Examining the Potential for Bacterial Build-up on Apparel Fabrics with Repeated Use

- A Laboratory Study

by

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Abstract

When clothing is worn multiple times by a consumer, a build-up of oily soils, dirt, odours, and bacteria can transfer from the wearer to the garment. The bacteria that have been transferred to the garment may result in unwanted odours. Over time, this accumulation of bacteria may cause persistent odours that remain in clothing even after laundering. In addition, fibre type may influence how textiles retain and transfer bacteria. The purpose of this *in vitro* laboratory study was to examine if bacteria can accumulate in apparel fabrics with repeated use/laundering cycles. Three inoculation/wash levels were used (1, 2, 5). Cotton and polyester fabrics were inoculated with *Staphylococcus aureus* and later laundered. After laundering, bacterial counts from cotton and polyester fabric specimens were calculated in colony forming units (CFU) per sample. Three groups of fabric specimens were used in this study, baseline, treatment, and control. Baseline specimens were used to provide an initial bacterial count of the treatment fabrics to investigate if there was a build-up of bacteria after laundering the treatment specimens. The control fabrics were used to examine the transference of bacteria between fabrics during washing. No differences were found in log CFU per sample of *S. aureus* on treatment fabrics as the number of inoculation/laundering cycles increased. This suggests that bacteria did not accumulate on either cotton or polyester fabrics as the number of cycles increased. The transfer of bacteria between fabrics during the wash cycle was also examined in this research. It was found that bacteria transferred from the inoculated fabrics to the control fabrics during laundering. The transference of bacteria was minimal for cotton and polyester fabrics. Despite the expectation that bacteria would build-up as the number of inoculation/wash cycles increased, there was no evidence for this in the current study. While laundering can reduce the bacterial counts laundering was not sufficient in removing all *S. aureus* colonies from both cotton and polyester specimens.

Preface

This thesis is an original work by Skylar Brown. No part of this thesis has been previously published.

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List of Abbreviations

ATCC	American Type Culture Collection
AATCC	American Association of Textile Chemists and Colorists
ANOVA	Analysis of Variance
CFU/mL	Colony Forming Units per millilitre
CGSB	Canada General Standards Board
log	logarithm transfer to the base of 10
S. aureus	*Staphylococcus aureus*
df	degrees of freedom
h	hours
mL	millilitre
R.H.	Relative Humidity
°C	Degrees Celsius
p	p value
%	percentage
<	less than
>	greater than
µL	microlitre

List of Definitions

In the context of this research, the following terms are defined:

Colony forming units (CFU)	A unit used to estimate the number of viable bacteria (Todar, 2020).
Gram negative bacteria	Double membrane bacteria which does not stain from the Gram stain test (Amyes, 2013).
Gram positive bacteria	Single membrane bacteria which stain when the Gram stain test is used (Amyes, 2013).
Bacteria	"A domain of life containing a diverse group of ubiquitous microorganisms all of which consist of only a single cell that lacks a distinct nuclear membrane and has a cell wall of a unique composition" (Hine, 2019, p. 24).
Biofilm	"A colony of bacteria and other microorganisms that adheres to a substrate and is enclosed and protected by secreted slime. Biofilms readily form on virtually any surface, whether nonliving or living, where there is moisture and a supply of nutrients" (Hine, 2019, p. 28).
Gram's stain	"Staining method used to differentiate bacteria. The bacterial sample is smeared on a microscope slide, stained with a violet dye, treated with acetone-alcohol (a decolourizer), and finally counterstained with a red dye" (Hine, 2019, p. 116).
Inoculation	"The placing of a small sample of microorganisms or any other type of cell into a culture medium so that the cells can grow and proliferate" (Hine, 2019, p. 137)
Log reduction	Calculation used to show how many viable microorganisms are eliminated after a treatment (Microchem Laboratory, 2022).
Percentage reduction	Proportion of bacteria removed relative to the baseline fabrics after their final wash cycle.
Percentage transfer	Number of bacteria transferred from the baseline fabrics which were inoculated with *S. aureus* to the uninoculated control fabrics during washing.
Persistent odour	An accumulation of odour-causing bacteria that are difficult to be remove from textiles (McQueen et al., 2007; McQueen et al., 2021).

Staphylococcus aureus	Gram positive bacteria that “inhabit the skin and mucous membranes, and some cause disease in humans and animals. *S. aureus* infection can lead to boils and abscesses in humans; this species also produces toxins that irritate the gastrointestinal tract and result in staphylococcal food poisoning. Certain strains are resistant to antibiotics, and infection with these is very difficult to treat” (Hine, 2019, p. 259).

Chapter 1

Introduction

Clothing is our nearest physical environment and is often worn close to the skin for extended periods. Sweat, body oils, skin flakes and bacteria can transfer to our clothing through continuous contact with the body (Munk et al., 2000). Bacteria can cause body odours when non-odorous sweat is converted into volatile compounds (Callewaert et al., 2014; Shelley et al., 1953). Therefore, body odours can also transfer to clothing due to the close contact of fabrics to odorous body sites (e.g., the axillae) (Urban et al., 2016). Clothing that becomes odorous and soiled will typically require laundering to remove malodours and soils and turn clothing that is "fusty, musty, or tired into things that are fresh, scented, fluffy, and 'ready' to wear" (Shove, 2003, p. 402). However, laundering may not be effective at eliminating all soils and odours and this can lead to a problem of "persistent odour" within textiles (Denawaka et al., 2016; McQueen et al., 2021, p. 1860; Abdul-Bari et al., 2020; Takeuchi et al., 2012).

The fibre content of clothing can influence odour intensity following wear (McQueen et al., 2014; McQueen & Vaezafshar, 2020). For example, polyester is often perceived as being more odorous than fabrics made from natural fibres such as cotton and wool (McQueen et al., 2007; Wang et al., 2019). Polyester tends to have more persistent odour than cotton (McQueen et al., 2014), due to the influence of fibre composition and chemical structure (Callewaert et al., 2014; McQueen et al., 2007). Since polyester is an oleophilic fibre, this can result in a build-up of odour caused by the attraction of non-polar soils and odorous compounds (Abdul-Bari et al., 2020; Munk et al., 2001).

Persistent odours that form in clothing may result not only from incomplete removal of odours and soils (Abdul-Bari et al., 2020), but also through a build-up of odour-causing bacteria

and the incomplete removal of bacteria during laundering (Monticello, 2019; Munk et al., 2000, 2001). In fact, Monticello (2019) described the bacterial build-up within clothing that is repeatedly worn and washed as responsible for this persistent odour. Over time, there may be a build-up of bacteria in the garment resulting in the formation of a biofilm. The quantity of bacteria remaining in the fabric eventually reaches a point where odour threshold has been met (Monticello, 2019) and laundering has "not fulfilled its purpose" (Laitala et al., 2014, p. 142). However, there have been limited studies that have directly examined the build-up of bacteria with repeated use and laundering cycles. Due to the relationship between bacteria and odour, investigating bacterial survival in everyday consumer textiles is warranted.

Multiple species of skin bacteria are found in the moist odorous body regions like the axillary vault (Grice et al., 2009) some of which have been directly implicated as being odour-causing (e.g., corynebacteria, *Staphylococcus hominis*) (McQueen & Vaezafshar, 2020; Rennie et al., 1990; Urban et al., 2016). Many studies have found that bacteria can persist in textiles for days and even months without laundering (Burden et al., 2011; Colclasure et al., 2015; McQueen et al., 2007; Neely & Maley, 2000; Treakle et al., 2009; Wiener-Well et al., 2011). The process of laundering may also be carried out with the intent to remove microorganisms from textiles (Gerba & Kennedy, 2007; Riley et al., 2017). Yet, many microorganisms can still survive the laundering process in washing machines (Honisch et al., 2014; Munk et al., 2001), particularly when lower wash temperatures are used (Riley et al., 2017). In addition, microorganisms can transfer from one textile to another during the wash cycle (Callewaert et al., 2015). Hence, examining the transfer of bacteria during washing from used clothing to other clothing fabrics can provide useful insight into bacterial cross-contamination during laundering.

Although, research has shown that odorous compounds and precursors to odour such as soils/sweat from the body are not always effectively removed from textiles by laundering (McQueen et al., 2014), there is still a need to better understand the role that bacteria can play in persistent odour. Therefore, the purpose of this research is to examine if bacteria builds up in apparel fabrics with repeated use/laundering cycles.

1.1 Objectives

The objectives of this study were to determine:

1. how effective laundering is at removing selected bacteria from fabrics that vary in fibre type (i.e., cotton and polyester);
2. if the bacterial load increases as the number of inoculation/wash cycles increase;
3. whether selected bacteria transfer from inoculated fabrics to control fabrics during wash cycles.

1.2 Research hypotheses

The following research hypotheses have been posed:

H1: For baseline fabrics the bacterial counts extracted from polyester will be significantly greater than bacterial counts extracted from cotton for each inoculation/wash cycle.

H2: For treatment fabrics the bacterial counts extracted from polyester will be significantly greater than the bacterial counts extracted from cotton fabrics following each inoculation/wash cycle.

H3: That as the number of inoculation/wash cycles increase the bacterial load will significantly increase on all fabrics for a) baseline fabrics; b) treatment fabrics, and c) control fabrics.

H4: That cotton fabrics will exhibit a greater bacterial percentage reduction than polyester fabrics.

H5: That polyester fabrics will exhibit a greater bacterial percentage transfer than cotton fabrics.

Chapter 2

Review of Literature

2.1 Introduction

Most individuals will wear a garment multiple times throughout its lifecycle and as a result the garment will undergo numerous launderings. Due to this repetitive wear, odours may develop within clothing and become persistent in some textiles where they are not completely removed through laundering (Abdul-Bari et al., 2020; McQueen et al., 2014; Munk et al., 2000).

Throughout this literature review, bacteria will be examined in the context of textiles while evaluating survival and transmission. Influencing factors of bacterial adhesion in textiles including the effect of fibre content, fabric structure, surface area and surface roughness will be discussed. Additionally, this chapter will survey prior literature relating to the build-up of odour and bacteria in clothing. The efficacy of laundering to remove and decrease bacterial load will also be analyzed. Finally, the literature surrounding consumer laundering behaviours will be reviewed.

2.2 Overview of bacteria

Bacteria can be defined as tiny single-cell organisms that do not have separate components within the cell and have an unusual cell wall structure (Amyes, 2013; Hine, 2019). Bacteria can be classified into two main groups: Gram-negative and Gram-positive. A staining procedure called the Gram-stain method is used to identify them. The cell wall structure of Gram-negative bacteria is more complex than Gram-positive bacteria due to the multiple layers of peptidoglycan among other unique features (Todar, 2020). Examples of Gram-positive bacteria include *Staphylococcus aureus, Streptococcus pyogenes,* and *Streptococcus*

pneumoniae. While *Escherichia Coli, Pseudomonas aeruginosa* and *Salmonella* are examples of Gram-negative bacteria (Todar, 2020).

A multitude of bacteria can be found on the skin surface, where approximately 1 million bacteria exist per square centimeter (Belkaid & Segre, 2014; Belkaid & Tamoutounour, 2016). In addition to the bacteria on our skin, there are a total of approximately 10,000 different bacterial species that occupy our bodies to aid in the digestion of our food and to create vitamins among serving other purposes for the body (Pennington, 2016). While most bacteria are not harmful to humans there are a few bacterial species that are responsible for disease such as parasites (Hine, 2019). Fortunately, these bacteria will typically not attack our immune systems. Bacteria that inhabit our skin, play numerous roles in the body such as preventing skin infections. Although this list is not exhaustive, many regions including the navel, groin, inside the forearms, behind the kneecaps, and between the fingers are known to host bacteria (Grice et al., 2009). In particular, high volumes of bacteria exist on the axillary region (Grice et al., 2009; Li et al., 2019; McQueen et al., 2014). Bacterial strains have been studied in previous research when examining the build-up of odour in garments (Callewaert et al., 2014; Munk et al., 2001). The odour-causing bacterial strains *S. aureus, Staphylococcus epidermidis, Escherichia coli*, and *Pseudomonas aeruginosa* were used in the Munk et al., (2001) study. Whereas, McQueen et al., (2007) evaluated the odour intensity of corynebacteria in textiles. Other bacteria, including *Micrococcus* species, and *Moraxella osloensis* have been found to generate odours in textiles (Kubota et al., 2012). Since *S. aureus* is an odour-causing bacteria that frequently resides on the skin, it will be used in the present research (James et al., 2004; Munk et al., 2001).

