca*	Antibiotic resistant nosocomial infections, particularly respiratory infections
home-laundered	*Pantoea* spp 3	Environmental isolate, plant contaminant
home-laundered	*Pantoea* spp 4	Environmental isolate, plant contaminant
home-laundered	*Serratia rubidaea*	Nosocomial infections mostly in neonates and infants

*The results were compared to the apiweb system database and identification was carried out using Biomerieux apiweb version 4.1. Percent significant taxa identification was above 90%, indicating "good identification" except for Pantoea spp 4 at 63% (acceptable identification to the genus) and *Serratia rubidaea* at 87.7% (very good identification to the genus).

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APPENDIX C:

BACTERIA CONTAMINATION AT COMMUNITY-USE LAUNDRY FACILITIES

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ABSTRACT

In 2003 the Centers for Disease Control and Prevention issued guidelines for treatment of hospital laundry to reduce infectious disease transmission between patients. No such guidelines have been issued for public laundry facilities. The recent emergence of community-acquired MRSA has prompted the need to determine potential exposure routes of infectious disease transmission in the community. The objective of this study was to determine the role of washing machines and common use areas (i.e., table top surfaces) of public and apartment laundromats in disease transmission. Swab specimens (n=60) were collected and analyzed for the presence of HPC and total coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and MRSA. HPC bacteria were isolated from all sampled surfaces. Numbers varied widely at both public and apartment laundromats (8 – 1156 CFU/cm^2, geometric mean 70 CFU/cm^2 and 2 – 107,080 CFU/cm^2, geometric mean 180 CFU/cm^2, respectively). Student t-tests were performed to compare public and apartment laundromat results. HPC numbers on apartment folding tables were significantly greater ($p<0.01$) than seen on public folding tables. No other comparisons were statistically significant.

Thirty-five percent of surfaces (21/60) tested positive for coliform bacteria with *Klebsiella pneumoniae* (spp. pneumoniae) and *Enterobacter cloacae* identified most frequently (7/21 samples for each species). No *E. coli* were found. One interior washer lid tested positive for MRSA (1/60). No other *S. aureus* was detected.

Little is known about the levels of pathogens, including MRSA, in public laundry facilities and the risk of transfer between users via the washing machine or other contact

surfaces. Although our study found a low prevalence of MRSA, more information is needed to fully identify the possible exposure hazard and better inform quantitative microbial risk assessment models. Improved disinfection strategies for laundromat owners, when applied routinely, are expected to be beneficial for reducing exposure risks related to random pathogen occurrence.

INTRODUCTION

Studies show that pathogenic microorganisms survive on environmental surfaces for extended periods of time providing an opportunity for the transmission of infectious diseases. (Kramer *et al.* 2006, Manangan *et al.* 2001, Reynolds *et al.* 2005, Rusin *et al.* 1998, Scott *et al.* 1982, Weber *et al.* 2001). Sanborn (1963) described several outbreaks in which contaminated surfaces were involved in causing gastrointestinal, cutaneous and respiratory diseases. Arnold (1938) was the first to study microbiological contamination of commercial washing machine interiors. He showed that using wash water temperatures equal to or higher than 71.1˚C for 25 minutes killed nearly all bacteria forms except spores. He also identified seasonal variation in bacteria counts of laundry water ranging from 325,000 CFU/mL of wash water during the coldest months to over 1.8×10^7 CFU/mL in the warm weather months of the year. His results formed the basis of the Centers for Disease Control hospital laundry recommendations (CDC, 2003).

Other laundry studies followed, but most focused on bacteria levels in wash water or on fabrics at hospital laundries (Barrie 1994, Blaser *et al.* 1984, Christian *et al.* 1983, Neely *et al.* 2000, Smith *et al.* 1987, Walter *et al.* 1975). Transfer of microbes between contaminated and uncontaminated items of clothing and linens during wash cycles that were only partially removed by subsequent rinsing was frequently noted by researchers (Kundsin 1966, Wiksell *et al.* 1973).

Studies enumerating bacteria levels on public laundry surfaces have rarely been performed. Buford *et al.* (1977) found bacteria ranging from geometric mean counts of 5 to 73,960 CFU/cm^2 in the interior tub surfaces of automatic washers in self-service

laundry facilities, with a count of 490,000 CFU/cm^2 obtained on one occasion. The researchers hypothesized that even greater numbers would be found if samples had been collected from less accessible areas that receive little abrasion, or from wet surfaces. Legnani and Leoni (1997) also tested interior washing machine surfaces and wash water in commercial launderettes. They concluded that bacteria contamination was highest in the most heavily used machines and those where customers, trying to reduce costs, overloaded them or used lower temperature programs to wash various kinds of clothing together (underwear, pants, shirts, shoes, etc.). Higher wash water temperature or using an oxygen-based bleach with a low temperature cycle provided a significant bacteria reduction ($p<0.001$) both in terms of percentages of positive samples and mean concentrations. However, both bleach and hot water were necessary to ensure a nearly complete elimination of bacteria from fabric, wash water and washer interiors, including the less accessible parts of the washing machine, such as the drum. No published data were found for bacteria numbers on home washing machine interiors.

Only a few reported cases have identified laundering as the responsible cause of disease transmission, and those have been in a hospital setting. Two patients developed *Bacillus cereus* meningitis, following neurosurgery, due to a contaminated continuous batch tunnel washer harboring large numbers of the organism causing incomplete disinfection of the linen (Barrie *et al.* 1992, Wilcox *et al.* 1995)**.** Another outbreak attributed to contaminated laundry involved a maternity ward where several babies contracted *Streptococcus pyrogenes*. The organism was traced to a heavily contaminated

dryer used during laundering of the babies' clothes. The outbreak ended once the clothes were autoclaved (Fijan *et al.* 2005).

Larson *et al.* (2001) was the first to suggest a potential link between using self-service laundromats and disease transmission within the home. Three hundred and ninety eight inner-city households (96.4% Hispanic) were surveyed and the only hygienic practices in a home setting associated with transmission of infectious disease symptoms among household members were use of communal laundries or a failure to use bleach when washing laundry. Other home hygiene practices that were not identified as being significant ($p<0.05$) to the prevalence of infection included personal hygiene habits such as bathing, frequency of general cleaning, duration of kitchen sponge use, wearing gloves when cleaning the toilet, and use of an automatic dishwasher.

This study characterizes the relative hygiene of laundromat surfaces based on indicators of HPC bacteria levels, occurrence of fecal and total coliform bacteria, and presence of MRSA. This information can be used to augment existing data in the development of a hazard characterization as part of a quantitative risk assessment for community laundromat use, as well as support the need for disinfection strategies for laundromat owners.

MATERIALS AND METHODS

Initial sample collection focused on identification of surfaces with greatest bacteria density. In this first phase, a total of 20 samples were collected from multiple surfaces within two public laundromats and analyzed for HPC bacteria. Sampling locations were ranked by HPC count per square centimeter of surface sampled and subsequent testing primarily focused (40/60 samples) at top ten ranked surfaces (interior drums, rims, and/or lids of top-loading washing machines). Top-load washing machine exteriors and table tops were also sampled at each laundromat since patrons often come into contact with these surfaces. Front-load washing machines were excluded during phase two testing due to low HPC counts found in preliminary testing. During the second phase, samples from six surfaces were collected one time from five public laundromats and five laundromats located at different apartment complexes with a range of 138 - 385 units each. A total of 60 samples were collected. Sampling was conducted during times when high-usage was expected (mid-Saturday mornings). Total coliforms, *E. coli* and HPC bacteria were used to evaluate the overall hygiene of sample surfaces. Each sample was also tested for *Staphylococcus aureus*, with positives samples analyzed further to determine if isolates were methicillin-resistant.

