Efficacy of a silver treated textile for prevention of microbial activity

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A thesis submitted for the degree of
Masters of Science
at the University of Otago, Dunedin
New Zealand

May 2016

Abstract

The antimicrobial effect of cationic silver has been well established; however evidence of bacteria interactions with antimicrobials has seen limited investigation. This study aimed to investigate the growth and attachment behaviour of resident human skin bacterial strains on an antimicrobial silver fabric. A silver treated textile was supplied by a New Zealand apparel company.

The antimicrobial effect of the silver treated textile was investigated in two parts. Firstly the efficacy of the antimicrobial was tested against a selection of known residential human skin bacterial strains. Secondly the bacterial growth behaviour of the bacterial strains on fabric was examined against the silver treated textile (nonwashed and washed), alongside a woven and knit control of similar structural fabric properties. A novel humidity chamber was designed that enabled the fabrics to be suspended in a controlled environment where the temperature and humidity simulated that of the microclimate at the skin surface. Fabrics were inoculated with bacteria and suspended in the chamber for varying lengths of time that coincide with normal wear of clothing. Bacterial cells were removed from the fabrics and grown over 24 hours in order to quantify bacterial growth on the fabrics. Growth was represented as a percentage of the colonies originally inoculated on the fabric in attempts to quantify bacterial adherence.

The silver treated fabric sold and marketed for antimicrobial purposes was shown not to exhibit any bactericidal effect using the methods set out in this study, regardless of the selected bacterial strain or concentration of bacterial populations. The bacterial activity of the strains on the fabrics did not produce results that were expected. Due to the absence of an antimicrobial effect, the activity of the natural skin bacterial strains on the fabric did not reduce with the addition of the "antimicrobial" treatment. In some cases bacterial activity was increased with the addition of the antimicrobial treatment. Although washing the antimicrobial fabric resulted in a tighter sett, there was no change in bacterial activity. Gram positive and gram negative cell membrane structure differences did not influence bacterial interaction with the fabrics. Anaerobic bacteria did not display fabric interaction until after 6 hours as opposed to one hour for the aerobic bacterial strains. In general the longer the fabric was exposed to the simulated wear environment, the more bacteria grew and the greater the interaction with the fabric. Overall, fabric structure had no affect on bacterial interaction. Majority of the differences recorded in this study were in the order of one log or less which is minimal in terms of bacterial growth.

There is an issue in the textile sector of antimicrobials being marketed as such, with limited proof of bactericidal effect. Although the issue regarding bacterial interactions on antimicrobials still stands, this study brings to light the challenges faced by apparel companies reliant on fabric suppliers. There is the need for transparency in supply chains and standardised international requirements for demonstrating antimicrobial efficacy of antimicrobials. Antimicrobial resistance is an international concern. Manufacturers and consumers need to take care in the use of antimicrobials.

Acknowledgements

There are many people, who without their assistance, support and encouragement, this Masters would not have been completed and I will be forever thankful.

My supervisors Professor Raechel Laing and Associate Professor Cheryl Wilson, for your guidance and knowledge. Thank you for sticking out the distance with me and believing in me until the end.

Professor Phil Bremer, for your knowledge of bacteria and microbial techniques. Thank you for giving me your time.

Michelle Petrie, for laboratory guidance and going above and beyond to help with experimental work. Thank you for investing your time and interest in me and helping me develop my skill set.

The staff and students of the Clothing and Textiles Department, for the advice, support, morning teas and friendship and to the Food Science Department, for sharing your laboratory space with me. Thank you.

Catherine Smith and Veronica Steadman Smith, for their unprecedented love and support, for becoming my family away from home. Thank you Ronnie for your beautiful advice and laughter. Catherine, there are no words in the world to express the gratitude I feel towards you. I am incredibly humbled and forever indebted.

To my beautiful friends, thank you for your support and acceptance. Becky Blain thank you for believing in me and seeing my future even when I couldn't. To Marguerite Larvin, Lauren Dennehy, Elvina Lawrenson, Karina Phillips, Sarah Pearson, Liani Baylis, Charlotte Haselden and the girls of Cosy Dell, thank you for the wines (and Scrumpy), food, laughter and class A banter, you are all the reason I remain sane.