2.2.1 Bacteria in clothing and laundry environments

Bacteria can thrive in humid environments, transfer from the human body, and other environmental sources to textiles (McQueen et al., 2014; Munk et al., 2001). During laundering, bacteria that is on textiles may transfer to the washing machine. As a result, these bacteria could accumulate and transfer bacteria to other textiles in the washing machine (Callewaert et al., 2015; Gattlen et al., 2010; Lakdawala et al., 2011; Munk et al., 2001). In fact, at least 94 microorganisms have been identified in used washing machines, with 30% of these microorganisms being potentially pathogenic (Gattlen et al., 2010).

Due to the close contact of textiles to the skin, bacteria transfers from the body to clothing (Callewaert et al., 2014). Bacterial density on textiles may be determined by sampling various locations on a garment where there is known to be high skin-to-textile bacterial exchange. The areas of clothing that have been commonly sampled for bacterial loads include the sleeve cuffs, pockets, chest area, sides of the body and collar region (Goyal et al., 2019; Janani & Santhosh, 2018; Wiener-Well et al., 2011). The bacterial densities have been found to vary depending on where the sample was obtained from the textile (Gerhardts et al., 2015; Janani & Santhosh, 2018). Because the armpit hosts a greater density of bacteria, this area of a garment is known to contain high bacterial loads in clothing (Bockmühl, 2017; Li et al., 2019; Munk et al., 2001; Urban et al., 2016). Multiple studies have shown evidence of bacteria transferring from the wearers' skin to clothing items (Hanczvikkel et al., 2019; Treakle et al., 2009; West et al., 2018; Wiener-Well et al., 2011) and have measured the odour development to consider if there is any connection between bacteria and odour (McQueen et al., 2007, 2014).

2.3 Survival and transmission of bacteria in textiles *in situ* and *in vivo*

Much of the research related to bacterial transference from the human body to clothing has been studied in healthcare settings where medical uniforms such as scrubs and whitecoats have been shown to be contaminated with potentially pathogenic bacteria (Riley et al., 2017; Sands & Fairbanks, 2019; Treakle et al., 2009; Wiener-Well et al., 2011). There is limited research surrounding everyday clothing that is used in a non-health related setting (Callewaert et al., 2014; Frosth et al., 2018; McQueen et al., 2014; Muthiani et al., 2012).

Textiles can be a source of infection in medical settings since clothing can harbour various types of bacteria from the wearer which can be transferred to and survive on textiles (Colclasure et al., 2015; Hanczvikkel et al., 2019; Neely & Maley, 2000; Treakle et al., 2009; Wiener-Well et al., 2011). Furthermore, many strains of bacteria have the capability to persist on textiles for extended time periods depending on the bacteria and conditions (Hanczvikkel et al., 2019). For instance, pathogenic microorganisms such as *Enterococcus faecium, Klebsiella pneumoniae,* and *Acinetobacter baumannii* have been found to survive on textile surfaces for months and even up to years (Rice, 2008). One study found that *S. aureus* persisted for at least one day on cotton textiles and up to 56 days on polyester (Neely & Maley, 2000).

Hospitals usually provide in-house or third-party laundering services for their staff, yet some healthcare workers still launder their medical uniforms at home. This could lead to cross contamination and ineffective sanitation of clothing as self-laundering of medical uniforms has been shown to be less effective than the use of a professional laundry service (Chiereghin et al., 2020; West et al., 2018). Pathogens, such as adenovirus, hepatitis A virus, and rotavirus have reportedly survived through healthcare workers' home laundering practices and cross-contaminated textiles that were uncontaminated prior to laundering (Gerba & Kennedy, 2007).

While the transmission of pathogenic bacteria is of concern in medical settings, non-pathogenic bacteria occur in everyday clothing since bacteria may transfer from the body to garments (Callewaert et al., 2014; McQueen et al., 2007; McQueen et al., 2014). Fortunately, non-pathogenic bacteria may not pose as much risk as pathogenic bacteria (Colclasure et al., 2015; Muthiani et al., 2012; Treakle et al., 2009). Wiener-Well et al, (2011) found that all the uniforms worn by healthcare workers examined in their study, were contaminated with non-pathogenic bacteria directly from the skin flora whereas fewer uniforms were contaminated with pathogenic bacteria. Second-hand undergarments have been found to contain high microbial loads (Muthiani et al., 2012). Used clothing and toys containing textile materials also host potential pathogens such as *S. aureus, Enterobacteriaceae*, and *Bacillus* species (Muthiani et al., 2012). Another example demonstrates that the equestrian bacteria, *Streptococcus equi subspecies equi (S. equi)* found in horse stables can be passed from one horse to another through garments worn by visitors and can survive on clothing for at least 24 hours (Frosth et al., 2018).

2.4 Influencing factors of bacterial adhesion in clothing

Various textile properties including fibre content, fabric structure, and surface area may influence bacterial adhesion in clothing. Furthermore, these differences among textiles may significantly affect the likelihood of fabrics retaining bacteria (Colclasure et al., 2015; Teufel et al., 2010; Varshney et al., 2019).

2.4.1 Effect of fibre content on bacterial adhesion

The preceding research has shown how fibre content of a textile can affect the overall ability of a fabric to support bacterial adhesion (Colclasure et al., 2015; Takashima et al., 2004; Teufel et al., 2010). When examining bacterial adhesion of different fibre types, it was found that each fibre type (cotton, silk and cotton blend) supported the adhesion of coliform bacteria in

varying loads (Colclasure et al., 2015). Following the application of coliform suspension, the highest volume, up to 99% of bacteria were found to have attached to cotton, while blended cotton and silk showed a bacterial adhesion of 86% and 73% respectively.

In addition to fibre content, the bacterial species may also influence how bacteria adhere to textiles (Teufel et al., 2010). Previously, *Staphylococcus* species have been found in the highest volumes on Tencel® (lyocell) and cotton, but in lower volumes on polyester, polyamide, and polypropylene. While strains such as *Enterobacteriaceae* were less viable on cotton and Tencel®, higher volumes of *Enterobacteriaceae* appeared on polypropylene, and polyester (Teufel et al., 2010). When a 100% cotton t-shirt sample was compared to a 92% polyester and 8% elastane jacket material, *S. equi* survived on the cotton sample for a longer duration (Frosth et al., 2018). Another study revealed that higher loads of *S. aureus* and *Pseudomonas aeruginosa* were in polyester, wool, and acrylic fibres compared to cotton (Takashima et al., 2004). An additional group of researchers found that *S. aureus* and *E. faecalis* adhered to polyester for a greater time period than cotton fabrics (Neely & Maley, 2000).

2.4.2 Effect of fabric structure on bacterial adhesion

Fabric structure and physical properties (e.g., fabric thickness, pore size) may impact how bacteria adhere to textiles (Colclasure et al., 2015). When the adherence of coliform bacteria to cotton, blended cotton and silk was examined, woven cotton was able to retain the highest volume of bacteria (Colclasure et al., 2015). The authors speculated that the 100% woven cotton was able to hold a larger amount of moisture due to the pore space between the fibres and yarns in the fabric. Whereas the tightly woven silk fabric was likely to have less pore space between the yarns which resulted in lower bacterial adhesion (Colclasure et al., 2015). Unfortunately, the

authors did not provide further details about the mass, density, or surface areas of their test fabrics.

Modifying the weave of yarns to be looser in a fabric could reduce volume of bacteria that may be transferred and persist on clothing (Varshney et al., 2019). Nevertheless, it is difficult to state how bacteria adhesion will be impacted based on fabric structure alone. In the study of McQueen et al. (2007) it seems that smaller fabric structural differences among knitted fabrics (single jersey, 1x1 rib, interlock) did not account for differences in bacterial adhesion. Instead, bacterial adhesion was more clearly linked to fibre type (wool, cotton, polyester) with differences observed in survival as the number of days increased (as initially no differences in the quantity of bacteria transferred was found). Even so, Teufel et al., (2010) suggested that polypropylene was more conducive to higher bacterial growth than polyester due to its increased thickness when comparing bacterial counts.

2.4.3 Effect of surface area on bacterial adhesion

It has been suggested that fabrics with a large surface area will support higher rates of bacterial binding (Varshney et al., 2019). Colclasure et al., (2015) speculated that the woven cotton fabric has a greater surface area than the nonwoven blended cotton fabric. It is likely that the higher surface area woven fabric could support the adhesion of more bacteria than the lower surface area of the nonwoven fabric. However, the authors did not provide any evidence on the surface areas of the two test fabrics.

2.4.4 Effect of surface roughness on bacterial adhesion

In addition to the surface area of a textile, surface roughness may further affect how bacteria adhere to clothing via friction since friction has been found to increase the volume of bacteria that is transferred between textiles (Gerhardts et al., 2015; Varshney et al., 2019). When

dynamic friction occurs between two of the same fabrics, and where one fabric has been exposed to bacteria, there may be a 5% to 61% increase in the volume of bacteria that is transferred to the second fabric. Bacterial counts were found to be higher when the fabric receiving the bacteria was damp since wet fabric is reportedly more admissible to the donor of motile bacteria (Gerhardts et al., 2015; Varshney et al., 2019). Bacterial transference rates seemed to be lower when there is no dynamic friction between fabrics. When polyester had been treated with a polydiallyl-dimethyl-ammonium chloride and polyacrylic acid nanocoating, the ability of *S. aureus* to adhere to the fabric decreased up to 50% due to the increased surface roughness (Smith et al., 2017). However, a clear correlation between surface roughness and fabric structure has not been established as there is conflicting information on this subject (Smith et al., 2017; Varshney et al., 2019).

2.5 Build-up of odour and bacteria in clothing

Since clothing is in direct contact with the body, bacteria and odour can be easily transferred to a garment (McQueen & Vaezafshar, 2020; Teufel et al., 2010; Urban et al., 2016; Van Herreweghen et al., 2020). Sterile sweat produced from the body is initially odorless, and it is upon microbial action of specific types of bacteria that odour is generated (James et al., 2004). In general, many types of bacteria that are present on the skin will not produce a strong odour (Li et al., 2019; Rennie et al., 1990). More specifically, volatile fatty acids have been linked to forming odour in the axillary region (Taylor et al., 2003) whereas bacterial species of staphylococci and aerobic *Bacillus* have been found to generate odour from the feet (Ara et al., 2006). Strong body odours may be more noticeable on some people than others as this is dependent upon type of bacteria present on the body, the available nutrient source for the

bacteria and specific area of the body (Urban et al., 2016). Since some parts of the body are more conducive to bacterial growth, clothing that is in contact with the skin can play an important role.

If sweat and bacteria become trapped in the fabric, then a build-up of odorous compounds within the textile can occur. When worn during exercising, polyester that had been contaminated with bacteria and sweat produced a more potent malodour than that produced on cotton fabrics (Callewaert et al., 2014). Odours from contaminated polyester can be described as a sour, strong, sweaty, musty or have an ammonia-like scent (Callewaert et al., 2014). Polyester has been found to be significantly more odorous than either cotton or wool, with wool typically being the least odorous out of the three different fibre types (Klepp et al., 2016; McQueen et al., 2007).

Clothing and textiles may carry certain odours that may be permanent despite laundering (Van Herreweghen et al., 2020; Yin et al., 2019). It has been hypothesized that persistent odours in textiles that are unable to be removed during laundering are caused by bacterial build-up from repetitive wear (Monticello, 2019). This build-up in bacteria indicates that the odour threshold has been reached and a biofilm has formed. Permanent odours emanating from laundered clothing may also be caused by the build-up of bacteria in washing machines. This build-up of bacteria can cause a formation of a biofilm on the drum of a washing machine (Gattlen et al., 2010). Microorganisms that form a biofilm can survive ultraviolet radiation, antibiotic treatments, extreme temperatures, lack of nutrients and severe pH levels (Yin et al., 2019). It is possible that cleaning chemicals may not be an effective means to remove biofilms (Gattlen et al., 2010).

In Japan, clothing frequently carries “acidic or sweaty odour” post laundering (Kubota et al., 2012, p. 3317). This may be due to bacteria not being entirely washed out of the textile since Japanese consumers generally use low washing temperatures and sometimes hang-dry garments

in often humid indoor environments. The source of unpleasant odour in textiles has been linked to the compound, 4-methyl-3-hexenoic acid (4M3H) (Takeuchi et al., 2012). In addition, the odour-causing bacteria, *Moraxella osloensis*, has been shown to metabolize the compound 4M3H which results in laundry malodor (Kubota et al., 2012). Certain species of odour-causing bacteria are more likely to adhere to certain types of clothing than other species (Callewaert et al., 2014). Finally, Tsuchiya et al., (2008) identified *Micrococcus* species that were able to create a biofilm-like structure on a non-sterilized fabric.