During both phases, Spongesicle (Biotrace, Forest City, IA) swabs, containing ten mL of letheen broth, were used to sample surface areas ranging in size from 0.17 m^2 to 1.3 m^2. After sampling, Spongesicles were transported on ice back to the laboratory for processing within six hours. Liquid was eluted from the Spongesicles via hand agitation for 60 seconds, then serially diluted and assayed on R2A agar (Hardy Diagnostics, Santa

Maria, CA) using the spread plate technique. The plates were then incubated at 37°C for 5 days to determine HPC bacteria numbers. For phase two locations, samples were also assayed for *Staphylococcus* spp. on Trypticase Soy Agar (TSA) amended with 5% sheep blood (Hardy Diagnostics, Santa Maria, CA), 0.015 g/L of nalidixic acid (Sigma, St. Louis, MO) and 0.01 g/L of colistin (Sigma, St. Louis, MO) and incubated at 37°C for forty-eight hours. Creamy-white and yellow colored colonies demonstrating β-hemolysis were further subjected to a series of biochemical tests to confirm *S. aureus*. Biochemical tests included slide coagulase (Becton Dickinson, Sparks, MD), tube coagulase, catalase, and polymixin-B (Becton Dickinson, Sparks, MD) sensitivity. Confirmed *S. aureus* isolates were then plated on two different types of MRSA media, the selective and differential CHROMagar (Becton Dickinson, Sparks, MD) containing cefoxitin and a the selective MRSA screening plates (Hardy Diagnostics, Santa Maria, CA) containing oxacillin. If full growth occurred on the MRSA media, isolates were confirmed as MRSA. If no growth or only residual growth occurred, the strains were considered methicillin susceptible *S. aureus*. Colilert (IDDEX, Westbrook, MN) was used to determine the presence and absence of total and *Escherichia coli*. Samples testing positive for coliforms were plated on MacConkey agar (MAC) (Difco, Sparks, MD) agar and incubated for 24 hours at 37ºC. Lactose fermenting colonies were then reassayed on TSA so further coliform identification could be done using BioMerieux API-20E test strips (BioMerieux, St. Louis, MO). A diagram summarizing experimental procedures is presented in Figure 3.1.

Geometric mean and standard deviation values were performed using Microsoft Office Excel software (2003). Geometric means were calculated instead of arithmetic means due to the five $\log_{10}$ range of surface contamination values found. Student t-tests were conducted to determine if contamination levels on specific surfaces were significantly higher than others.

RESULTS

Phase one HPC results are shown in Table 3.1. Sample surfaces were ranked by total colony forming units per square centimeter, with the greatest HPC number designated as ranking first (overall range of 32 – 2470 CFU/cm^2). HPC bacteria were isolated from all surfaces. Eight of the top ten ranked HPC count sampling locations were interior drums, rims, and/or lids of top-loading washing machines. Interiors of top-load washers generally had the highest numbers (range 155 – 1696 CFU/cm^2, geometric mean 657 CFU/cm^2) while table tops and interiors of front-load large capacity washers had the lowest numbers (32 – 374 CFU/cm^2, geometric mean 122 CFU/cm^2). The overall geometric mean HPC value for phase one samples collected was 361 CFU/cm^2. The two front-loading washing machine interiors tested had the lowest HPC counts detected (32 – 65 CFU/cm^2, geometric mean 46 CFU/cm^2), therefore front-loading washing machines were excluded during phase two testing.

Table 3.2 shows HPC summary data for phase two sampling surfaces. HPC bacteria were again isolated from 100% of tested surfaces and numbers varied widely at both public and apartment laundromats (8 – 1156 CFU/cm^2, geometric mean 70 CFU/cm^2 versus 2 – 107,080 CFU/cm^2, geometric mean 180 CFU/cm^2, respectively). Student t-tests were performed to compare public and apartment laundromat results. HPC numbers on apartment folding tables were significantly greater ($p<0.01$) than seen on public folding tables. No other comparisons were statistically significant.

Thirty-five percent of surfaces tested (21/60) were positive for total coliform bacteria; however no *E. coli* were detected. Identification of the species from the API

tests are shown in Table 3.3 with one-third of the samples (7/21) identified as *Klebsiella pneumoniae* (spp. pneumoniae) and another third of the samples (7/21) identified as *Enterobacter cloacae*. Coliforms were found more often in apartment laundromats than in public laundromats (13 versus 7 surfaces).

One surface out of the sixty tested, an interior top-load washer lid at a public laundromat, was positive for MRSA.

DISCUSSION

Although HPC and coliform bacteria are typically not human pathogens, they were surveyed as potential markers of general hygiene and relative contamination of laundromat surfaces. Bacteria indicators used to evaluate surface hygiene were chosen based on similar studies performed on a variety of public surfaces (Buford *et al.* 1977, Reynolds *et al.* 2005, Gerba 2007).

The geometric mean of phase two HPC bacteria was 116 CFU/cm^2 (range 2 – 107,080 CFU/cm^2). In a similar study, Buford *et al.* (1977) found comparable HPC numbers (geometric mean 359 CFU/cm^2, range 5 – 490,000 CFU/cm^2) on 160 washing machine interior surfaces. These data show that surfaces of laundromats can be contaminated with substantial numbers of bacteria and the potential exists for transfer of bacteria from a past user to the next laundromat patron.

The only environmental surface contamination guidelines found for HPC numbers were published in 1967 by Pryor *et al.* for hospitals, and based on the use of direct agar contact plates rather than swabs. The guideline states that floors with microbial contamination greater than 50 counts per plate relate to poor cleanliness (Nelson *et al.* 2006). How HPC counts from contact plates compare with those obtained from swab samples is not known. Also unknown is how HPC concentrations relate to the potential for various pathogen survival on surfaces.

Of the 35 percent of surfaces testing positive for coliform bacteria (21/60), one third of those were identified as *Enterobacter cloacae* and another third of those were identified as *Klebsiella pneumoniae*. *E. cloacae* and *K. pneumoniae* are common

nosocomial pathogens, but they have also been associated with community-acquired infections that can cause respiratory, urinary tract, wound, and skin and soft-tissue infections (Fraser *et al.* 2008), particularly in persons with weakened immune systems (Brochert 1999). The presence of these Enterobacteriaceae species is an indicator of poor surface hygiene and represents the potential likelihood that if a pathogen is present and transmission occurs, an infection may result.

Within the laundromat industry, store owners choose between providing front-load, top-load or a combination of both models of washing machines. Many customers prefer the top-loading machine because they are more familiar and the lid can be opened to add additional laundry items after the machine has started (modernlaundromat.com 2008). All ten laundromats sampled had the same top-load model from a single manufacturer (Neptune, Maytag, Benton Harbor MI). Front-load washers offer several advantages to self-service customers as well as the laundromat owner. They come in a variety of capacities allowing the customer to choose the washing equivalent of 2, 3, or more loads at once resulting in the capacity to wash large items such as comforters and blankets. They also are significantly more energy efficient and use less water (Bluejay 2007). Forty percent of businesses sampled had at least one front-load washer available (2 of 5 apartment laundromats and 2 of 5 public laundromats).

During phase one of the study, front-load washer interiors were found to harbor significantly less ($p<0.05$) HPC bacteria than top-load washing machine interiors. Front-load washing machines use entirely different mechanisms for washing clothes than top-

load machines and have longer rinse cycles which may account for the dissimilarity seen in HPC bacteria numbers.

Surface bacteria numbers may vary due to frequency of facility use. Generally, Friday, Saturday, and Sunday are peak use periods for self-service laundromats (Buford *et al.* 1977). During this study, all samples were collected on Saturday mornings between 9 a.m. and noon and every facility tested had at least one patron present during sampling. Other factors that may influence bacteria numbers reported include health status of patrons, type and amount of soiling, water temperature available and chosen, laundry additives used, and time since equipment was last used. These unknown risk variables require that routine hygiene maintenance be performed to achieve a reduction in exposure potential for laundromat staff and patrons.