To my beautiful family Janine, Bob, Hayden, Nick, Max and Zac Palmer, thank you for the abundance of love and laughter you bring to my life regardless of the hardships we face. I am so proud of our little unit and incredibly lucky to call you mine.

In memory of my brother, Brendan Palmer, how I wish you were here with me to celebrate these accomplishments. I will forever endeavour to make you proud.

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aerobic	an organism, environment, or cellular process that requires oxygen (Campbell and Reece, 2002)
anaerobic	an organism, environment, or cellular process that lacks oxygen (Campbell and Reece, 2002)
aliquot	a portion of a larger whole, especially a sample taken for analysis (Soanes and Stevenson, 2008)
antibacterial	treatment designed to prevent or mitigate the growth of bacteria, or to kill bacteria (International Organization for Standardization, 2013)
antimicrobial	compounds that kill or inhibit the growth of microorganisms (Schaechter, 2009)
argyria	a greyish discolouration of the skin and blueing of the lips caused by the use of silver (Trop, Novak, Rodl, Hellbom, Kroell and Goessler, 2006)
biocide/bactericidal	agents that kill bacteria and fungi (Simoncic and Tomsic, 2010)
biostat/bacteriostatic	agents that inhibit the microorganisms' growth (Simoncic and Tomsic, 2010)
biofilm	adherent three-dimensional bacterial communities surrounded by a secreted matrix of extracellular polymeric substance (Wirth, Bertuccio, Cao, Lowry and Tilton, 2016)
biofouling	formation of a biofilm (Yu, Cho, Shivapooja, Ista and López, 2013)
cytotoxic	toxin of bacterial nature that causes cell damage (Schaechter, 2009)
finish	a substance or mixture of substances added to a substrate at any stage in the process to impart desired properties (Denton and Daniels, 2002)
gram negative	group of bacteria with a structurally more complex cell wall made of less peptidoglycan (Campbell and Reece, 2002)
gram positive	group of bacteria with simpler cell walls with a relatively large amount of peptidoglycan (Campbell and Reece, 2002)

inoculate	deliberate transfer of material from one source, such as a laboratory culture, to another (Hsieh and Merry, 1986)
inoculum	the material used to inoculate a culture (Heritage, Evans and Killington, 1996)
microbe/microorganism	any species of bacteria, archaea, fungi, algae, protozoa and viruses (Pelczar, 2014)
nanoparticle	a particle that has three dimensions in the nanoscale between 1 and 100nm (International Organization for Standardization, 2010)
nitrification	the assimilation of atmospheric nitrogen by certain prokaryotes nitrogenous compounds that can be directly used by plants (Campbell and Reece, 2002)
pathogen	an organism capable of causing disease (Heritage, et al., 1996)
pellet	compacted bacteria following centrifugation (Peterson, Sharma, van der Mei and Busscher, 2012)
species	a group whose members possess similar anatomical characteristics and have the ability to interbreed (Campbell and Reece, 2002)
strain	cells descended from a single isolation in pure culture, usually from a single colony (American Type Culture Collection, 2010)
supernatant	the fluid that lies above the pellet after separation of a mixture by centrifugation (Heritage, et al., 1996)
symbiosis	an ecological relationship between organisms of two different species that live together in direct contact (Campbell and Reece, 2002)

List of abbreviations

®	registered trademark
°C	degrees Celsius
AATCC	American Association of Textile Chemists and Colorists
Ag^+	cationic silver
Ag^O	elemental silver
AgCl	silver chloride
Ag/kg	silver per kilogram
ANOVA	analysis of variance
ATCC	American Type Culture Collection
BS	British Standard
C.V.	coefficient of variation
CFU	colony forming units
CFU/cm^2	colony forming units per square centimetre
CFU/ml	colony forming unites per millilitre
Da	Daltons
df	degrees of freedom
DNA	deoxyribonucleic acid
EN	European Standard
F	F-statistic
g	gram/grams
g/m^2	grams per square metre
ISO	International Organization for Standardization
kPa	kilo Pascals
log_{10}	logarithmic base 10
$mgkg^{-1}$	milligrams per kilogram
ml	millilitre/millilitres
mm	millimetre/millimetres