2.6 Laundering methods to remove bacteria

Laundering of clothing and textiles can greatly reduce bacteria, soiling, sweat, dead skin cells, odour and dirt (McQueen et al., 2014; McQueen & Vaezafshar, 2020; Munk et al., 2001). However, the ease and proficiency of laundering to remove bacteria is dependent on several variables including fibre content, wash temperature, time length of wash cycle, detergent type, use of bleach and antimicrobials (Honisch et al., 2014; Riley et al., 2017). Another component of the laundering process that must be examined is the method of drying the textile since this can impact the level of decontamination (Munk et al., 2001; Wiksell et al., 1973). The original degree of contamination may also affect the volume of bacteria on textiles after laundering (Nordstrom et al., 2012).

2.6.1 Fabric parameters

Through a comparison of odour intensity and bacterial counts after clothing has been worn and laundered, previous literature has shown that the fibres from which apparel fabrics are made can influence the effectiveness of the laundering process (McQueen et al., 2014; Munk et al., 2001). Fabric structure can also affect the ease with which odours and bacteria can be removed (Riley et al., 2017; Teufel et al., 2010). Therefore, the effectiveness of laundry

parameters on removing odour and bacteria via laundering can depend on both fibre content and fabric structure. This may be further influenced by wash water temperature, the hydrophilicity of the fibre and the types of detergents that are used (Van Herreweghen et al., 2020).

In studies examining the effectiveness of laundering on removing odour and bacteria, comparisons between cotton and polyester fibres are often of interest (McQueen et al., 2014; Munk et al., 2001). In the case of the McQueen et al. (2014) study, they found that fabrics made from cotton were less odorous than polyester after being worn and washed twenty times. In another study, odours were more readily removed from cotton fabrics than polyester when temperatures of 30 °C were used (Munk et al., 2001). This is most likely explained by polyester's hydrophobic properties, which differ from the hydrophilic fibres of cotton. Thus, oily soils are more readily removed from cotton than polyester fibres since cotton is hydrophilic (Kadolph & Marcketti, 2017). Water and detergent are able to wet cotton fabrics more quickly and begin the process of releasing soils into the wash water (Abdul-Bari et al., 2020). No notable differences in the quantity of aerobic bacteria remaining between the two fibre types were found in the study by McQueen et al. (2014). Thus, while oily soils and odorants are removed from cotton more readily than from polyester, removal of bacteria through laundering has not shown the same differences (McQueen et al., 2014).

The initial structure of a fabric before laundering and the structural changes that occur during laundering may affect the ease with which odours and bacteria are removed (Riley et al., 2017; Teufel et al., 2010). The decontamination process could be affected since a fabric's structure can be altered during laundering due to shrinkage or distortion over multiple laundering cycles (Çoruh, 2017; Masteikaitė et al., 2013). Woven fabrics composed of cotton can undergo dimensional changes after each laundering cycle (Masteikaitė et al., 2013). Further, weft knitted

fabrics seem to be more affected as they are susceptible to significant distortion after repeated launderings (Çoruh, 2017). These severe dimensional changes in knitted fabric structures may be caused by the properties of the yarn, the knitting process, as well as finishes that may be added to the fabric. Additional research needs to be conducted to confirm the possible effects on fabric structure and the impact of laundering regarding dimensional change on bacterial and odour removal.

2.6.2 Effect of wash temperature

The bacterial load on textiles has been found to decrease as the water temperature of a wash cycle is increased (Davis & Ainsworth, 1989; Honisch et al., 2014; Rehberg et al., 2017). Decreases in bacterial counts were more evident when higher wash temperatures around 66 °C were used in comparison to lower wash temperatures around 31 °C (Smith et al., 1987). Rehberg et al., (2017) reported that if water temperatures under 50 °C were set, then antibiotic-resistant bacterial strains may survive through laundering (Rehberg et al., 2017).

Wash water temperature has been found to be more effective in reducing bacterial load than elapsed time in a laundering cycle (Honisch et al., 2014; Riley et al., 2017). For example, at 52 °C only 15 minutes was required to eliminate *S. aureus* bacterial colonies from cotton fabrics. In contrast, a wash cycle of 90 minutes at 42 °C was required to achieve the same level of bacterial reduction (Honisch et al., 2014). However, the impact of wash temperature on laundry can vary depending on the type of bacterial strain since some types of bacteria can survive in higher water temperatures. As Honisch et al. (2014) found *S. aureus* to be eliminated at a temperature of 52 °C, other studies have found that some bacterial strains (*S. aureus, P. aeruginosa, E. aerogenes E. faecium and, K. pneumoniae*) can be chemothermal-resistant up to 60 °C (Fijan et al., 2007; Riley et al., 2017; Tano & Melhus, 2014). Hence temperatures of 60 °C

at a minimum are recommended in clinical settings to prevent cross-contamination and to remove all microorganisms. Nevertheless, in domestic laundering much lower wash water temperatures are typically used for washing everyday clothing (Laitala et al., 2012; Yates & Evans, 2016). Even in a study examining the behaviours of healthcare workers laundering methods, Riley et al., (2017) found that 44% of 265 healthcare workers surveyed, laundered their work uniforms below 60 °C.

2.6.3 Effect of length of wash cycle

Increasing the length of the wash cycle at lower wash temperatures can further improve bacterial removal, particularly at very low wash temperatures (e.g., 20.5 °C) (Honisch et al., 2014). For example, a greater bacterial log reduction of *S. aureus* was found as the wash cycle increased from 15 to 45 minutes, and again when it was extended to 90 minutes at 32.3 °C (Honisch et al., 2014). Similarly, in the case of *Pseudomonas aeruginosa,* the log decrease varied depending on temperature and length of wash cycle. The log reduction value of *P. aeruginosa* at 32.3 °C for 15 minutes was 5·6, between 6·0 and 7·2 at 45 minutes, and between 6·9 and >7·2 at 90 minutes (Honisch et al., 2014). Therefore, when cooler wash water temperatures are used, it is recommended the laundry wash cycle is increased to facilitate removal of bacteria.

2.6.4 Effect of detergent

Detergent type used in the laundering process, has been shown to affect bacterial removal from textiles (Riley et al., 2017; Wiksell et al., 1973). Riley et al., (2017) outlined the three types of detergents as nonbiological, biological, and antimicrobial. Despite the expectation that an antimicrobial detergent would eliminate bacterial populations at low wash temperatures, researchers found that the reduction of *S. aureus* at 40 °C showed no difference when an antimicrobial detergent was used versus biological and nonbiological detergents (Riley et al.,

2017). One drawback of this article is the type of antimicrobials contained in the detergents were not stated by the authors. Efficacy of laundering at cold water temperatures is important as many consumers, including healthcare workers, wash using cold water. In their study, Riley et al., (2015) also found that biological detergents were the most popular type of detergent used by healthcare workers who laundered their work uniforms at home.

Activated oxygen bleach (AOB) has been incorporated into detergent formulations as it can be more effective in decontaminating clothing when washed at lower wash temperatures, thereby, acting as a substitute for higher washing temperatures (Honisch et al., 2014; Rehberg et al., 2017; Riley et al., 2017; Showell, 2019; Tavčer, 2020). The bacterial load of *P. aeruginosa* was significantly reduced (78–95%) when laundered with an AOB containing detergent at 20 °C when compared to laundering at 40 °C without the use of AOB (Rehberg et al., 2017). Using a washing temperature of 50 °C and without AOB, demonstrated a similar level of reduction of 20 °C with AOB. The use of detergents containing bleach during wash cycles has been shown to remove all contaminants of *S. epidermidis* (Munk et al., 2001). However, detergent without bleaching agents have allowed the survival of *S. epidermidis*, *P. aeruginosa* and *E. coli.* Incorporation of an AOB to a detergent was shown to facilitate the removal of bacterial loads of *S. aureus* and *Ent. hirae* on cotton fabrics (Honisch et al., 2014). The use of an AOB reduced *S. aureus* and *Ent. hirae* in 15 minutes at 32 °C. When an AOB was not used, a wash cycle temperature of 47 °C was required for 15 minutes to have the same log reduction (Honisch et al., 2014). Therefore, the use of a washing detergent that contains AOB could be a viable option for reducing bacteria and act as a substitute for hotter temperatures.

The importance of a detergent during laundering can be best illustrated by Davis and Ainsworths' (1989) study. The researchers discovered that the majority of *Streptococcus faecalis*

bacteria that contaminated the cotton/polyester fabric samples were removed when laundered at 50 °C without detergent, however, cold wash temperatures of 15 °C used in combination with a household detergent, were more effective than a hot water only wash. The removal of bacteria in clothing through effective laundering methods can help to reduce the likeliness of cross-contamination to other garments.

2.6.5 Effect of bleach and antimicrobials

Since there has been a trend by consumers to use cooler wash water when doing laundry, additives to detergents and use of bleach may be required (Laitala et al., 2012; Miilunpalo & Räisänen, 2019). The use of bleach as a method of decontamination is dependent upon fibre type. Muthiani et al. (2012) found that the use of a household bleach containing 3.85% of sodium hypochlorite decreased the number of pathogenic bacteria in clothing. Despite the inclusion of bleaching agents in detergent formulas, consumers still tend to use separate bleaching agents such as hypochlorite and hydrogen peroxide bleaches as a method to disinfect, remove dirt and stains, and to whiten textiles (Showell, 2019).

While bleach has been used widely for many years on textiles as an agent for decontamination, antimicrobials have gained popularity (Balakumaran et al., 2016; Rehberg et al., 2017). As a finish, antimicrobials are added to some fabrics to decrease the build-up of bacteria and, by association, odours. As well, antimicrobials may be incorporated into synthetic textiles as a component of the fibres. Silver nanoparticles have previously been added to cotton fabrics and have displayed excellent durability and antimicrobial effectiveness after laundering (Balakumaran et al., 2016). Initially, the silver-treated cotton fabric was 99.9% effective in reducing bacteria, but after 15 laundering cycles this efficacy decreased to 93% in pathogenic reduction (Balakumaran et al., 2016). Another downfall of antimicrobial treated fabrics are the

environmental considerations as antimicrobial finishes can slow down the degradation process of textiles (Stuart & Ueland, 2017).

As mentioned previously, antimicrobials may be added to detergents. For example, Kathon is a biocide which is sometimes added to detergents to provide antimicrobial properties to fabrics. The effectiveness of this biocide was evaluated in the study by Munk et al. (2001) in which its impact on cotton and polyester were compared. Despite the likelihood of antimicrobials reducing odour and bacteria in textiles, overuse could result in antimicrobial resistance (Teufel et al., 2010). Thus, laundering methods without antimicrobials should be explored for use on textiles that are intended for long term wear while employing numerous washing cycles.

2.6.6 Drying laundered clothing

Drying conditions including whether clothing is tumble dried or line dried can have a significant impact on the bacterial load and odours present in a textile (Honisch et al., 2014; Pugliese et al., 2020). Line drying reportedly impacts the odour of textiles. Some differences were found when 100% cotton towels were line-dried indoors versus outdoors. The towels were compared with regard to odour and the chemical reactions that occurred during drying (Pugliese et al., 2020). It has been postulated that when drying outdoors, textiles may undergo oxidation ozone or photochemical reactions which result in a more apparent fresh line-dried laundry scent. In contrast, when the cotton towels were line-dried indoors the smell of freshness was minimal compared to the outdoor dried towels.

Still, tumble drying appears to be more effective at decreasing bacterial counts in textiles. This was evident in the research by Tano and Melhus (2014) where bacterial log reduction was examined after just one wash cycle and then after the tumble-dry cycle. In their study, fabric swatches (50% cotton/50% polyester) were contaminated with *E. faecium* and washed at 70 °C

for 15 mins. A decrease in bacteria of around 5 $\log_{10}$ colony forming units (CFU) was found after this procedure. However, using the same wash conditions and then following with a 78 °C tumble dry cycle for 22 minutes, the bacterial reduction decreased by up to 9 $\log_{10}$ CFU (Tano & Melhus, 2014).

2.7 Consumer laundering behaviour

The primary reasons for laundering clothing are to remove stains, soiling, odour, and bacteria. Since there is an abundance of laundry settings, detergents, and other miscellaneous laundering products such as bleach and fabric softeners available on the market, individual processes, and habits to launder clothing may differ vastly among consumers (Laitala et al., 2012; Yates & Evans, 2016).