Because of the many factors affecting bacteria numbers and types in laundromats, the public health risk associated with presence of surface bacteria is not known. Survival rates of bacteria are highly variable and may even be strain specific (Noskin *et al.* 1995). Neely *et al.* (2000) tested 22 different species of staphylococci and enterococci and found that they survived for days to months after drying on inoculated hospital plastics and fabrics. Larger inocula tended to have longer survival times. Although collection of viruses was not done as part of this study, viruses also can survive on hard surfaces, remaining infectious for hours to days (Ansari *et al.* 1988, Cheesbrough *et al.* 2000, Dowell *et al.* 2004). Non-enveloped viruses such as norovirus and astrovirus are fairly stable in the environment (Barker *et al.* 2001, Abad *et al.* 2001), surviving on surfaces longer than enveloped viruses such as influenza A (Bean *et al.* 1982). Dowell *et al.*

(2004) concluded that severe acute respiratory syndrome (SARS) corona virus can contaminate environmental surfaces and inanimate objects (fomites) should be considered a possible mode for the transmission of SARS.

The drying cycle can be effective in removing remaining bacteria and other pathogens from laundry (Blaser et al. 1984). However, microbes remaining on interior surfaces of washing machines can be transferred to clothing, introducing an opportunity for exposure to the laundry handler who transfers the load to the dryer. Pathogenic bacteria and viruses are easily transferred from surfaces to hands and visa versa (Bean *et al.* 1982, Rusin *et al.* 2002, Manning *et al.* 2001). Microorganisms are transferred from nonporous surfaces more effectively than from porous surfaces (Scott *et al.* 1990a, Sattar *et al.* 2000, Rusin *et al.* 2002). Rusin found that up to 65% of viruses were transferred from fomites to uncontaminated hands and up to 34% were transferred to the mouth. Rusin also determined that rates of transfer of Gram positive bacteria from fomites to uncontaminated hands and to the mouth were nearly identical (42% versus 41%, respectively). In an earlier study by Scott *et al.* (1990a), *S. aureus* and *E. coli* transferred from a laminate surface to fingertips at a similar rate. Rusin concluded that commonly handled objects can serve as reservoirs of bacteria and viruses and easily transfer to the hands through direct contact, which in turn can be easily transferred to the lip. All of these factors also point to the need for regular laundromat disinfection.

Epidemiological studies have identified fomites as a potential vehicle for disease transmission (Boone *et al.* 2007). The risks associated with exposure to microorganisms in community laundromats are difficult to quantify and will depend on many factors

including the frequency of exposure, type and virulence of the microorganisms present at the time, the health status of the laundry handler, and the probability of pathogen transfer from surface to potential infection site of the patron. However, the risk is apparent due to the multiple users with unknown infection rates.

The coliform bacteria species identified during this limited study are naturally-occurring and do not generally represent a significant risk to healthy individuals (Washtenaw County Environmental Health Fact Sheet 2004). The MRSA isolate found will also primarily be a risk factor for a limited population including those who are immunocompromised or may have skin trauma. However, even though all laundromats tested were visibly well maintained, hygiene practices were unknown and two out of the 60 surfaces tested had HPC bacteria numbers that exceeded 100,000 CFU/cm^2. In a healthy adult, the infective dose of *Salmonella* and *E. coli*, for example, may be as high as $10^6 – 10^7$ CFU, or as low as $10^2 – 10^3$ CFU depending on the strain involved (Bloomfield *et al.* 1997).

Communal laundry users have no control over the environmental conditions, including attainable water temperatures found in self-service laundromats, so it is important that they use bleach in their wash loads whenever possible and dry their clothes completely to minimize the potential for transmission of infection, particularly when using top-load washing machines. It is recommended that laundromat owners perform daily cleaning of laundromat surfaces, including washing machine interiors with a disinfectant detergent to reduce the number of potentially pathogenic microorganisms present and thereby the risk for transmission of infection.

This research provided a snapshot of the relative hygiene in public and apartment self-service laundry facilities. Additional studies expanding the sample size and targeting the identification and distribution of other pathogens in laundromats are needed. Such data could be used in quantitative microbial risk assessment modeling to develop disinfection strategies for laundromat operators in order to minimize exposure risks to consumers.

Figure 3.1. Flowchart of laundromat experiments

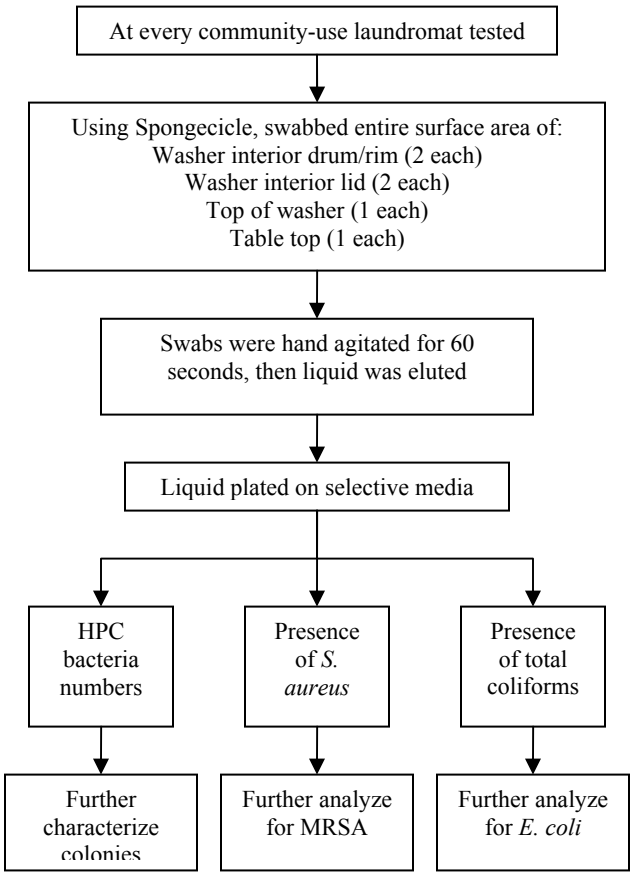

Table 3.1 Phase one survey results

Rank	Sample surface (site)	Public Laundromat Location	Square cm sampled at site	CFU recovered/ sq cm
1	top-load exterior washer top	1	4515	2470
2	top-load interior drum & rim only	2	4644	1696
3	top-load interior lid only	1	1451	1522
4	top-load interior - drum, rim & lid	2	6450	1258
5	top-load interior - drum, rim & lid	1	6450	1103
6	top-load interior - drum, rim & lid	2	6450	748
7	top-load interior - drum, rim & lid	2	6450	581
8	top-load interior lid only	2	1451	490
9	top-load exterior washer top & front	1	9675	458
10	top-load interior - drum, rim & lid	1	6450	439
11	"dirty" laundry table	2	41925	374
12	top-load interior - drum, rim & lid	1	6450	290
13	folding table at back of facility	1	13932	232
14	folding table at front of facility	1	13932	187
15	top-load exterior top, front & buttons	2	9675	168
16	top-load interior drum & rim only	1	4644	155
17	top-load exterior top & buttons	2	4515	148
18	"clean" laundry table	2	29025	97
19	front-load interior – drum & window	2	7740	65
20	front-load interior – drum & window	1	9030	32

Table 3.2 Public and apartment laundromat HPC counts summary

Location	Sampled surface	Number of samples	Geomean (CFU recovered/ sq cm)	Standard deviation	Range (CFU recovered/sq cm)
Public	drum/rim	10	53	351	8 - 1156
Laundromats	interior lid	10	84	49	28 - 176
	top washer	5	119	82	39 - 225
	table top	5	51	21	36 - 159
Apartment	drum/rim	10	26	38	2 - 106
Laundromats	interior lid	10	1352	43080	28 - 107,080
	top washer	5	138	264	38 - 575
	table top	5	201	144	50 - 401