mm^2	square millimetre/millimetres
MIC	minimum inhibitory concentration
n	number
NaCl	sodium chloride
nm	nanometre
NS	no statistically significant difference
p	significance level
P. acnes	*Propionibacterium acnes*
Ps. aeruginosa	*Pseudomonas aeruginosa*
QAC	quaternary ammonium compounds
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute
s.d.	standard deviation
SEM	scanning electron microscopy
S. epidermidis	*Staphylococcus epidermidis*
sig.	statistical significance
t	t-statistic
T_0, T_1, T_6	time 0, 1, and 6 hours
TEWL	transepidermal water loss
TSA	tryptic soy agar
TSB	tryptic soy broth
UV	ultraviolet
UVC	ultraviolet C

Chapter 1

Introduction

Antimicrobial agents have many desirable benefits for consumers in Western society. Antimicrobials give us the ability to control the abundance of microorganisms, in particular bacteria, which reside in the human body alone, at ten times that of human cells (Tannock, 1995). Although some microorganisms are inherently pathogenic, most organisms on the skin are benign and are in natural equilibrium with the body. However, a break in the skins integrity or an overturn in the equilibrium, can cause these resident organisms to become pathogenic and sickness to occur (Campbell and Reece, 2002; Dastjerdi and Montazer, 2010). Antimicrobial treatments can be applied to fibres, yarns and fabrics and function to kill or prevent the proliferation of microbes (Dastjerdi and Montazer, 2010). Due to the sensitive balance of the skin's microbial flora, the effect of use of antimicrobials in next to skin applications, particularly long term, is of great concern.

Antimicrobials function for one of two end-use purposes: either preservation of the textile or protection of the user i.e. infection control (Windler, Height and Nowack, 2013). Bacteria can cause damage to fibre integrity as well as discolouration of a textile, inevitably reducing its lifespan (Gao and Cranston, 2008). Antimicrobials included in textile applications such as, curtains, carpets, tents, awnings and upholstery can prevent the growth of bacteria within the textile increasing their durability over the duration of its use (Windler, et al., 2013). Antimicrobials have also held importance in healthcare for preventing infection and disease (Windler, et al., 2013). In the healthcare sector antimicrobials have been incorporated in dressings, bandages, implantables, uniforms, curtains and linen, all to prevent the spread and proliferation of microbes that contribute to infection in compromised individuals, i.e. those with open wounds (Rajendran and Anand, 2006). Many strains of bacteria also produce an offensive odour through the process of breaking down sweat from the axillary glands, commonly referred to as body odour (McQueen, Laing, Wilson, Niven and Delahunty, 2007b). The production of body odour is a major reason for inclusion of antimicrobials in next-to-skin clothing products. Manufacturers finish clothing items with antimicrobials in order to prevent bacteria from residing within the clothing and producing the odour that Western society deems offensive, having connotations of poor hygiene and health (Labows, McGinley and Kligman, 1982).

In recent years, there have been attempts to investigate the detrimental effects antimicrobials may be causing to the environment and consumer alike. Antimicrobials are nonspecific in their bacterial target, therefore not only do they reduce the prevalence of pathogenic or undesirable bacteria but may also reduce beneficial bacteria and those necessary for the continuation of life. Through laundering and the disposal of antimicrobial products, the finish used can leach into the environment and have negative effects on waterborne organisms and the nitrification process in waste systems (Limpiteeprakan and Babel, 2016; Reed, Zaikova, Barber, Simonich, Lankone, Marco,

Hristovski, Herckes, Passantino, Fairbrother, Tanguay, Ranville, Hutchison and Westerhoff, 2016). Just as antimicrobials can affect natural bacterial populations through waste and end-of-life disposal, they may affect prevalence and diversity of bacteria throughout the use of the product also. Of particular concern is the use of antimicrobials in next-to-skin products. A reduction in the skin's microbial flora could lead to infection or a diseased state (Hannigan and Grice, 2013). Another concern with next-to-skin antimicrobials is the potential for these agents to be absorbed through the skin and into the bloodstream. Particularly with the development of nanoparticle applications, the scale of the molecules enhances the ability of agents to cross the skin barrier and increase the potential of physiological toxicity (Rovira, Nadal, Schuhmacher and Domingo, 2016; Stefaniak, Duling, Lawrence, Thomas, LeBouf, Wade and Virji, 2014).