Traditionally, higher laundering temperatures were used by consumers to launder textiles when compared to consumers of today (Gattlen et al., 2010). This may be due to environmental concerns, possible alterations in laundering products, or new wash settings offered on washing machines (Klint et al., 2022). A Norwegian survey revealed that the average wash temperature used by consumers is around 48 °C (Laitala et al., 2012). While another study in the United Kingdom (UK) found that 50% of their participants used temperatures of 40 °C to launder most of their clothing (Yates & Evans, 2016). However, 60% of consumers laundered their delicate garments using temperatures below 30 °C. In terms of laundering products, the average consumer in the UK uses about three laundry aids (e.g., stain removers, softener, colour enhancers) (Yates & Evans, 2016).

The frequency at which an individual chooses to launder their clothing varies and is based on the intended use of the garment. In the UK, 91% of consumers will launder their underwear after one to two wears, while 51% of the same respondents will launder their regular

clothing in the same time frame (after one to two wears) (Yates & Evans, 2016). The majority of Finish consumers (80%) did laundry at least once a week (Miilunpalo & Räisänen, 2019). Miilunpalo and Räisänen, (2019) discovered that 71.7% of their participants chose to wash their clothing items by hand at least one to two times per year despite having the option to use the delicate washing cycle feature on their laundry machine. Based on these studies it is evident that while consumers have a vast range of selections to make when laundering their garments, they all want the same result of clean, odour-free textiles (Laitala et al., 2012; Miilunpalo & Räisänen, 2019).

2.8 Summary

The overall scope of literature surrounding bacterial build-up in everyday clothing is limited except in medical settings where there is a risk of infections being transmitted via textiles. Most of the prior literature is based on bacteria in textiles and focuses on pathogenic bacteria and to a lesser extent odour retention. While it is widely known that laundering can reduce the bacterial load in garments, consumer habits in laundering can greatly affect levels of bacteria and odours present in clothing (Kubota et al., 2012; McQueen et al., 2014; Munk et al., 2001; Riley et al., 2017). The literature provides evidence that lower wash temperatures are less effective in completely removing bacterial populations, however, lower wash temperatures are often used to launder clothing within households. Due to the incomplete removal of bacteria in clothing there is the potential for build-up of bacteria with repeated use and laundering. Therefore, more research is required to examine the relationship between bacteria build-up in garments over time in conditions that may reflect domestic laundering.

Chapter 3

Methods

3.1 Experimental fabrics

The fabrics used in this study were 100% cotton and 100% polyester interlock knit fabrics of similar weight and thickness. Fabric properties are detailed in Table 3.1. The fabrics selected were measured in accordance with the test methods CAN/CGSB-4.2 No.5.1-M90 (Canadian General Standards Board, 2013) for the unit mass and thickness, CAN/CGSB 4.2 No.37-M87 (CGSB, 2002).

Table 3.1 Experimental fabrics

Properties	100% cotton	100% polyester
Mass per unit area (g/m^2)	234	224
Thickness (mm)	1.28	1.31
Fabric structure	Interlock knit	Interlock knit
Wales (stitches/cm)	18	16
Courses (stitches/cm)	14	14

3.2 Specimen preparation

In preparation for the experiments, test specimens were cut using a 50 mm circular die fabric punch. Two circles of fabric were then sewn together on two opposite ends of the specimen as shown in Figure 3.1. Following preparation, the specimens were wrapped in tinfoil and then placed into labeled plastic Ziplock bags until use. Prior to the inoculation process, the fabrics were placed into labelled Petri dishes as shown in Figure 3.2.

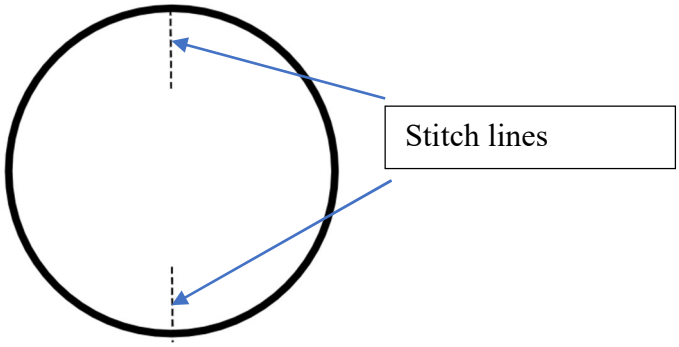


Figure 3.1 Diagram of fabric specimen preparation

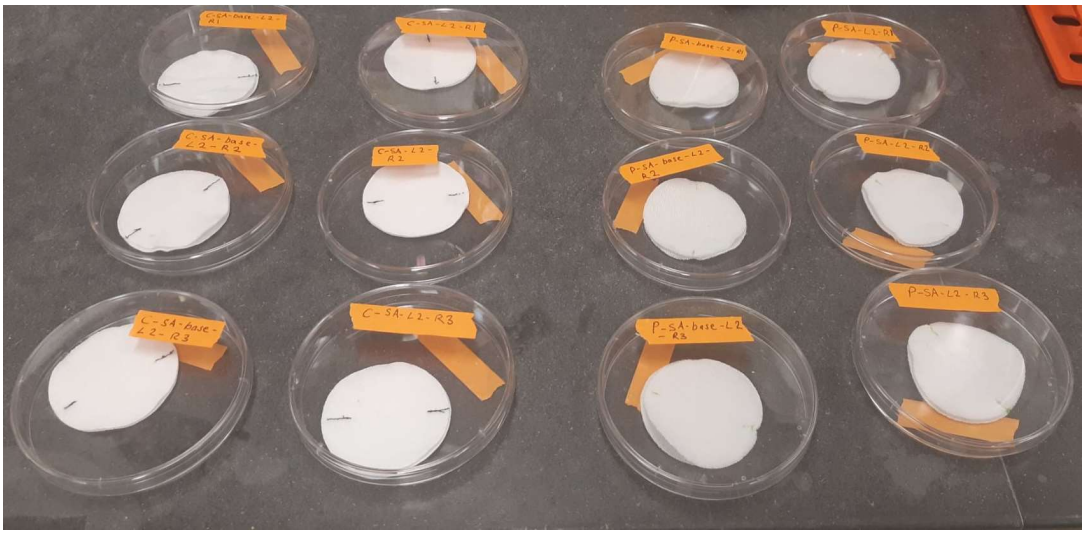


Figure 3.2 Fabric specimens in Petri dishes in preparation for inoculation and storing during experimental work

3.3 Fabric specimens and experimental treatments

The experiment involved preparing three types of fabric specimens which included the baseline, treatment, and control fabrics that were subjected to different numbers of inoculation and wash cycles as shown in Table 3.2. The baseline specimens were used to provide a measurement of the bacterial counts present on the specimen prior to the final wash cycle. The baseline specimens for the L1 fabrics were inoculated once but not laundered; the L2 baseline specimens were inoculated twice and laundered once; and the L5 baseline specimens were

inoculated five times and laundered four times. The treatment specimens were inoculated and laundered once for L1, twice for L2 and five times for L5. Finally, the control fabrics were not inoculated, they were only included in the canisters for laundering, and were laundered once, twice and five times for L1, L2 and L5 respectively.

Table 3.2 Number of inoculation and laundering cycles for each cycle treatment

	Experiment	No. of inoculations	No. of wash cycles
L1	Baseline	1	N.W.
	Treatment	1	1
	Control	N.I.	1
L2	Baseline	2	1
	Treatment	2	2
	Control	N.I.	2
L5	Baseline	5	4
	Treatment	5	5
	Control	N.I.	5

N.W. = not washed; N.I. = not inoculated

3.4 Experimental design

The experimental design was a 2 x 3 factorial design. Two fibre types (cotton, polyester), and three inoculation/wash levels (1, 2, 5) were the independent variables under investigation. The dependent variables were the log colony forming units (CFU) per sample of *S. aureus* extracted from cotton and polyester fabrics, and also the percentage reduction and the percent transfer. Each experimental treatment was conducted in triplicate.

3.5 Experimental procedure

3.5.1 Inoculation of fabrics

Staphylococcus aureus, ATCC No. 6538 was used in this study as the test microorganism. *S. aureus* was chosen since it is representative of Gram-positive aerobic bacteria

that is found frequently on the skin (Belkaid & Segre, 2014). The culture media was nutrient broth and nutrient agar and obtained from Oxoid (Ottawa, ON).

The 24-hour culture broth was prepared by swiping two *S. aureus* colonies using an inoculating loop and then inserting the loop into 10 mL of nutrient broth solution contained in 15 mL corning tubes. Three control broths for each replicate and one sterile control broth without the addition of *S. aureus* were created. The inoculated broths and sterile "control" broth were then incubated for 24 hours at 37 °C. After the 24-hour incubation period, the broths were removed from the incubator. The broths were evaluated to confirm the sterile culture broth was not contaminated and that the inoculated broths were cloudy. The culture broths were vortexed for 30 seconds to agitate the corning tube to ensure the bacteria was evenly incorporated throughout the solution.

To create the test inoculum, 250 µL of each of the 24 h *S. aureus* culture broths were vortexed with 5 mL of distilled water containing 0.05% Triton X 100 solution in 50 mL corning tubes. This resulted in a 1:20 or 5% ratio of nutrient broth to water and 0.05% Triton X 100. Then 1 mL of the inoculum was pipetted in triplicate onto the treatment and baseline specimens for each replicate. The control fabrics were not inoculated.

Baseline and treatment fabric specimens were incubated for 3 hours at 37 °C. After the incubation period, the control, baseline, and treatment specimens were left in labelled Petri dishes and left to stand semi-covered by the Petri dish lid in a room set at 20 ± 2 °C and relative humidity (R.H.) of 65 ± 5% for 72 hours prior to laundering.

3.5.2 Laundering procedure

Fabrics were laundered in the Launder-O-meter (Atlas Electric Devices Co.; Model B-5; Type LHD-EF) following the test method CAN/CGSB-4.2 No. 19.2-2003/ ISO 105-C06:1994.

The laundry protocol conducted by Abdul-Bari et al. (2020) was followed where some modifications were made to the original standard test method. To be more representative of household laundering, the temperature was modified to 30 °C, rather than using 40 °C as suggested in the original test method. Tide® Free and Gentle Liquid detergent was used to be consistent with the detergent used by Abdul-Bari et al. (2020) for odour related research. As well, the original test standard uses a duration of 45 minutes of washing to simulate 5 wash cycles of domestic laundry. As per the protocol of Abdul-Bari et al. (2020) the Launder-O-Meter was stopped after 10 minutes of use to be representative of 1 domestic wash cycle.

Reverse osmosis water was used to create the wash liquor and for the rinsing of specimens after laundering. The water was boiled for 15 minutes, covered with tinfoil, and then left to cool to room temperature to reduce the likelihood of contamination. 10 steel balls and the fabric specimens were added to each canister. The steel balls were used to agitate the fabric specimens during washing. The washing liquid was prepared using 5 mL of detergent per 1 L of water for a detergent concentration of 0.5%. The wash liquid was then heated to 30 °C. 250 mL of the wash liquor was poured into each canister before capping and securing into the Launder-O-meter. The water bath temperature of the Launder-O-meter was set to 30 °C.

The washing cycle was carried out for 10 minutes using 40 ± 2 rotations/minute. After the 10-minute wash cycle was completed, the canisters were removed from the Launder-O-meter. The washing liquid was poured into a beaker while the fabric specimens and steel balls were caught in a sieve. The fabrics were removed from the sieve using sterilized tweezers and then rinsed in 250 mL of distilled water. Clean paper towels were used to blot the fabric specimens before each specimen was placed back into its assigned Petri dish. Fabric specimens were left to air dry in standard room conditions (i.e., 20 ± 2 °C and a R.H. of 65 ± 5 %) for 72

hours if undergoing another wash cycle, or for 48 hours if this was the final wash cycle before extraction.

3.5.3 Negative controls

Negative control fabrics were implemented to identify if any cross-contamination occurred during the laundering process since there was cross-contamination in the L1 wash cycle. These negative controls were laundered in individual canisters separate from the inoculated and control fabrics and then underwent identical washing and drying processes as the other specimens. The negative controls were included at each wash cycle for the L2 and L5 wash cycles.

3.5.4 Inoculation/multiple wash cycles

The entire procedure of inoculation, washing, drying and bacterial extraction was completed once, twice and five times for the multiple wash cycles as shown in Figure 3.3. Baseline fabrics did not undergo a wash cycle directly prior to the extraction process while the control and inoculated fabrics were laundered and then left for 48 hours to dry after the final wash cycle.