Table 3.3 Public and apartment laundromat coliform identification

Location	Coliform identification
apartment laundromat 1 interior lid	*Klebsiella pneumoniae spp pneumoniae*
apartment laundromat 1 top of washer	*Serratia plymuthica*
apartment laundromat 1 table top	*Enterobacter cloacae*
apartment laundromat 3 top of washer	*Enterobacter cloacae*
apartment laundromat 4 interior lid	*Klebsiella pneumoniae spp pneumoniae*
apartment laundromat 4 interior lid	*Klebsiella pneumoniae spp ozaenae*
apartment laundromat 4 top of washer	*Klebsiella pneumoniae spp pneumoniae*
apartment laundromat 4 table top	*Enterobacter aerogenes*
apartment laundromat 5 drum/rim	*Klebsiella pneumoniae spp pneumoniae*
apartment laundromat 5 drum/rim	*Citrobacter freundii*
apartment laundromat 5 interior lid	*Klebsiella pneumoniae spp pneumoniae*
apartment laundromat 5 interior lid	*Enterobacter cloacae*
apartment laundromat 5 table top	*Klebsiella pneumoniae spp pneumoniae*
public laundromat 1 drum/rim	*Enterobacter cloacae*
public laundromat 1 table top	*Serratia ficaria*
public laundromat 1 table top	*Enterobacter cloacae*
public laundromat 2 interior lid	*Enterobacter cloacae*
public laundromat 2 interior lid	*Enterobacter cloacae*
public laundromat 3 table top	*Escherichia vulneris*
public laundromat 4 top of washer	*Enterobacter aerogenes*
public laundromat 5 table top	*Klebsiella pneumoniae spp pneumoniae*

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APPENDIX D:

QUANTITATIVE RISK ASSESSMENT FOR HOME LAUNDERING FABRICS CONTAMINATED WITH *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

MRSA infections result in 19,000 deaths per year. Community acquired infections are dramatically increasing with 2% being asymptomatic carriers of MRSA. Little is known about the primary routes of MRSA in the home or the role of asymptomatic carriers in both infection as well as re-infection. Increased frequency of homecare for the elderly, immunocompromised, and/or known and unknown carriers of MRSA as well as a poorer hygienic quality of laundering by lower wash temperatures and fewer rinse cycles raise concerns regarding human health risks of contaminated laundry. A quantitative risk assessment was calculated using published dose response data to determine the annual risk of infection from handling laundry contaminated with *S. aureus*. The worst case risk estimate from handling unwashed laundry was 0.59 infections per person per year. The risk of developing a *S. aureus* infection when handling laundry washed with detergent or detergent and bleach was 5.9×10^{-6} infections per person per year. With the high colonization rate of *S. aureus* and increasing MRSA rate, it remains important to consider the potential for infection from handling unwashed laundry that may be highly contaminated, particularly when family members are ill or the person who comes in contact with the contaminated laundry is at increased risk for infection or has skin abrasions. *S. aureus* colonization in skin affected by atopic dermatitis or from an exuding wound can result in transfer of up to 10^7 CFU/cm^2 to fabric. Contaminated hands are known to introduce *S. aureus* to the nose or an abraded skin site, setting up conditions for a possible infection at those locations. Washing with detergent reduces *S. aureus* and MRSA by 5 – 6 $\log_{10}$, if complete drying and/or bleach are also employed, a 6 – 7 $\log_{10}$

reduction is achieved and few *S. aureus* remain. Handling of contaminated laundry in the home may be an unrecognized risk that can be greatly reduced by using simple risk reduction strategies such as 1) hold laundry away from your body during transport, 2) launder contaminated items separately, 3) wash hands after handling laundry, and 4) launder soiled items within 24 hours to minimize microbial regrowth.

INTRODUCTION

Pathogens are brought into homes via people, food, water, insects, pets, and the air (Bloomfield *et al*. 2003) and can contaminate a variety of surfaces, including fabrics. Infection risks within the home may be less than in health care settings such as hospitals and nursing homes, but are still present. To date, few reports of nosocomial infections transmitted via a fabric route (Standaert *et al*. 1994) are documented, however, increased frequency of homecare for the elderly, immunocompromised, and/or known and unknown carriers of methicillin resistant *Staphylococcus aureus* (MRSA) raise concerns regarding human health risks of contaminated laundry. New fabric types, increasing numbers of healthcare workers washing scrubs at home, and changing laundering practices (i.e. lower wash temperatures, energy and water efficient appliances, and reduction in bleach use) have yet to be fully evaluated and may impact the effectiveness of current home-laundering habits (Scott 1999).

Quantitative microbial risk assessment is a process of mathematical modeling used to evaluate the likelihood of adverse human health effects occurring after exposure to a pathogenic microorganism. In a quantitative risk assessment, the risk is expressed as an estimation of the chance of infection (or other outcome) after exposure to the pathogen, and it incorporates the cumulative probabilities as well as uncertainties of specific events over a designated period of time. This type of analysis is used to determine the number of cases of disease that can be prevented by implementing certain control strategies.

Information needed to estimate the likelihood of infection transmission incurred from handling unwashed laundry includes: 1) concentration of pathogens in soiled laundry; 2) the effectiveness of laundering practices used, determined by wash water temperature, additives, type of washing machine, and drying technique; 3) potential of cross-contamination of other laundry items in the same load or to the inside of the washing machine for transference to subsequent loads (communal laundries have been found to be particularly problematic, Larson *et al*. 2001); 4) potential for contamination of nose or abraded skin surface; and 5) health status of laundry handler.

The purpose of this risk assessment was to estimate the number of cases of *S. aureus* infection that can be expected and to improve the quality of public health decisions by informing risk managers of practices to minimize disease transmission during handling laundry.

MATERIALS AND METHODS

Quantifying the probability that *S. aureus* infection will occur from handling soiled laundry cannot be done using typical epidemiological studies because of the low rate expected (Gerba 2001, Gibson *et al*. 1999). Risks less than 1 in 10,000 require a very large study population and epidemiological studies are not able to evaluate risks over time. Also, risks associated with unwashed laundry are difficult to document by using standard disease surveillance and epidemiologic tools since most cases of disease in the home would not be immediately reported and the route of exposure would not be clear. Alternatively, a four step risk assessment involving hazard identification, exposure assessment, dose-response, and risk characterization can be employed to increase sensitivity of the endpoint analysis.

Hazard identification

Hazard identification includes gathering information about which pathogens are present in the laundry and whether or not they are harmful. For this assessment, *S. aureus* was chosen as the specific pathogen to be evaluated since it is an important cause of infection in the hospital and in the community and because of the existence of available data. *S. aureus* is a common bacterium normally found on the skin and in the nose of some healthy people, with the nares being the most common carriage site. *S. aureus* carrier status is known to be dynamic (Kluytmans *et al.* 1997). At any one time approximately 30 percent of the population is colonized with *S. aureus*. Twenty percent of that group is always colonized and the other 10 percent are considered transient carriers (Wertheim *et al*. 2005). Carriers of *S. aureus* typically shed organisms from the skin surface. The

extent of shedding varies widely by individual and over time, with extensive shedders and persons being treated with antibiotics having the highest rates. Some carriers, however do not appear to shed at all (Sheretz *et al*. 2001). Singh *et al.* (1971) determined that recovery success on skin is variable relative to culture methods used with *S. aureus* recovered 10 - 20% of the time using a single swab but 82% of the time if the same site was tested for eight consecutive weeks. Native microflora is best adapted to normal skin and their presence is a deterrent to the introduction of bacteria from foreign sources (Singh *et al*. 1971) such as laundry. For *S. aureus* to colonize or cause a skin infection it must enter via an opening in the skin such as a cut, abrasion, surgical wound or other abnormal skin condition like psoriasis or eczema. On eczeous skin pathogens can be just as successful at forming stable communities as native microflora. Catheters and shunts, used during invasive medical procedures, are readily colonized by *S. aureus* (Bamberger *et al*. 2005). Once *S. aureus* gets into the body it may produce no symptoms, cause self-limiting infections such as boils or pimples, or occasionally produce serious infections such as pneumonia or blood infections. Normal host defenses are usually able to limit the *S. aureus* infection location to its portal of entry. Asymptomatic nasal colonization with *S. aureus* appears to be an important factor in the development of most infections caused by this organism (Laupland *et al.* 2003).