An important issue that has arisen with the use of antimicrobials (and antibiotics alike) is the potential for antimicrobial resistance amongst microbial populations. Microbes by nature and evolution are highly adaptable organisms, and with over exposure of lethal agents many strains have shown the ability to adapt and become resistant to antimicrobials (Tenover, 2006). This leaves society without defence towards this organism which in some cases i.e. pathogenic organisms, is a grave threat to the human population (Hoffman, Outterson, Røttingen, Cars, Clift, Rizvi, Rotberg, Tomson and Zorzet, 2015). The textile sector currently produces antimicrobial clothing and fabric applications on a large scale often without regulation of the exposure of these agents to the consumer. This poses the question whether the textile sector alone is contributing to a mass prescription of antimicrobial agents and therefore potentially mass resistance.

Concerns relating to antimicrobial resistance combined with the potential for physiological toxicity, and damage to beneficial bacterial populations, requires the textile market and consumers to use antimicrobial agents responsibly. Do the benefits of using an antimicrobial agent outweigh the potential detriments? At the end of 2015, a major healthcare provider in the United States of America decided the disadvantages of using antimicrobials were too great and banned the use antimicrobial agents from use in their facilities, with silver included (Saito, 2015). Of the antimicrobial agents banned, silver was the most interesting as it is one of the most prevalent antimicrobial agents used in textiles (Windler, et al., 2013).

The objective of this work is to investigate the antimicrobial efficacy of an antimicrobial silver treated textile against human skin's resident microbial flora. A silver treated textile was chosen due to its prevalence in the textile sector. Through a novel simulated wear environment, over varying lengths of time, the interactions of the resident bacterial strains on fabrics is evaluated against fabric structure and the addition of the antimicrobial agent. This work aims to assess any potential damage this antimicrobial silver textile causes to healthy skin bacteria populations and whether antimicrobials are efficient at preventing adherence of bacteria. Interactions of bacteria on antimicrobials, whether they remain live or have perished, has effects on the effectiveness of the

antimicrobial and therefore may not behave as marketers and consumers expect they would for example limiting odour prevention and infection control.

This project aims to investigate the attachment behaviour of resident human skin bacterial strains on an antimicrobial silver fabric. The objectives of this work were to determine whether:

- an antimicrobial silver treatment on textiles affects natural skin bacterial interactions with fabrics under simulated wear conditions?
- fabric structure affects the growth of natural skin bacteria on textiles under simulated wear conditions?
- washing an antimicrobial silver treated textile affects natural skin bacterial interactions under simulated wear conditions?
- length of exposure affects interactions of natural skin bacteria on textiles under simulated wear conditions?

This investigation is limited to the efficacy of one commercially available silver treated textile. The bacterial attachment behaviours on the fabrics will be compared as an amount of bacteria on the textile as opposed to examining the way they are attached. Only live removed bacteria are evaluated, this study does not investigate whether any perished bacteria remain attached to the antimicrobial or whether they are being removed.

Chapter 2

Literature review

2.1 The Skin

Textile products, in particular clothing, are often designed to sit against the skin. Clothing therefore may be termed a "second skin," having an important relationship to the physical skin and in turn with internal physiology (Elsner, Hatch and Wigger-Alberti, 2003). Skin is essential for providing a physical barrier to protect the internal physiological environment; however the skin must be maintained within narrow parameters in order to optimise this function.

The outermost layer of the skin, the stratum corneum, is the surface in direct contact with clothing. It is composed of approximately 12 to 15 layers of dead keratinised skin cells. These keratinised skin cells contribute to the physical barrier function of the skin, aiding to the control of the body's percentage hydration via the passage of water and protecting the internal environment from external chemicals and pathogens (Hatch, Markee and Maibach, 1992; Hatch, Markee, Maibach, Barker, Woo and Radhakrishnaiah, 1990). The body can either lose moisture through the skin to the external environment, via sweating, or can absorb moisture from the environment in cases of dehydration (Hatch, et al., 1990). The passage of water through the stratum corneum must be maintained between certain parameters (specific to the individual) in order to maintain appropriate hydration. Hydration, which can be indicated by the water content of the stratum corneum, is linked to the skin's pH, elasticity and trans-epidermal water loss (Cua, Wilhelm and Maibach, 1990; Kownatzki, 2003; Schmid-Wendtner and Korting, 2006). The pH must be maintained within a narrow acidic range (pH of 5.4-5.9) ensuring optimisation of the enzymic activity in the skin and the regeneration of the barrier function (Schmid-Wendtner and Korting, 2006).