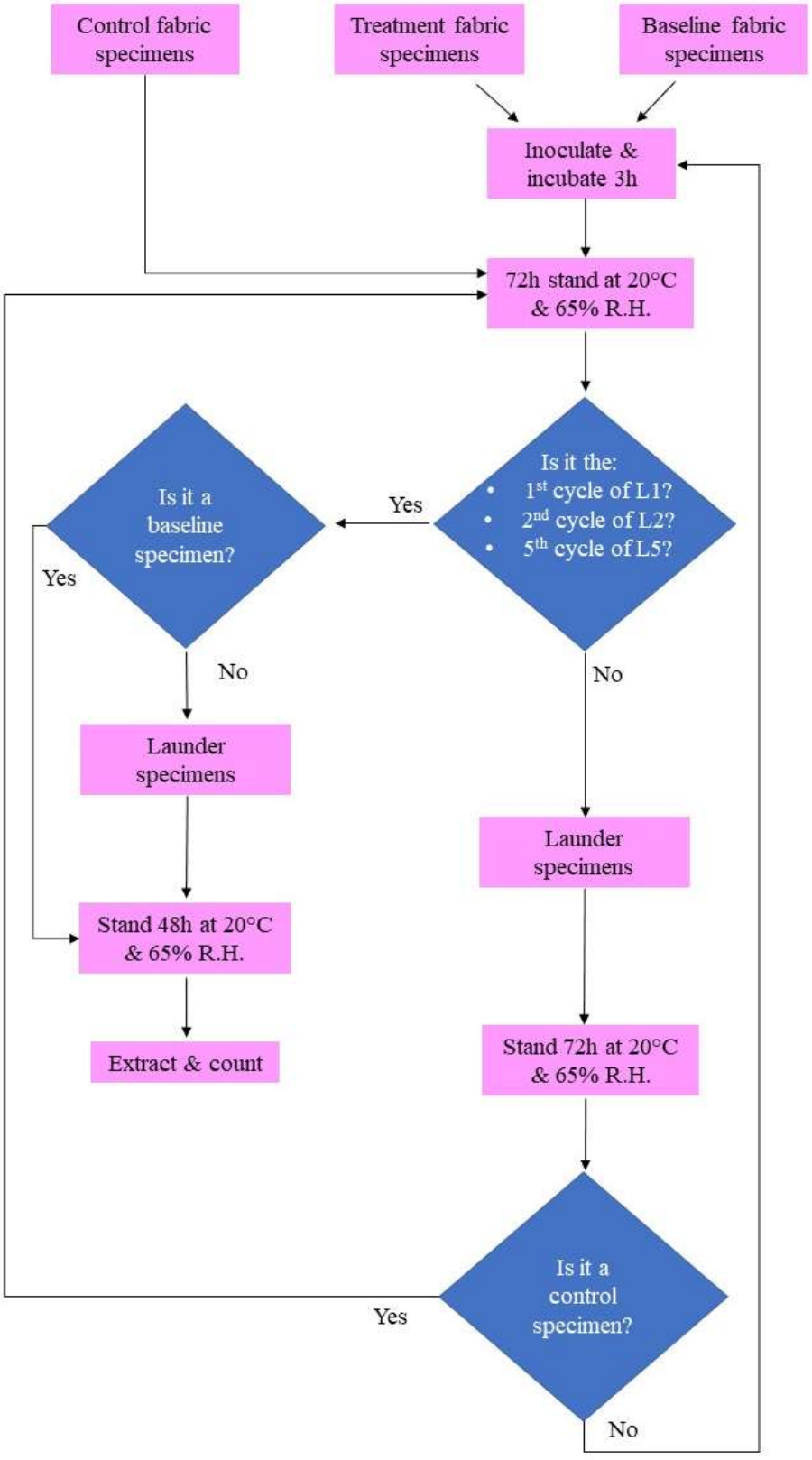


Figure 3.3 Inoculation to extraction process overview

3.5.6 Bacterial extraction protocol and colony analysis

Each specimen (baseline, treatment, and control) was placed into a 50 mL conical tube using sterilized tweezers containing 30 mL of saline phosphate buffer solution (PBS) and then vortexed for 30 seconds. Serial dilutions were made from the vortexed PBS. Then aliquots of 15 µL were drop-plated onto nutrient agar plates in triplicate for the appropriate dilutions. The agar plates were then incubated at 37 °C for 24 hours before the bacterial colonies were counted. The data recorded for the viable bacteria counts were calculated into colony forming units per millilitre (CFU/mL) and then converted to CFU/sample by multiplying by 30. The limit of detection was <22.22 CFU/per mL. The calculation used to calculate the CFU/per mL is given in Equation 3.1.

$$\left(\frac{Average\ number\ of\ colonies}{dilution}\right) \times \left(\frac{1000}{15}\right) = CFU/mL \tag{3.1}$$

The percentage reduction was calculated to provide information on the proportion of bacteria relative to the baseline that were removed from the fabrics after their final wash cycle. The calculation is provided in equation 3.2 below.

$$\frac{(Baseline\ CFU/mL - Treatment\ CFU/mL)}{Baseline\ CFU/mL} \times 100 = \%\ reduction \tag{3.2}$$

The transfer percentage was used to calculate how much bacteria is being transferred during the wash cycle from the baseline fabrics which were inoculated with *S. aureus* to the uninoculated control fabrics. Percentage transfer was calculated using Equation 3.3.

$$100 - \left(\frac{(Baseline\ CFU/mL - Control\ CFU/mL)}{Baseline\ CFU/mL} \times 100\right) = \%\ transfer \tag{3.3}$$

3.6 Statistical analysis

Descriptive statistics (i.e., mean, standard deviation (SD), minimum and maximum values) were calculated for the bacterial counts (i.e., control, treatment, baseline) for $\log_{10}$ CFU/sample. For the baseline fabrics, a two-way analysis of variance (ANOVA) was used to determine the significant differences between the cotton and polyester baseline fabrics for each cycle (i.e., L1, L2, L5) were carried out followed by Tukey's range post-hoc tests when significant differences were found. Due to contamination experienced on the cotton L1 fabric specimens they were excluded from further analysis for the treatment and control fabrics (see Section 4.1.2). Therefore, for the treatment and control fabrics a two-way ANOVA was conducted to determine whether there were differences between fibre type and two of the inoculation/wash cycles (i.e., L2 and L5). An additional one-way ANOVA was performed to determine the significance differences among the three cycles for polyester fabrics.

For the percent reduction and percent transfer the data did not meet the assumptions of normality, therefore non-parametric tests were used instead of ANOVAs (Willard, 2020). To calculate the significant differences for both the percent reduction and percent transfer, Kruskal-Wallis, Mann-Whitney and Wilcoxon tests were used. Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS), Version 28.

Chapter 4

Results

This chapter is arranged in two sections. The first section presents results from the bacterial counts per sample including the baseline, treatment, and control fabrics. In the second section, the results of the percentage reduction and transfer of bacteria for the test fabrics are presented.

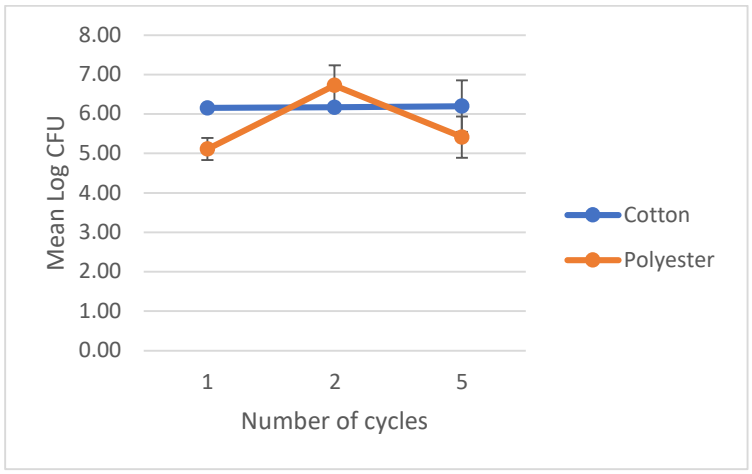
4.1 Bacterial counts per sample

Bacterial counts obtained from each fabric sample were reported as the mean log colony forming units (CFU) per sample. The summary of the log CFU data for baseline, treatment and control cotton and polyester fabrics are displayed in Table 4.1 and Figure 4.1. Baseline, treatment, and control bacterial counts were obtained from fabric specimens at the three selected cycles.

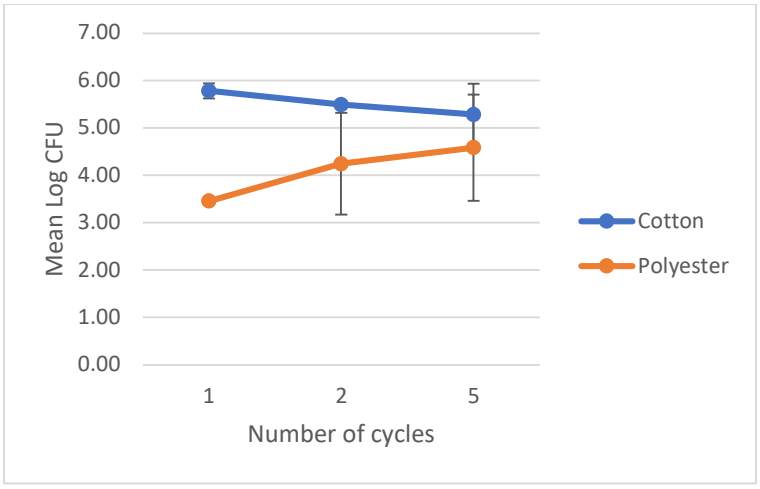
Table 4.1 Summary of fabric bacterial counts (log CFU per sample)

		Cotton				Polyester			
Number of cycles		**Mean** (n=3)	**SD**	**min**	**max**	**Mean** (n=3)	**SD**	**min**	**max**
L1	Baseline	6.16	0.04	6.12	6.20	5.12	0.28	4.80	5.32
	Treatment	5.79	0.16	5.66	5.96	3.46	0.06	3.43	3.52
	Control	5.67	0.33	5.33	5.98	3.57	0.39	3.12	3.87
L2	Baseline	5.77	0.69	4.96	6.17	6.73	0.51	6.14	7.03
	Treatment	5.49	0.08	5.40	5.56	4.25	1.07	3.30	5.41
	Control	3.02	0.35	2.82	3.43	3.29	0.81	2.82	4.22
L5	Baseline	6.20	0.65	5.78	6.95	5.42	0.53	5.01	6.01
	Treatment	5.29	0.65	4.86	6.03	4.59	1.12	3.30	5.36
	Control	3.91	1.39	2.82	5.48	3.49	1.15	2.82	4.81

a. Baseline fabrics



b. Treatment fabrics



c. Control fabrics

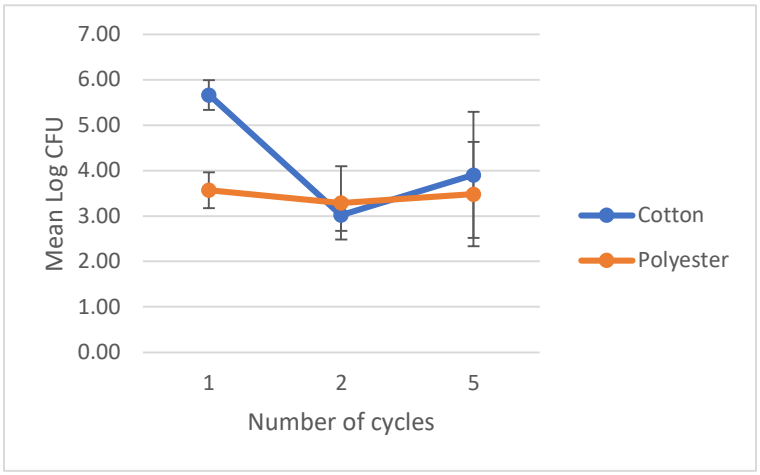


Figure 4.1 Mean log/CFU per sample for cotton and polyester fabrics

4.1.1 Baseline fabrics

For baseline fabrics that were not washed for the final cycle, the mean log CFU among all the fabric samples ranged from 5.12±0.28 to 6.73±0.51, for polyester L1 and polyester L2 respectively. For the L1 baseline specimens, the highest mean log CFU was found on cotton at 6.16±0.04, whereas on polyester L1 baseline specimens the log CFU was 5.12±0.28. Baseline L2 cotton specimens were lower than the L1 mean at 5.77±0.69 log CFU. In contrast, the L2 polyester baseline specimens increased in bacterial counts compared to L1 counts at 6.73±0.51 log CFU. The mean L5 cotton baseline specimens displayed the highest bacterial counts of the three time periods among cotton specimens at 6.20±0.65 log CFU. However, the L2 polyester baseline specimens had a higher mean than both polyester L1 and L5 with mean counts of 6.73±0.51 log CFU.