Drug resistant strains of *S. aureus* have developed a resistance to commonly prescribed beta-lactam antibiotics, such as penicillin, amoxicillin, ampicillin, and methicillin. Whereas colonization rates of *S. aureus* have decreased from 32.4% in 2001 – 2002 to 28.6% in 2003 – 2004, colonization rates with MRSA increased from 0.8% to

1.5% during these same time periods (Gorwitz, *et al.* 2008). The CDC estimated that in 2005, more than 94,000 people developed a serious MRSA infection and approximately 19,000 people died from MRSA in the United States (Klevens *et al*. 2007).

Exposure assessment

Exposure assessment data has a greater impact on a risk assessment than dose-response data because it can vary by many orders of magnitude (Gerba 2001, Gibson *et al.* 1999). *S. aureus* colonization in skin affected by atopic dermatitis can reach 10^7 CFU/cm^2 (Lim *et al.* 2007) which easily transfers to fabric. Studies have shown that bacterial contamination of laundry varies considerably (Bloomfield *et al*. 2002). Reports have dealt primarily with hospital-based laundry (Walter *et al*. 1975, Battles *et al*. 1981, Christian *et al*. 1983, Blaser *et al*. 1984, Tompkins *et al*. 1988), and no data are available regarding *S. aureus* specific concentrations in home laundry. Items in the home, including laundry, can become contaminated especially when a family member is ill (Bloomfield *et al*. 2003). Saltzman *et al*. (1967) determined total bacteria levels to be approximately 10^5 CFU/cm^2 in used articles of clothing. Similar levels (10^5 – 10^6 CFU/cm^2) were found in hospital laundry by Blaser *et al*. (1984). Christian *et al*. (1983) analyzed hospital laundry specifically for *S. aureus* and reported concentrations of <0.1 to 4 x 10^3 CFU/cm^2. For this risk assessment, a worst-case starting concentration of 10^6 *S. aureus*/cm^2 based on previous studies and the author's unpublished results was chosen. Actual *S. aureus* concentrations are expected to be lower in most instances.

The greatest potential for exposure most likely occurs during initial laundry handing, and later when moving wet items from the washer to the dryer or hanging them

out to dry. Hands and/or broken skin locations may become contaminated during these tasks. Once on the hands, *S. aureus* may be transferred to the nose or an abraded skin site, setting up conditions for a possible infection at those locations. While no studies have been conducted to specifically assess the transfer rate of *S. aureus* from hand to nose or visa versa, Tammelin *et al.* (2003) showed that self-inoculation of the nose accounted for half (9/18) of the individuals who had *S. aureus* on their hands. The enhanced risk of having *S. aureus* on the skin (Goldblum *et al.* 1982) and in the blood (von Eiff *et al.* 2001) as a nasal carrier has been confirmed. In one hospital-based study, *S. aureus* isolates from a patient's anterior nares were identical their blood isolates 82.2% of the time (180/219) and to areas other than their nares 94.3% of the time. Ears and sinuses have also been reported as *S. aureus* reservoirs (Bertin, *et al.* 2006).

The amount of *S. aureus* transferred from fabric to hands also varies widely. Studies that use culture techniques have reported that anywhere from 10% to 100% of bacteria present on contaminated wet fabric was transferred to hands of study volunteers (Gibson *et al*. 1999). Two separate studies tested *Staphylococcus* spp. transference from fabric to hands. Macintosh *et al*. (1984) evaluated the transfer rate of several different microorganisms, including *S. saprophyticus*. An average of 1.67% *S. aureus* organisms were transferred in this study. Sattar *et al*. (2001) used an identical contact time, smaller surface area (3 cm^2 versus 300 cm^2), and varied the parameters of moisture and friction levels. Sattar reported 0.1% to 2.5% of *S. aureus* was transferred between laundry inoculated with 10^5 CFU/cm^2 and hands. No data was found that specifically documented transfer of *S. aureus* from fabric to broken/abraded skin. In this current

study, a 2.5% transfer rate was used to calculate the amount of *S. aureus* transferred from laundry to hands and broken/abraded skin, again to represent a worst case scenario.

Previous studies in our laboratory showed *S. aureus* reduction in laundry is achieved by a combination of removal through agitation, dilution, drainage and killing either by detergent use or heat (unpublished data). Inoculated laundry washed with detergent, but no bleach, provided a 5 – 6 $\log_{10}$ reduction. Drying and/or adding bleach to the laundry process reduced *S. aureus* counts by 6 – 7 $\log_{10}$. However, the validity of microbial reduction when inoculated swatches are used to contaminate laundry loads may be overestimated because in a normal dirty laundry load, microorganisms occur within soil aggregates making them more difficult to remove (Terpstra 1998). Table 4.2 shows the expected concentrations of *S. aureus* based on information from previous laundry experiments. A likely framework for the transmission of *S. aureus* via contaminated home laundry is shown in Figure 4.2.

Dose-response assessment

The dose-response assessment, or hazard characterization step, estimates the frequency of infection or other adverse outcome based on different exposure levels of *S. aureus* or MRSA. The size of *S. aureus* inoculum required to initiate an infection varies depending on a number factors including immune status, exposure route, and size of initial inoculum. To demonstrate this point, experimental inductions performed by Elek *et al*. (1957) required 6.6 – 6.9 $\log_{10}$ *Staphylococcus pyrogenes* organisms to cause infection when injected intradermally to human volunteers. This number was reduced by 2.7 – 4 $\log_{10}$ if the organism was introduced via silk sutures that had been immersed in a *S.*

aureus culture (Elek 1956). Foster *et al*. (1960) was able to infect an experimentally created human forearm lesion with as few as 15 staphylococci bacteria if, after induction, the lesion was sealed with a coverslip and adhesive tape. Thus, an infection could potentially be caused by inoculum containing anywhere from fifteen to several million colony forming units (Dancer 2008).

Mathematical models for the probability of infection for *S. aureus* have been developed for human food and water ingestion studies, but not specifically for handling *S. aureus* in contaminated laundry. Singh *et al*. (1971) inoculated the forearms of volunteers with known doses of *S. aureus* (number of organisms per centimeter of skin) then measured the incidence of infection (Table 4.1) and bacterial population kinetics over a six day period. Singh determined that the severity of infection was dose dependent and subsequently developed a growth model of the bacteria on skin based on the inoculum size. Because there was a great deal of initial bacteria die off attributed to transplantation shock, a secondary study was done where several sites on the forearms of volunteers were inoculated with 10^6 *S. aureus* organisms to determine bacteria die off rates during the first 48 hours. After one hour, levels were reduced by 76 to 86 percent and after six hours, the *S. aureus* numbers decreased by 79 to 97 percent. Die off peaked at 24 hours with a reduction of 76 to 99 percent but levels rebounded and 24 hours later levels were back to between 5 and 75 percent of the initial value. During these studies, test sites were occluded after dosing to promote bacteria growth. Actual growth of inoculated *S. aureus* will most likely be lower since actual exposure site will not normally be purposefully closed off.