2.1.1 Skin microflora

The skin is home to a host of resident microbial flora, a natural population of microorganisms in and on the body. Microorganisms include any species of bacteria, fungi, and viruses (Pelczar, 2014). The body's microbial flora population exists at approximately ten times the number of human cells (Tannock, 1995). Most organisms located on the skin are benign or beneficial and are in natural equilibrium with the body (Campbell and Reece, 2002). It is when this equilibrium is overturned, or a foreign microbe enters the body, that sickness occurs (Dastjerdi and Montazer, 2010).

The skin surface varies anatomically, ranging from wet to dry, as well as being slightly acidic and containing many different invaginations, follicular surfaces and sebaceous areas (Hannigan and Grice, 2013). These different environments allow the skin to be colonised by a range of bacterial

and fungal species, the majority of which live in symbiosis with the host (Grice and Segre, 2011) (Table 2.1). Bacterial populations may also vary vastly in composition and quantity between individuals (McQueen, Laing, Brooks and Niven, 2007a).

The resident microbial flora are an important feature of the protective function of the skin. The bacteria and fungi present break down the apocrine fluids, lower the skin surface pH and protect the body from pathogenic microorganisms (Gao and Cranston, 2008). Their residence not only prevents the colonisation of pathogenic microorganisms but their presence also aids the immune system by training host T-cells to become antibiotic to pathogens (Grice and Segre, 2011).

The most common species of flora populating the skin are given in Table 2.1. Bacteria and fungi (in particular *Staphylococcus epidermidis* and *Corynebacterium*) are mostly found in areas of high moisture content, for example in anatomical creases i.e. the umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow) (Grice and Segre, 2011). *Staphylococcus epidermidis*, residing mostly in these areas, is the most populated bacteria on human skin, comprising 90% of the microbial flora population and is therefore the most important species for preventing the colonisation of pathogenic bacteria (Baviera, Leoni, Capra, Cipriani, Longo, Maiello, Ricci and Galli, 2014). It is in these areas however, that the habitation and growth of pathogenic species (e.g. *Staphylococcus aureus* and *Streptococcus pyogenes,* see Table 2.1) may occur and therefore must be taken into account in the treatment and management of wounds to prevent infection.

Lipophillic species (e.g. *Propionibacterium acnes)* (Table 2.1) tend to proliferate in sebaceous areas of the skin. Sebaceous glands are located by the hair follicles and secrete an oily fluid known as sebum, which acts to protect and soften the skin and hair, however also provides a perfect environment for lipophillic bacteria and fungal species (e.g. *Malassezia globosa, furfur and restricta*) (Grice and Segre, 2011).

Dry areas of the skin, for example the forearm, hands and buttock, have the most varying microflora in terms of species types and population amounts (Grice, Kong, Conlan, Deming, Davis, Young, Program, Bouffard, Blakesley, Murray, Green, Turner and Segre, 2009). Areas of high moisture content and secretory substances tend to be those which are in most contact with next-to-skin clothing and textiles i.e. clothing apparel and dressings/healthcare products. It is the species that populate these areas that may then be of most concern and require the most attention when developing antimicrobial agents to be included in these textile substrates.

Table 2.1

Common microbial strains found on human skin, their relationship with the body and typical anatomical region.