A two-way analysis of variance (ANOVA) to determine differences among baseline fabrics in fibre content (cotton and polyester) and cycles (L1, L2 and, L5) are shown in Table 4.2. There were no significant differences found for fibre content, which meant that the quantity of bacteria remaining on cotton and polyester fabrics could not be considered different. However, there was a significant difference for cycle ($F_{2,18}$ =6.334, $p < 0.05$) and a significant difference in the interaction between fibre and cycle ($F_{2,18}$ =6.409, $p < 0.05$). Since there were significant differences in cycle and the interaction between fibre and cycle, then Tukey's range test post-hoc tests were used to determine which levels differed from one another. The Tukey range tests for the baseline fabrics are shown in Table 4.3. For main effects, the wash cycle L2 was significantly different from L1. For the interactions between fibre and wash cycle, polyester L2 was significantly different from polyester L1 and L5. No other significant differences were found between the two groups.

Table 4.2 Two-way ANOVA significance of fibre content (cotton and polyester) and wash cycles (L1, L2 and L5) on baseline fabrics

Source of variation	d.f.	SS	MS	F	p<
Fibre	1	0.801	0.801	4.629	NS
Wash cycle	2	2.193	1.096	6.334	0.05
Fibre vs. wash cycle	2	2.219	1.110	6.409	0.05
Error	12	2.077	0.173		
Total	8	647.594			

NS = non-significant at p>0.05

Table 4.3 Significance differences of wash cycles and comparison of baseline fabrics fibre vs. wash cycle significance. Tukey's range tests.

Source	Mean	n	Tukey groupings
Wash			
L1	5.64	6	
L5	5.81	6	
L2	6.45	6	
Fabric/wash			
Polyester/L1	5.12	3	
Polyester/L5	5.42	3	
Cotton/L1	6.16	3	
Cotton/L2	6.17	3	
Cotton/L5	6.20	3	
Polyester/L2	6.73	3	

Means grouped by vertical lines are not-significantly different at $p \leq 0.05$

Revisiting the first hypothesis (H1), *that for baseline fabrics the bacterial counts extracted from polyester will be significantly greater than bacterial counts extracted from cotton for each inoculation/wash cycle*, the research hypothesis 1 was rejected. As Table 4.2 indicates there were no significant differences in bacterial counts due to fibre content for each inoculation/wash cycle.

For the hypothesis H3a, it was proposed *that as the number of inoculation/wash cycles increase the bacterial load will significantly increase on all fabrics for baseline fabrics*. Research hypothesis 3a was rejected. Although the wash cycle L2 was significantly different from L1 for the baseline fabrics, L1 was not significantly greater than L5. Therefore, there was no consistent trend with increasing bacterial numbers as the number of cycles increased.

4.1.2 Treatment fabrics

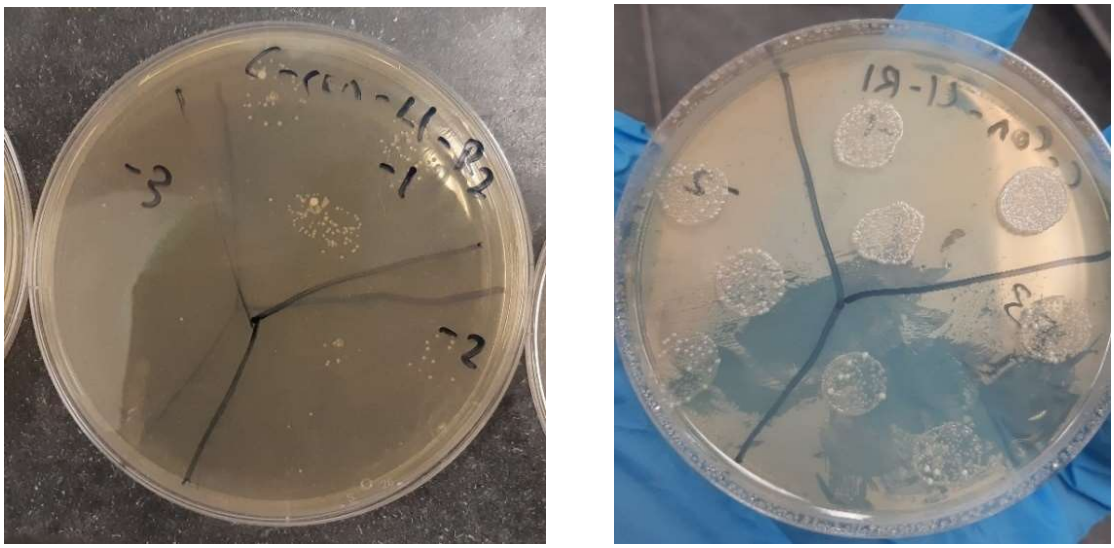
Bacterial counts from the treatment fabric specimens were extracted after the final wash cycle that specimens were subjected to. That is, the L1 specimens had been inoculated and washed once, L2 specimens were inoculated and washed twice, and L5 specimens were inoculated and washed five times. The mean log CFU data is represented in Table 4.1 and Figure 4.1b.

The mean log CFU for the treatment fabrics ranged from 3.46±0.06 (polyester L1) to 5.79±0.16 (cotton L1). Between the specimens that had been inoculated and washed once (L1) cotton exhibited the highest mean log CFU per sample at 5.79±0.16 compared to polyester L1 treatment specimens at 3.46±0.06 log CFU. Bacterial counts obtained from the cotton L2 specimens were lower than L1 specimens with a mean of 5.49±0.08. The polyester L2 specimens displayed an increase in bacterial counts compared to L1 polyester at 4.25±1.07 log CFU. The mean log CFU for cotton L5 specimens presented the lowest bacterial counts among the cotton fabrics at 5.29±0.65 log CFU. However, the polyester L5 specimens increased compared to L1 and L2 with a mean 4.59±1.12 log CFU.

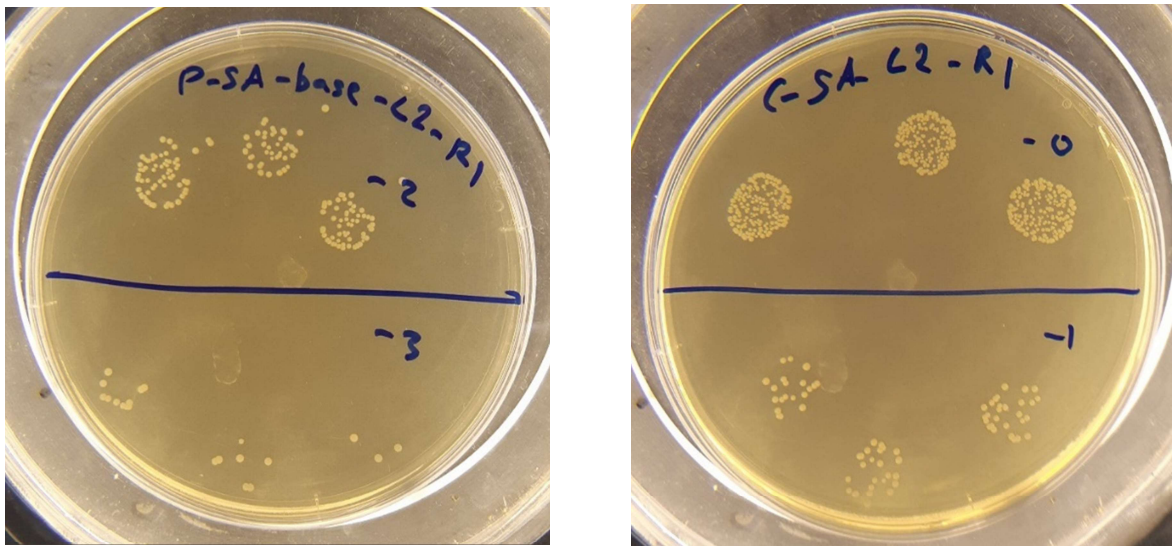
There appeared to be some contamination on the L1 cotton specimens. However, it was difficult to determine whether some of the colonies that were present were the ATCC *S. aureus* strain that had been inoculated onto the fabrics intentionally, or if they were a result of external

contamination. Some of the contamination had clearly arisen from external sources. For example, many of the colonies from the contamination appeared as small off-white pin pricks at more diluted levels whereas the *S. aureus* colonies had a glossy appearance, were larger in size, circular, creamy, and yellow. Therefore, only the colonies that appeared to be *S. aureus* were counted. It was suspected that the external contamination may have arisen from the wash water used to launder the specimens during the first laundering cycle, since this contamination was not present on the baseline L1 cotton samples. Subsequently a negative control was introduced for L2 and L5 laundering treatments, but no external contamination occurred in the latter parts of the study. Because of the contamination that impacted the treatment cotton L1 data were excluded from further analysis. Examples of agar plates with and without contamination are displayed in Figure 4.2. Polyester L1 was repeated because initially the undiluted extract was not plated. No colonies were present at a dilution factor of 10^{-1} for the polyester fabrics, however, as countable colonies were present at 10^{-1} for the cotton L1 fabrics these were not repeated.

Due to the contamination found on the cotton L1 specimens, the cotton L1 fabrics were excluded from further analysis. Therefore, a two-way ANOVA for fibre type and wash cycle was conducted for only the L2 and L5 cotton and polyester treated specimens. This was followed by a one-way ANOVA that was carried out for the polyester treatment fabrics. The two-way ANOVA results are shown in Table 4.4. No significant differences among fibre, cycle and fibre x cycle were found (see Table 4.4). Additionally, in the one-way ANOVA no significant differences were found between the inoculation/wash cycles among the polyester treatment fabrics (see Table 4.5).



a) Agar plates with contamination



b) Agar plates without contamination

Figure 4.2 Agar plates with (a) and without contamination (b)

Table 4.4 Two-way ANOVA significance of cotton vs polyester treatment fabrics (L2, L5)

Source of variation	d.f.	SS	MS	F	p<
Fibre	1	2.843	2.843	4.009	NS
Wash cycle	1	0.013	0.013	0.018	NS
Fibre vs. wash cycle	1	0.223	0.223	0.315	NS
Error	8	5.673	0.709		
Total	12	297.333			

NS = non-significant at p>0.05.

Table 4.5 One-way ANOVA significance of polyester treatment fabrics (L1, L2, and L5)

Source of variation	d.f.	SS	MS	F	p<
Between Groups	2	2.011	1.005	1.251	NS
Within Groups	6	4.823	0.804		
Total	8	6.834			

NS = non-significant at p>0.05.

Revisiting the research hypotheses, for the second hypothesis (H2) it was proposed *that for treatment fabrics the bacterial counts extracted from polyester will be significantly greater than the bacterial counts extracted from cotton fabrics following each inoculation/wash cycle*. The bacterial counts for the polyester treatment fabrics were not significantly higher than the bacterial counts from the cotton treatment fabrics after each inoculation/wash cycle (see Table 4.4). Therefore, Hypothesis 2 was rejected.

For the third hypothesis for treatment fabrics (H3b) it was proposed *that as the number of inoculation/wash cycles increase the bacterial load will significantly increase on all fabrics for treatment fabrics*. Hypothesis 3b was therefore rejected as the inoculation/wash cycle increased, the bacterial load of the treatment fabrics did not significantly increase (see Tables 4.4 and 4.5).

4.1.3 Control fabrics

Bacterial counts obtained from the control fabric specimens at each wash cycle are also represented as log CFU per sample (see Table 4.1 and Figure 4.1c). The mean log CFU for the control fabrics ranged from 3.02±0.35 (cotton L2) to 5.67±0.33 (cotton L1). The highest mean log CFU among the L1 control specimens was cotton at 5.67±0.33. The L1 cotton control mean was unexpectedly high compared to the other specimens, however, upon reflection the high counts obtained from the cotton control L1 fabric specimens were again likely due to the contamination that was observed in the treatment fabrics. The L1 log CFU on the polyester control specimens were 3.57±0.39. Control cotton L2 specimens has a mean at 3.02± 0.35 log CFU. The polyester L2 control specimens showed a decrease in bacterial counts compared to the polyester L1 counts at 3.29±0.81 log CFU. The mean cotton L5 control specimens displayed a mean of 3.91± 1.39 log CFU, whereas the polyester control L5 specimens had a mean of 3.49±1.15 log CFU. As a result of the contamination that appeared on the cotton L1 fabrics the cotton L1 control counts were not used further in the analysis.