Rose and Haas (1999) developed a risk assessment framework evaluating the impact of washing with an antibacterial soap on probability of acquiring a *S. aureus* skin infection. They incorporated the effect of contact time using an "area under the curve" strategy to revise Singh's original dose response data from "number of organisms per square centimeter of skin" to "days of exposure multiplied by the number of organisms per square centimeter of skin" (Table 4.1). These integrated dose values were used to construct a dose response curve which was fitted to a simple and commonly used exponential dose response model expressed as

$$\pi = 1 - \exp(-d/k)$$

where π is the risk of infection, d is the integrated dose (days x number of *S. aureus* organisms/cm^2), and k is a measure of the potency of the *S. aureus* evaluated from the data set using a likelihood fitting (Hass 1983). Rose and Haas determined k to be **"**1.31 x 10^7 days · number of *S. aureus* organisms/cm^2**".** The resulting model plotted the risk of infection based on the area under the curve and provided a predictive relationship for microbial growth on the skin. It was also found to describe Singh's data well (Figure 4.1).

Risk characterization

The annual risk of acquiring a *S. aureus* infection for persons handling contaminated laundry was determined using the dose-response equation developed by Rose *et al*. (1999), and is found in Table 4.3. Risks were calculated for various laundry treatments under a worst case scenario using an initial concentration in unwashed laundry of 10^6 CFU/cm^2. This value, representing a single exposure or point estimate for the risk

of acquiring a *S. aureus* infection, was 1.9×10^{-3} infections per person per year for individuals handling unwashed laundry. Once the laundry was washed with detergent or detergent and bleach, the risk became 1.9×10^{-8} and the addition of drying further reduced the risk. These point risk estimates do not take into account multiple exposures. An average family washes six loads of laundry per week (wholesomebabyfood.com 2009), equating to 312 loads per year. Affected persons can shed MRSA from weeks to years, with averages in various studies ranging from 7.4 months to greater than 3 years (Thompson *et al.* 1982, Scanvic *et al.* 2001, Marschall *et al.* 2006). Using an average shedding period of one year, this increases the worst case risk estimate for probability of acquiring a *S. aureus* infection from handling unwashed, contaminated laundry to 0.59 infections per exposed person per year (Table 4.3).

Risk estimates calculated for the general population, appear high. Few situations will produce *S. aureus* levels as high as 10^6 CFU/cm^2. Also, the majority of *S. aureus* illnesses are mild or asymptomatic, particularly in normally healthy individuals. However, segments of the population identified as having a higher susceptibility to *S. aureus* or other type of infection including elderly, diabetics, immunosuppressed, injecting drug users, or recently hospitalized (Kluytmans *et al.* 1997, CDC 2005), will be at higher risk for severe illness.

DISCUSSION

Minimization of risks associated with *S. aureus* and MRSA transmission in the home, community, and hospital are important in order to avoid both infection as well as re-infection. In general, a lower wash temperature and rinsing efficiency are likely to result in a reduced level of soil removal, poorer hygienic quality of the washing process and a less efficient removal of pathogens (Terpstra 1998). Our laundry studies showed that a 5 – 6 $\log_{10}$ reduction of *S. aureus* and MRSA occurs after washing with detergent alone. If complete drying and/or bleach are also employed, a 6 – 7 $\log_{10}$ reduction is achieved and few *S. aureus* remain. The method employed to recover *S. aureus* in our studies had an 8.2 percent efficiency rate, so actual *S. aureus* levels remaining on washed laundry may be higher. Microbial counts in laundry need to be low enough so that they will not cause infection. Christian *et al*. (1983) recommended that a washing technique should produce a minimum of 6 – 7 $\log_{10}$ bacteria kill to be effective in reducing the risk of hospital infection transmission. Walter *et al.* (1975) determined that 20 CFU/100 cm^2, the lower detectable limit of organisms in his study, appeared to be equivalent to complete pathogen removal. He found that this level could be achieved by using the proper combination of wash time, water temperature, and laundry additives. The probabilities of infection developed using this risk assessment support those recommendations for household contacts. The worst case annual risk of developing a *S. aureus* infection when handling washed laundry was estimated to be 0.059 infections per 10,000, below USEPA's (1991) 1 per 10,000 acceptable risk for acquiring a waterborne disease but approximately six times greater than the frequently cited 1 per million "acceptable risk",

most notably used for lifetime risk in the general public of developing cancer (Kelly 1991, Haas *et al.* 1999).

The 0.59 infections per person per year estimated risk of developing a *S. aureus* infection when handling unwashed laundry with an initial *S. aureus* level of 10^6 CFU/cm^2 fabric is lower than what Singh *et al.* (1971) found during his *S. aureus* exposure study. Fourteen out of 20 (70%) healthy volunteers inoculated with 10^5 CFU/cm on unbroken skin that was subsequently occluded for six days, developed moderate to severe lesions. Removal of the occluded dressing resulted in immediate resolution whether or not antibiotics were administered. When Singh placed inocula on skin striped of its outer horny layer barrier, then occluded the area, subjects developed fever, malaise, pain and swelling at the inoculation sites within hours. Singh concluded that on broken skin surfaces, *S. aureus* bacteria can occupy a relative large area which is not immediately accessible to normal tissue defenses, resulting in an infection. There are several dissimilarities in the scenarios which would account for the differences seen in risk estimates. Singh used only healthy volunteers, but then removed all normal human skin flora, directly inoculated, and then occluded the inoculation sites for several days.

Foster and Hall (1960) also performed dose response tests where 10^6 *S. aureus* organisms were introduced into covered as well as uncovered, artificially-induced lesions. The researchers concluded that keeping the lesion moist was important for growth. Similarly, dose response experiments performed by Marples (1973) showed that *S. aureus* can cause disease but conditions necessary for experimental infections are severe: a large inoculum or protection from drying if a lower dose is employed, mild skin

trauma, and use of a foreign body to promote growth at a credible dose. He concluded that although the degree of skin trauma is critical, it is not absolutely necessary for the induction of infection provided native microflora has been reduced and the traumatized area kept hydrated.

No other studies were found for risk estimates of handling laundry contaminated with *S. aureus* or other microorganisms commensally on the skin or nose. Studies to date have focused on risk estimates for handling laundry contaminated with enteric microorganisms such as *E. coli*, *Shigella*, and rotavirus (Gerba 2001). Gibson *et al.* (1999) calculated risk estimates for laundry contaminated with *Shigella* as high as 10 per million population, which became much lower when lower excretion rates of bacteria in feces were figured in the model. Approximately a 90% and 99% reduction in the probability of disease through laundering and use of a sanitizing detergent, respectively, were suggested by the models developed in Gibson's study.

However, a more recent study by Gerba (2001) estimated a 10,000 times greater risk for rotavirus infection from laundering contaminated home laundry. The initial virus concentration in this study was set higher at 10^{10} and a washing efficiency of only 99% was used for the assessment calculations. An overall risk for infection of 1:10 was hypothesized from these assumptions.

Because of the absence of data, a number of assumptions had to be made when developing this risk assessment. Better data are needed for determining the probability of infection under specific exposure scenarios to provide better public health safety information. The dose-response models developed by Singh (1971) and Rose *et al*.

(1999) lack data on short term exposures like those associated with handling contaminated laundry and only dealt with exposures on otherwise healthy individuals. In addition, this risk assessment only addressed potential for infection from *S. aureus*, however other pathogenic organisms such as *E. coli* or rotavirus may also be present in unwashed laundry and these pathogens have a higher risk for transmission of infection. Future exposure assessment studies may be improved by employing dermal transfer exposure models previously developed for measuring transfer of pesticides from surfaces to skin. Coefficients for pesticide transfer have been developed by the US. EPA that take into account garment and surface loading and aggregate the mass transfer associated with a series of contacts with a contaminated medium (Cohen Hubal *et al*. 2006) and may be applicable to microbial exposure assessment.