The two-way ANOVA to examine the effect of fibre (cotton, polyester) and cycle (L2, L5) for the control fabrics is shown in Table 4.6, and the one-way ANOVA which was carried out to examine the effect of the cycle (L1, L2, L5) for polyester fabrics is shown in Table 4.7. There were no significant differences among fibre and cycle when both fibre types were included (see Table 4.6) nor any among the three wash cycles for the polyester fabrics (see Table 4.7). This suggests that during the repeated inoculation wash cycles that there was no accumulation of bacteria that transferred to the control fabric as the number of cycles increased. Nor any differences in the amount transferred from fabric samples associated with the type of fibre the fabric was composed from.

Table 4.6 Two-way ANOVA of control fabrics fibre vs. wash cycle (L2 and L5) polyester and cotton fabrics

Source of variation	d.f.	SS	MS	F	p<
Fibre	1	0.019	0.019	0.019	NS
Wash cycle	1	0.876	0.876	0.871	NS
Fibre vs. wash cycle	1	0.355	0.355	0.353	NS
Error	8	8.046	1.006		
Total	12	150.261			

NS = non-significant at p>0.05.

Table 4.7 One-way ANOVA significance of polyester control fabrics (L1, L2, L5)

Source of variation	d.f.	SS	MS	F	p<
Between Groups	2	0.126	0.063	0.089	NS
Within Groups	6	4.244	0.707		
Total	8	4.37			

NS = non-significant at p>0.05.

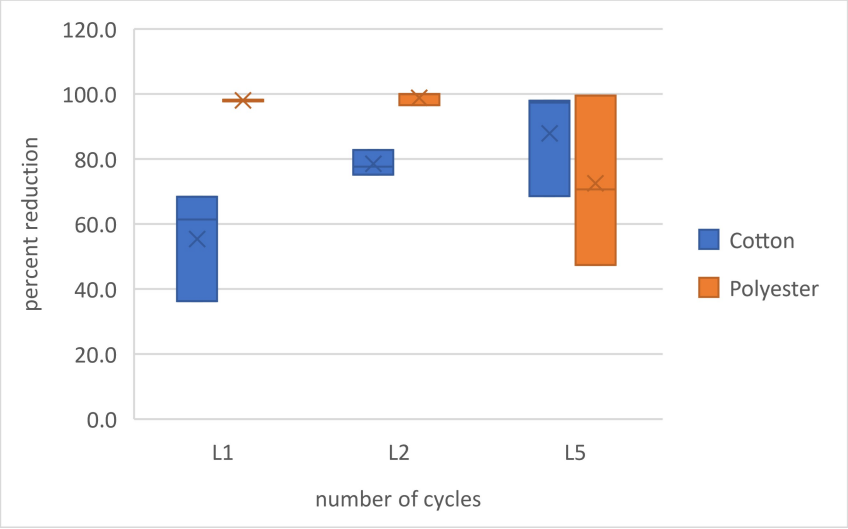
Revisiting the research hypotheses, the third hypothesis related to the control fabrics (H3c) proposed *that as the number of inoculation/wash cycles increase the bacterial load will significantly increase on all fabrics for control fabrics*. Therefore, research hypothesis 3c was rejected as there was no significant increases in bacterial load as the number of cycles increased for the control fabrics (see Tables 4.6 and 4.7).

4.2 Percent reduction and transfer of bacteria

4.2.1 Percent reduction of bacteria due to laundering

Percentage reduction was used to provide information on the proportion of bacteria relative to the baseline that has been removed from the fabric after its final wash cycle. The percent reduction data of the cotton and polyester specimens are shown in Figure 4.3. The percent reduction for the cotton L1 specimens varied largely as they ranged from 36.3% to

68.4%. The contamination in the L1 cotton specimens may be a reason for this large spread in percent reduction. Whereas the percent reduction for the cotton L2 specimens ranged from 75.2% to 82.8%. For the cotton L5 specimens the percent reduction ranged from 68.5% to 97.9%. There was less variability in the percent reduction for the polyester specimens. The polyester L1 specimens ranged from 97.7 % to 98.2%. The polyester L2 specimens ranged from 96.5 % to 100% and the polyester L5 specimens had a percent reduction ranging from 47.4% to 99.5%.



Note: Each bar represents the specimens from the same cycle and fibre type as per the legend. The horizontal line in the middle of each bar represents the median. The mean values are depicted by the X. The length of each bar depicts the specimen spread.

Figure 4.3 Percentage reduction per sample

The Kruskal-Wallis test was used to evaluate whether there were significant differences in the percent reduction among the three cycles for polyester fabrics (Table 4.8). No significant differences were found among the three cycles.

Table 4.8 Kruskal-Wallis comparisons for polyester percentage reduction

	Mean Ranks			Kruskal-Wallis test results			
	L1	L2	L5	N	H	df	p-value
polyester	5.00	6.67	3.33	3	2.241	2	NS

NS = non-significant at p>0.05.

The Mann-Whitney U and Wilcoxon test was used to determine whether significant differences were apparent in the percent reduction between fibre (cotton versus polyester) for L2 and L5 cycles, and between L2 versus L5 for cotton fabrics only (Table 4.9). For the L2 cycle a significant difference in the percent reduction between the two fibre types was found ($U = 0$, $p < 0.05$). However, no other significances were found among cotton and polyester (L5) for fibre type for the percentage reduction. Likewise, no significant differences were found between cycles L2 and L5 for cotton.

Table 4.9 Mann-Whitney and Wilcoxon comparison for percentage reduction

	L2		L5		L2	L5
	cotton	**polyester**	**cotton**	**polyester**	**cotton**	
Mean	2	5	3.67	3.33	3	4
Sum of ranks	5	15	11	10	9	12
Mann-Whitney U	0		4		3	
Wilcoxon W	6		10		9	
p<	0.05		NS		NS	

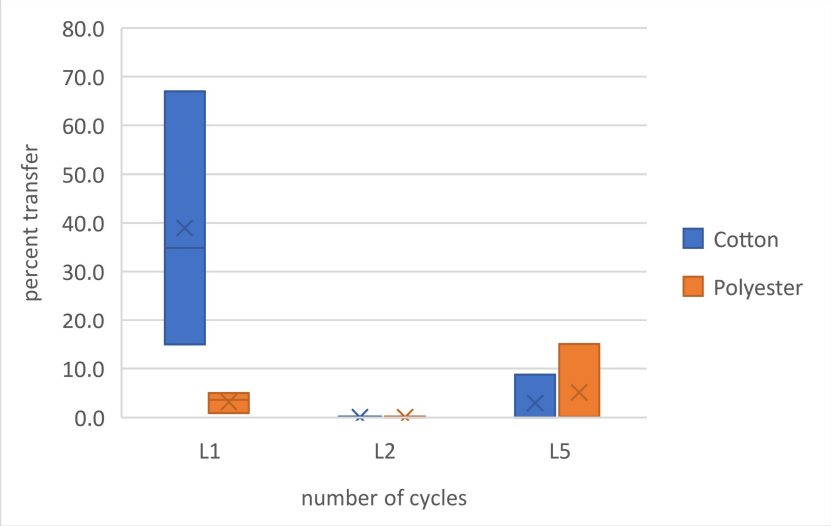
NS = non-significant at p>0.05.

Revisiting the research hypotheses, the fourth hypothesis (H4) proposed *that cotton fabrics will exhibit a greater bacterial percentage reduction than polyester fabrics.* Based on the results hypothesis H4 was rejected. This is because there was no significant difference found between the two fibre types for L5, and that cotton was significantly lower percent reduction than polyester.

4.2.2 Transfer of bacteria to the control fabrics

The percentage transfer calculates the relative proportion of bacteria that transferred during the wash cycle from the fabric samples inoculated with *S. aureus* to the uninoculated control fabrics. The calculation was based on the quantity of bacteria extracted from the baseline fabrics, although in reality bacteria would have transferred from the treatment fabrics as well.

Figure 4.4 presents the percent transfer from the inoculated fabrics to the control fabrics. The bacterial transfer was minimal except for the cotton L1 specimens which ranged from 14.9% to 67.0%. Again, this is likely caused by the contamination and relatively high numbers that were initially counted as *S. aureus* on the control fabrics. The L2 and L5 cotton specimens display less variability as the range spanned from 0% to 0.2% and, 0% to 8.7% respectively. The polyester percentage transfer varied less among the three difference wash cycles. The L1 polyester specimens extended from 0.9% to 5.0%, the L2 specimens from 0.0% to 0.2% and the L5 specimens ranged from 0.0% to 15%.



Note: Each bar represents the specimens from the same cycle and fibre type as per the legend. The horizontal line in the middle of each bar represents the median. The mean values are depicted by the X. The length of each bar depicts the specimen spread.

Figure 4.4 Percentage transfer per sample

Table 4.10 shows the Kruskal-Wallis test the polyester fabrics among the three wash cycles (i.e., L1, L2, L5). No significant differences were found in the percent transfer as the number of cycles increased. Mann-Whitney U and Wilcoxon tests were used to determine whether significant differences were apparent in the percent transfer between fibre (cotton versus

polyester) for L2 and L5 cycles, and between L2 versus L5 for cotton fabrics only (Table 4.11). No significant differences were found in the percent transfer between the two fibre types for either L2 or L5 cycles. Furthermore, no significance differences were found in the percent transfer between cycles L2 and L5 for cotton.

Table 4.9 Kruskal-Wallis comparisons for polyester percentage transfer

	Mean Ranks			Kruskal-Wallis test results			
	L1	**L2**	**L5**	**N**	**H**	**df**	**p-value**
polyester	7	2.67	5.33	3	3.887	2	NS

NS = non-significant at p>0.05.

Table 4.10 Mann-Whitney and Wilcoxon comparisons for percentage transfer

	L2		**L5**		**L2**	**L5**
	cotton	**polyester**	**cotton**	**polyester**	**cotton**	
Mean	4	3	2.67	4.33	3.33	3.67
Sum of ranks	12	9	8	13	10	11
Mann-Whitney U	3		2		4	
Wilcoxon W	9		8		10	
p<	NS		NS		NS	

NS = non-significant at p>0.05.

Revisiting the research hypotheses, the final hypothesis (H5) proposed *that polyester fabrics will exhibit a greater bacterial percentage transfer than cotton fabrics.* The H5 hypothesis was rejected as there were no significant differences found between the fibre types for any wash cycle.

Chapter 5

Discussion

5.1 Effects of fibre type on bacterial build-up

There is evidence that different bacterial species can persist on fabrics that vary by fibre content in different quantities or for varying lengths of time (Callewaert, De Maeseneire, et al., 2014; McQueen et al., 2007; Teufel et al., 2010). When studying the longevity of bacteria on different fibres, McQueen et al., (2007) observed a faster decline in bacteria on polyester than on both wool and cotton. Whereas Callewaert et al. (2014) found during an *in vitro* study that polyester retained higher loads of *Micrococcus* species than cotton. This group of researchers speculated that this increase in retention of *Micrococcus* on polyester could be credited to polyesters' "poor adsorbing properties" (Callewaert et al., 2014, p. 6617). In the current study, fibre content appeared to have no effect on the quantity of bacteria prior to laundering regardless of the number of cycles that *S. aureus* was inoculated onto the fabrics. This was evident as there were no significant differences in bacterial counts between polyester and cotton baseline fabrics after two cycles, and also after five cycles. As the extraction from the baseline fabrics was carried out 72 hours after the final bacteria inoculum on the fabrics then this may be one reason why differences between fibre types were not apparent. Possibly if the bacteria were left on the fabrics for longer where bacteria proceeded through their typical growth cycle (i.e., exponential growth, stationary phase and decline phases) (Cooper, 2012) then a decline in bacterial numbers may have occurred that might have resulted in differences between the two types of fabrics. A greater difference over time due to fibre type may have been observed if the bacteria were left on the fabrics for a longer period like in the research conducted by McQueen et al (2007) where bacterial populations were measured after 1, 7, and 28 days following use.

The main purpose of the current study was to determine whether there was a build-up of bacterial populations after repeated use (inoculation) and laundry cycles. Therefore, examining the quantity of bacteria on polyester fabrics and cotton fabrics following multiple inoculation then laundering cycles (i.e., the treatment fabrics) was pertinent to the study. When drawing comparisons between the bacterial counts (log CFU per sample) of the cotton and polyester treatment fabrics, cotton had higher loads of *S. aureus* consistently. Again, like the baseline fabrics, the bacterial counts on the treatment fabrics were not significantly different between polyester and cotton after two cycles, nor after five cycles. The present research aligns with another work where the researchers did not find significant differences in bacterial counts between cotton and polyester after 20 repeated wash and wear cycles were implemented (McQueen et al., 2014). As well, following laundering, fibre content appeared to have no effect on the bacterial counts from the control fabrics. This was not unexpected given that there were no differences in the number of bacteria for the treatment fabrics and that the control fabrics were not inoculated with *S. aureus*. Rather the bacteria on the control fabrics were *S. aureus* colonies that had transferred from the inoculated fabrics to the control fabrics during the wash cycles.