In 2008, MRSA accounted for over 60% of *S. aureus* infections, up from 2% in 1972 (APIC 2008). Because of this increase, it remains important to consider the potential for infection from expanded routes, including handling laundry that may be highly contaminated, particularly if the person who comes in contact with the wash is in a high risk group or has skin abrasions. Concentration of *S. aureus* as high as 10^7 CFU/cm^2 have been reported in exuding human wounds (Lim et al. 2007), and as high as 10^{10} in animal wounds in studies evaluating the efficacy of antibacterials (Yah *et al*. 2008). Direct handling of laundry soiled at those levels would result in a risk of infection 10 – 1,000 times greater than estimated here.

Using a 10^6 CFU/cm^2 concentration on soiled laundry, the risk of acquiring a *S. aureus* infection when handling unwashed contaminated laundry is estimated to be 0.59

infections per person per year through this risk assessment. General precautions for handling unwashed laundry are 1) hold the laundry away from your body during transport, 2) launder contaminated items separately, 4) wash hands after handling laundry, and 4) launder soiled items within 24 hours to minimize microbial re-growth (Scott *et al.* 1990).

The International Scientific Forum on Home Hygiene (Bloomfield 2006) published the following specific guidelines when washing items which may be contaminated with MRSA:

Wash at 40˚C using a bleach-containing laundry product, or

Wash at 60˚C or above using any laundry product.

Each of these recommended procedures may be problematic. The current generation of fabrics has new fibers, construction, quality of dyes and special finishes that are not suitable for using traditional bleach, and most home water heaters are set at 55˚C for energy conservation and to prevent scalding.

In future risk assessments, additional information is needed to inform the exposure assessment particularly related to the rate of transfer of *S. aureus* and MRSA from contaminated laundry to broken/abraded skin and from contaminated hands to the nose, as well as *S. aureus* and MRSA levels expected in unwashed, contaminated laundry.

Table 4.1 Dose response data for *S. aureus* infections after six days, and dose response data using an "area under the curve" (AUC) strategy

Dose (number/cm^2)	Integrated dose - AUC (days x number/cm^2)	Total subjects	Subjects with infections
40	2,428,000	20	4
220	6,266,500	20	8
2000	12,732,000	20	13
105,000	24,983,000	20	14
1,600,000	33,440,000	20	19
10,000,000	39,136,000	20	20

*Dose response data from Singh *et al*. (1971).
^Singh *et al*. data revised by Rose *et al*. (1999).

Figure 4.1 Comparison of Rose *et al*. (1999) best fit model (line) to Singh *et al*. (1971) observed data (symbols) by using AUC and exponential dose response model

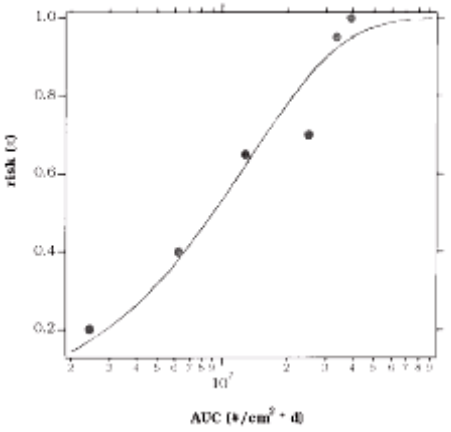


Table 4.2 Basis of assumptions for concentrations of *S. aureus* in laundry and on hands or broken/abraded skin. Worst-case values (bolded) chosen for risk estimate calculations

Before laundering:
Concentration of pathogens in home laundry based on:
<0.1 to >4 x 10^3 CFU/cm^2 (Christian *et al*. 1983) *S. aureus* in hospital laundry
10^5 CFU/cm^2 (Saltzman *et al*. 1967) bacteria in used articles of clothing
10^4 - **10^6** CFU/cm^2 (Blaser et al. 1984) bacteria in hospital laundry
After washing with detergent*:
Percent *S. aureus* reduction laundry washed with detergent but no bleach, based on:
99.999% reduction (Gerba *et al*. unpublished data) *S. aureus* inoculated laundry
99.999% - 99.9999% reduction (Nordstrom *et al*. unpublished data) *S. aureus* inoculated laundry in top- or front-load washer
After washing with detergent then drying*:
Percent *S. aureus* reduction in laundry washed with detergent but no bleach then dried, based on:
99.9999% reduction (Gerba *et al*. unpublished data) *S. aureus* washing then dried for 28-43 minutes
99.9999% reduction (Nordstrom *et al*. unpublished data) *S. aureus* washing in top- or front-load washers then dried for 28-50 minutes
After washing with detergent and bleach*:
Percent *S. aureus* reduction laundry washed with detergent and bleach, based on:
99.9999% reduction (Gerba *et al*. unpublished data) *S. aureus* inoculated laundry
99.9999% reduction (Nordstrom *et al*. unpublished data) *S. aureus* inoculated laundry in top- or front-load washer
After washing with detergent and bleach then drying*:
Percent *S. aureus* reduction in laundry washed with detergent and bleach then dried, based on:
99.9999999% reduction (Gerba *et al*. unpublished data) *S. aureus* washing then dried for 28-43 minutes
99.9999% reduction (Nordstrom *et al*. unpublished data) *S. aureus* washing in top- or front-load washers then dried for 28-50 minutes
Percent S. aureus transferred to hands before washing based on:
1.67% (Macintosh *et al*. 1984) *S. saprophyticus*, 300 cm^2 dry fabric for 10 second contact time
0.1% - **2.5%** (Satter *et al*. 2001) *S. aureus*, 3 cm^2 for 10 second contact time, moisture and friction varied

*Washing done using normal wash cycle with approximately 20˚C wash water.

Table 4.3 Estimated probability of infection for *S. aureus* after laundering with different treatments, based on an initial contamination of 10^6 CFU/cm^2

Scenario	Concentration of *S. aureus* in home laundry (CFU/cm^2)[1]	Conc. of *S. aureus* transferred to hands or broken/abraded skin (dose)[1]	Number of infections based on dose-response model[2]	Exposures per person per year[3]	Annual risk of infection per person per year[4]
Before laundering	10^6	25,000	0.0019	312	0.59
After washing with detergent	10	0.25	1.9×10^{-8}	312	5.9×10^{-6}
After washing with detergent then drying	1	0.025	1.9×10^{-9}	312	5.9×10^{-7}
After washing with detergent and bleach	10	0.25	1.9×10^{-8}	312	5.9×10^{-6}
After washing with detergent and bleach then drying	<1	<0.025	1.9×10^{-9}	312	$<5.9 \times 10^{-7}$

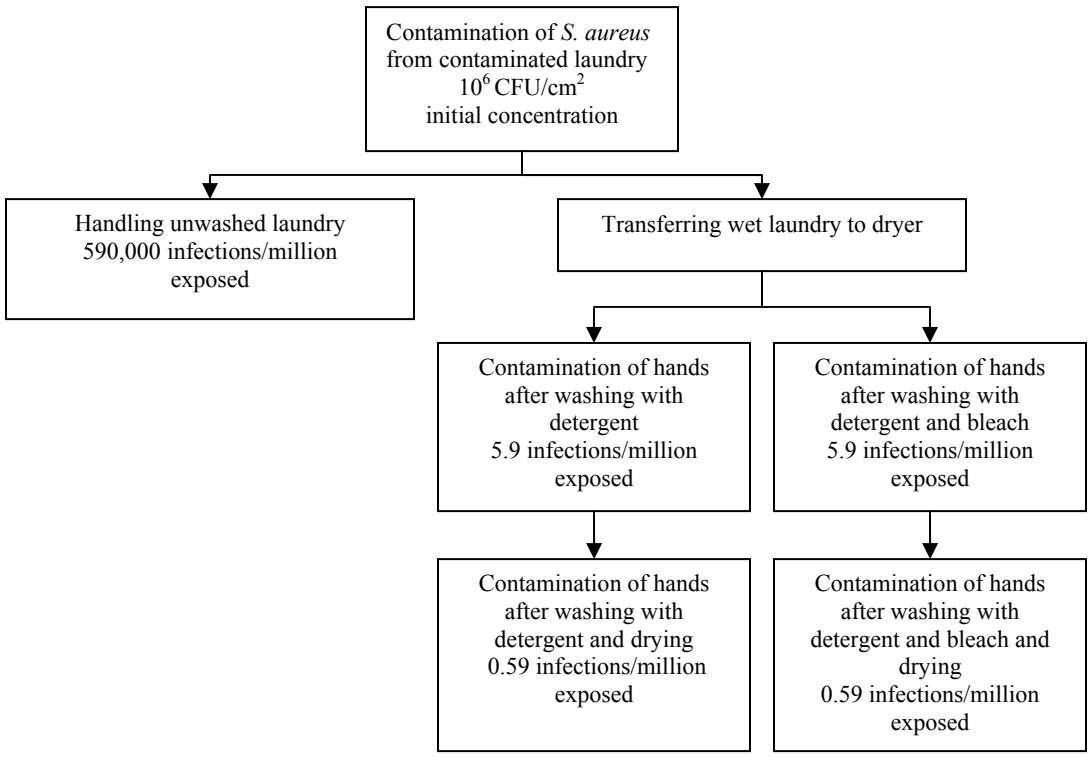
[1]Support data for values in Table 4.2

[2]Based on Rose et al. dose-response model in Figure 4.2 using risk of infection equation $(\pi) = 1\text{-}e^{-(d/k)}$ assuming 1 exposure day. $k = 1.31 \times 10^7$ days · "number *S. aureus*/cm^2 , d = dose (number *S. aureus*/cm^2).

[3]Average household does 6 loads per week, or 312 loads per year

[4]Sample calculation: number of infections using $[1\text{-}e^{-(dose/k)}]$ formula x 312 = annual risk if infection per person per year

Figure 4.2 Estimated worst case probability of infection for at-risk persons from handing laundry contaminated with *S. aureus*



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APPENDIX E:

ADDITIONAL DOCUMENTATION

Table 5.1 Log_{10} reduction after washing and after washing with drying*

Organism & Temperature	Laundry conditions	After washing: Log_{10} reduction ± SD (range)	After washing and drying: Log_{10} reduction ± SD (range)
***S. aureus* 27˚C**	Top load - no additives, water only	2.88 ± 0.59 (1.85 - 3.87)	4.45 ± 0.67 (3.48 - 5.51)
	Top load - detergent	6.13 ± 1.24 (3.24 - 7.62)	6.95 ± 0.55 (5.32 - 7.51)
	Top load - detergent and bleach	6.78 ± 0.36 (6.04 - 7.04)	6.43 ± 0.90 (5.0 - 7.04)
	Front load - no additives, water only	3.85 ± 0.47 (2.85 - 4.67)	4.66 ± 0.35 (4.15 - 5.50)
	Front load - detergent	5.98 ± 0.73 (4.46 - 6.98)	6.21 ± 0.45 (5.32 - 6.67)
	Front load - detergent and bleach	>6.58	>6.58
MRSA 27˚C	Top load - no additives, water only	3.37 ± 0.96 (1.98 - 5.01)	6.17 ± 0.71 (4.35 - 6.83)
	Top load - detergent	6.02 ± 0.62 (4.84 - 6.79)	6.39 ± 0.50 (5.32 - 6.79)
	Top load - detergent and bleach	>6.85	>6.85
	Front load - no additives, water only	2.70 ± 0.52 (2.11 - 3.84)	5.73 ± 0.40 (4.78 - 6.04)
	Front load - detergent	6.22 ± 0.94 (3.60 - 7.08)	6.49 ± 0.53 (5.60 - 7.30)
	Front load - detergent and bleach	6.43 ± 0.00 (6.20 - 6.66)	6.43 ± 0.00 (6.20 - 6.66)
***S. aureus* 17˚C**	Top load - no additives, water only	2.96 ± 0.29 (2.66 - 3.21)	5.45 ± 0.90 (4.66 - 6.74)
	Top load - detergent	5.92 ± 1.27 (2.65 - 6.79)	6.75 ± 0.03 (6.73 - 6.79)
	Top load - detergent and bleach	6.44 ± 0.76 (4.73 - 6.73)	>6.73
	Front load - no additives, water only	3.92 ± 0.78 (2.68 - 5.30)	4.68 ± 0.75 (3.78 - 6.83)
	Front load - detergent	6.46 ± 0.28 (5.81 - 6.81)	6.46 ± 0.14 (6.26 - 6.64)
	Front load - detergent and bleach	6.04 ± 0.29 (5.11 - 6.15)	6.12 ± 0.02 (6.11 - 6.15)
MRSA 17˚C	Top load - no additives, water only	2.37 ± 0.60 (1.77 - 3.64)	3.69 ± 1.13 (2.28 - 6.68)
	Top load - detergent	5.72 ± 1.08 (4.11 - 7.43)	6.21 ± 1.23 (3.19 - 7.08)
	Top load - detergent and bleach	6.04 ± 1.33 (4.04 - 6.70)	6.67 ± 0.06 (6.59 - 6.70)
	Front load - no additives, water only	3.77 ± 0.36 (3.23 - 4.44)	5.69 ± 0.58 (4.87 - 6.79)
	Front load - detergent	6.41 ± 0.45 (5.13 - 6.79)	6.29 ± 0.57 (5.13 - 6.79)
	Front load - detergent and bleach	>6.53	>6.53

*Wash loads each included 3.2 kg sterile cotton t-shirts, standardized dirt load on 1 pillowcase, and six seeded swatches with initial bacteria inoculum ranging from 6 to 7 logs per swatch.

">" values indicate log reduction exceeded limit of detection for experiment.

Table 5.2 Public and apartment laundromat HPC numbers

Location	Sample site	Square cm sampled at site	CFU recovered/ sq cm
Public Laundromat 1	drum/rim	4644	1156
	drum/rim	4644	19
	interior lid	1651	55
	interior lid	1651	86
	top washer	3548	39
	table top	11610	58
Public Laundromat 2	drum/rim	4644	10
	drum/rim	4644	147
	interior lid	1651	176
	interior lid	1651	106
	top washer	3548	216
	table top	11610	88
Public Laundromat 3	drum/rim	4644	40
	drum/rim	4644	68
	interior lid	1651	28
	interior lid	1651	88
	top washer	3548	80
	table top	11610	41
Public Laundromat 4	drum/rim	4644	177
	drum/rim	4644	80
	interior lid	1651	159
	interior lid	1651	38
	top washer	3548	225
	table top	11146	36
Public Laundromat 5	drum/rim	4644	8
	drum/rim	4644	16
	interior lid	1651	128
	interior lid	1651	86
	top washer	3548	159
	table top	13003	45
Apartment Laundromat 1	drum/rim	4644	68
	drum/rim	4644	106
	interior lid	1651	363
	interior lid	1651	756
	top washer	3548	504
	table top	11610	401
Apartment Laundromat 2	drum/rim	4644	43
	drum/rim	4644	85
	interior lid	1651	655
	interior lid	1651	373
	top washer	3548	575
	table top	11610	326

Apartment Laundromat 3	drum/rim	4644	6
	drum/rim	4644	18
	interior lid	1651	176
	interior lid	1651	242
	top washer	3548	110
	table top	8772	157
Apartment Laundromat 4	drum/rim	4644	2
	drum/rim	4644	4
	interior lid	1651	28
	interior lid	1651	100781
	top washer	3548	38
	table top	9288	323
Apartment Laundromat 5	drum/rim	4644	73
	drum/rim	4644	74
	interior lid	1651	23684
	interior lid	1651	107080
	top washer	3548	42
	table top	9288	50