When the influence of fibre type on percent reduction was assessed, there was a significant difference between cotton and polyester after two cycles, However, after five wash cycles there was no significant differences between cotton and polyester in percent reduction. It was somewhat unexpected that no difference in bacterial reduction between fibre types were found after five cycles since fibre properties vary between cotton and polyester and previous studies have illustrated that polyester binds in higher volumes of *S. aureus* than cotton (Takashima et al., 2004). Whilst cotton is a hydrophilic fibre, polyester fibres are hydrophobic (Kadolph & Marcketti, 2017).

5.2 Repeated inoculation and wash cycles

After clothing is worn multiple times there can be a build-up of odour, bacteria, sebum and sweat from contact with skin (Callewaert, De Maeseneire, et al., 2014; Munk et al., 2000). If bacteria accumulation occurs in textiles this can result in unpleasant odours (Abdul-Bari et al., 2018; McQueen et al., 2014; McQueen et al., 2021; McQueen & Vaezafshar, 2020). Although an increase in bacterial build-up in the treatment fabrics with additional wash cycles was expected in this study given the report from Monticello (2019), this was not the case. No bacterial build-up in the treatment specimens was detected as the number of wash cycles increased from one up to five washes. Despite L2 polyester baseline fabrics being significantly greater than those found on L1 cycle, since the bacterial counts on the baseline polyester L2 specimens were also significantly higher than L5 baseline specimens then it is unlikely that this significant difference was due to an accumulation of bacteria over repeated use. Rather, this statistically significant difference may be due to initially higher bacterial counts among all the *S. aureus* 24 h culture broths inoculated onto the baseline polyester L2 fabrics attributing to the higher accumulation in the L2 specimens. Given there was an increase in bacterial counts on polyester L2 and then a decline in L5, this means it is unlikely there was an accumulation of bacteria in the L2 polyester specimens related to repeated use.

The incomplete removal of bacteria in textiles has been shown in multiple experiments as bacteria may remain on textiles after laundering (Nordstrom et al., 2012; Riley et al., 2017; Wiksell et al., 1973). Although, many of the cotton and polyester specimens displayed a decrease in bacteria after washing, there were *S. aureus* that remained on both the treatment and control fabrics in the present study. Laundering has been shown to have a substantial impact in lowering the number of bacterial counts on both cotton and polyester (McQueen et al., 2014). The

reduction in bacterial numbers after laundering has been well documented (Colclasure et al., 2015; Honisch et al., 2014; Munk et al., 2001; Wiksell et al., 1973). Therefore, in the current research it was expected that bacterial levels for the treatment fabrics would be lower after laundering than their respective baseline fabric specimens which had not gone through the final laundering cycle. Throughout this study, there was no contrast in bacterial counts from fibre type and the number of wash cycles except in the baseline fabrics. Therefore the results from the current study do not align with prior speculation that theorizes that bacteria build-up increases with time and after multiple washings (Monticello, 2019) since there was no significant differences in bacterial load with increasing inoculation/wash cycles. Additionally, no significant differences were found for either percent transfer or percent reduction with the increasing number of cycles. Based on the findings from this research, it seems that there is no bacterial build-up as number of use cycles increase.

In the current study, bacterial colonies remained on many of the fabrics post-laundering. The fact that bacterial colonies did remain on the fabric following laundering could be to do with the low temperature of the wash water. The temperature of the wash water used in laundering will affect the number of bacteria that survive the laundering process (Riley et al., 2017; Wiksell et al., 1973). *S. aureus* has been found to remain on textiles even when a wash water temperature of 50 °C was used (Munk et al., 2001). Based on this, it was expected that *S. aureus* would be present on the specimens after washing since a temperature of 30 °C was used in this study. Thus, for this reason it was not entirely surprising that all the treatment fabrics were still contaminated with *S. aureus* post laundering. Higher wash temperatures are generally used to launder textiles that are expected to have been contaminated with *S. aureus* including medical textiles such as scrubs and white coats (Munoz-Price et al., 2012; Riley et al., 2017; Tano &

Melhus, 2014). Therefore when lower wash temperatures are used, it is expected that higher levels of bacteria will remain on specimens post laundering (Munk et al., 2001).

The transference of microorganisms from inoculated to uninoculated fabrics during washing is more likely to occur when low wash temperatures are used (Davis & Ainsworth, 1989; Honisch et al., 2014; Munk et al., 2001). As expected, the ability of fabrics to transfer bacteria to other garments may vary due to fibre type. In the existing research, the bacteria transferred from the treatment fabrics to the control fabrics was very minimal except in one group of specimens where contamination was present (i.e., L1 cotton). Since bacteria transferred from the treatment to the control specimens for both cotton and polyester fabrics, this supports other works where the transference of microorganisms to other textiles during laundering has been reported (Davis & Ainsworth, 1989; Riley et al., 2017). Aside from microorganisms that may be exchanged among textiles during washing, bacteria from the washing machine can be transferred to textiles via washing (Callewaert et al., 2015; Gattlen et al., 2010). When bacteria from the washing machine transfers to textiles, odour-causing bacteria may be distributed throughout the washing machine and contaminate textiles further (Callewaert et al., 2015). Nonetheless, based on the results of the current study it is difficult to assume that repeated wear and wash cycles will contribute to malodour based on bacterial transference alone.

5.3 Limitations to the study

There were some limitations to this study that may have influenced the results. As well, there were some practical barriers that impacted how the current research was carried out. Initially, for the research wear trials were planned for the experimental work. Conducting wear trials would have reflected more realistic circumstances of bacterial build-up in textiles, where not only bacteria was transferred but also sebum and other nutrient sources present in human

sweat. However, due to the beginning of the pandemic and subsequent COVID-19 restrictions a laboratory based *in vitro* study was carried out instead. This was because working with human participants became more challenging given public health guidelines and restrictions. Furthermore, the current study was limited to only one microorganism due to having limited access to the laboratory and subsequently a reduced time to conduct the experimental work (again this laboratory access was impacted by the public health and social distancing restrictions necessary during the pandemic). Other limitations of the present study included contamination of some specimens, and a low number of inoculation/wash cycles.

A major limitation to this research occurred as a result of having insufficient data for the L1 cotton samples. Although, there was a problem with the data collection for all of the L1 specimens, the L1 polyester specimens were repeated while the experimental work was still underway. However, additional experimental work repeating the three replicates for the cotton L1 specimens were not conducted. The reason for repeating the polyester L1 specimens was because initially the undiluted extract was not plated, and no colonies were present at the 10^{-1} level of dilution. Therefore, the decision was made early to repeat all three replicates of the polyester L1 specimens. However, despite the contamination observed on the cotton L1 specimens, it was not initially deemed necessary to repeat them since it looked like it was possible to differentiate between the colonies that were *S. aureus* and those that were not. Furthermore, countable colonies appeared at the 10^{-1} level of dilution for the cotton L1 treatment and control specimens. The obvious contamination that affected the L1 cotton specimens were tiny, glossy, greyish coloured colonies that were clearly different from the *S. aureus* colonies that were large, circular, creamy, and light-yellow in colour. However, retrospectively, since the colony counts on the treatment and control fabrics were too high it was clear that some of the

additional colonies that were originally assumed to be the ATCC *S. aureus* strain were likely to be other *Staphylococcus* species that were likely contamination. This problem was not identified until after the experimental work had been completed. Again, due to COVID-19 restrictions it was not possible to gain access to the laboratory to repeat the cotton L1 experimental work.

As a result of the problem of contamination that was identified after the first round of L1 specimens had been completed, negative controls were introduced into the study to check for contamination that appeared to have occurred during the laundering phase. Fortunately, no further contamination was observed during the latter part of the experimental work for the L2 and L5 wash cycles as the negative control samples did not have countable colonies. However, another limitation to the current study was that negative control specimens were not factored into the research from the beginning.

In future research, additional bacterial species should be included as this study only used one strain of bacteria. Other researchers have used the various types of bacteria such as corynebacteria, *Micrococcus* species, *Moraxella osloensis*, *E. coli* and *A. calcoaceticus* to investigate odour causing bacteria and build-up in textiles (Callewaert, De Maeseneire, et al., 2014; Kubota et al., 2012; Varshney et al., 2019). In the healthcare industry, potentially pathogenic organisms have been used frequently in the study of bacteria on textiles (Neely & Maley, 2000; Riley et al., 2017). While applicable in a healthcare setting, potentially pathogenic organisms would likely be less suitable for researching odour build-up over time. Bacterial strains including odour causing strains, and gram-negative strains could be used in addition to other potentially pathogenic bacterial strains.

This study only went up to five repeated inoculation/wash cycles. Based on the data collected, more than five cycles would be needed to determine the impact that bacterial

accumulation and removal has on repeated use cycles, since there were no significant differences in bacterial increase up to five cycles. Increasing the number of inoculation/wash cycles would determine whether there is in fact an accumulation of bacteria in fabrics. Based on other researchers who have made observations regarding bacteria and odour build-up with repeated use, perhaps 10 and up to 25 repeated wash cycles would be a more suitable number to determine if there is an accumulation of bacteria (Abdul-Bari et al., 2020; Chen-Yu et al., 2007; McQueen et al., 2014). In a previous laundering study, Abdul-Bari et al. (2020) examined the accumulation of odorants in cotton and polyester fabrics up to 10 wash cycles. Another researcher conducted a wear trial that examined the effectiveness of laundering up to 20 wash cycles to remove bacteria and odours from cotton and polyester t-shirts (McQueen et al., 2014). However, it has been found that some garments may be worn by the consumer for approximately on average 75 times throughout its life cycle, therefore additional wash cycles would be needed for a more accurate representation (Klepp et al., 2020).

In future research these limitations should be addressed by repeating any replicates that had contamination, using multiple strains of odour causing bacteria and using more than five inoculation/wash cycles. A wear trial rather than a laboratory-based study could also be implemented as this might be more representative of bacterial build-up within textiles.

Chapter 6

Summary, conclusions, and recommendations

6.1 Summary

The purpose of this study was to investigate whether bacteria accumulated in apparel fabrics with repeated use/laundering cycles. An *in vitro* laboratory study was employed to compare bacterial counts in polyester and cotton fabric specimens.

Cotton and polyester fabrics were inoculated with *Staphylococcus aureus* and subsequently laundered. The experimental design was a 2 x 3 factorial design, with two fibre types (cotton, polyester) and three inoculation/wash levels (1, 2, 5) as the independent variables. The dependent variables were the *Staphylococcus aureus* counts in colony forming units (CFU) per sample on the cotton and polyester fabrics. Baseline specimens were compared to the treatment fabrics to examine the build-up of bacteria from laundering the treatment specimens. There were no differences in log CFU per sample of *S. aureus* on treatment fabrics as the number of inoculation/laundering cycles increased. This indicates that bacteria did not accumulate on either cotton or polyester fabrics as the number of cycles increased. Some transfer of bacteria from the treatment fabrics to the control fabrics occurred during laundering, however, this transference of bacteria from the treatment to control specimens was minimal for both fibre types.

6.2 Conclusions

The findings of this research are restricted to the bacterial strain, fabrics, wash cycles, and experimental conditions of the present research. Consequently, based on the results the following conclusions can be drawn:

1. Laundering is effective at reducing *S. aureus* from cotton and polyester. However, the incomplete removal of bacteria was consistent across all fibre types and wash levels as *S. aureus* colonies were present on all treatment specimens after laundering.
2. There appeared to be no build-up of *S. aureus* in cotton and polyester fabrics as wash cycles increased as the various wash cycles contained similar bacterial loads.
3. *S. aureus* can transfer between fabrics during the wash cycle and contaminate other fabrics as control specimens for cotton and polyester were contaminated with *S. aureus* at every cycle.
4. There was no difference between cotton and polyester in bacterial build-up as both fibres contained similar bacterial loads.

6.3 Recommendations

The following recommendations are suggested for further understanding of the interactions between fibre type, use/wash cycle and bacteria build-up, based on the findings and limitations of this study:

1. Extend the number of cycles used when conducting a repeated use/laundering study to determine if there is a point at which the number of use/wash cycles does influence bacteria build-up.
2. Use multiple strains of microorganisms to gain a thorough understanding of how different bacteria build-up, transfer and may be removed from textiles.
3. Investigate the effect of microorganism removal during laundering by using a variety of wash temperatures.
4. Employ a wear trial with human participants to examine microorganism build-up and transfer from the body to textiles.

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