Microbial Species	Relationship with the body	Anatomical Region	Points to note	References
***Staphylococcus epidermidis* (bacteria)**	Commensal	Areas of high humidity i.e. umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow)	Gram positive Comprises 90% of the aerobic resident flora The most important bacteria for preventing the colonisation of pathogenic bacteria	(Hannigan and Grice, 2013). (Grice and Segre, 2011) (Baviera, et al., 2014).
***Propionibacterium acnes* (bacteria)**	Commensal	Lipophillic therefore predominantly in sebaceous areas, i.e. forehead, chest and back the retroauricular crease (behind the ear) and the alar crease (side of the nostril)	Gram positive	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Corynebacterium* (bacteria)**	Commensal	Areas of high humidity i.e. umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow)	Gram positive	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Staphylococcus aureus* (bacteria)**	Pathogenic	Areas of high humidity i.e. umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow)	Gram positive Growth favoured in high pH (>5) and occlusion of *Staphylococcus epidermidis* and *Corynebacterium*	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Streptococcus pyogenes* (bacteria)**	Pathogenic	Areas of high humidity i.e. umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow)	Gram positive Growth favoured in high pH (>5) and occlusion of *Staphylococcus epidermidis* and *Corynebacterium*	(Hannigan and Grice, 2013) (Grice and Segre, 2011)

Microbial Species	Relationship with the body	Anatomical Region	Points to note	References
***Proteobacterium i.e. Pseudomonas aeruginosa* (bacteria)**	Commensal	Dry areas e.g. forearm, buttock and various parts of the hand, and some representation in sebaceous areas	Gram negative	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Flavobacteriales* (bacteria)**	Commensal	Dry areas e.g. forearm, buttock and various parts of the hand and some representation in sebaceous areas	Gram negative	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Malassezia globosa, furfur and restricta* (fungi)**	Commensal	Sebaceous areas i.e. face, chest, back	Information on fungal species resident on the skin is limited due to lack of development in technology inhibiting extensive research to be completed	(Hannigan and Grice, 2013) (Grice and Segre, 2011)
***Candida* spp. (fungi)**	Pathogenic	Unclear	Thought to cause clinical infection in cases of immune deficiency or infection following antibiotic use Research limited due to technology requiring development	(Grice and Segre, 2011)

In order to maintain health of the skin, the resident microbial flora population needs to remain in stable and consistent populations. If the flora population becomes too small, infections and diseases may develop (Hannigan and Grice, 2013). For example, a decreasing population of *Staphylococcus epidermidis*, may result in the habitation of *Staphylococcus aureus* (a pathogenic bacteria), whose presence has been linked to skin diseases such as atopic dermatitis and its associated lesions (Hannigan and Grice, 2013). Over-population may cause over-acidification of the skin as well as the risk of potential infection, such as that from pathogenic species like *Staphylococcus aureus* and *Streptococcus pyogenes* (Lambers, Piessens, Bloem, Pronk and Finkel, 2006).

It is well known that bacteria adhere strongly to solid surfaces, and this has been investigated for a number of years (Hsieh and Merry, 1986). This phenomenon is termed biofouling and incorporates the formation of bacteria films on solid substrates (Yu, et al., 2013). However, research into how bacteria attach themselves to textiles and fibre-based surfaces is minimal. As bacteria come into contact with textiles they initially adhere to the fibre, proliferate on the surface, cause fibre deterioration, then disseminate from the textile. The way bacteria attach to the surface of the fibre is dependent on the bacteria strain as well as the chemical composition of the fibre, therefore it can also be deduced that chemical finishes will also alter attachment (Hsieh, Timm and Merry, 1987; Hsieh and Merry, 1986). The bacteria cell membrane and textile interface is subject to many confounding factors including those influenced by the bacteria: cell membrane structure and charge, the centrifugation of the inoculums and concentration of the suspension; and factors influenced by the textile: surface roughness and structure, fibre content and fabric structure, finishes on the fabric and the temperature and relative humidity of the environment (Bajpai, Bajpai, Jha, Dey and Ghosh, 2011).

Hsieh, et al. (1987) researched bacterial adherence on non-finished textiles and found that *Staphylococcus aureus* and *epidermidis* were more likely to adhere to textile fibres (regardless of fibre type) than *Escherichia coli*. This could be due to *Escherichia coli* having a more complex cell membrane (being gram negative) than *Staphylococcus aureus* and *epidermidis* (gram positive) therefore requiring more complex chemical attachment methods (Campbell and Reece, 2002). The chemical interactions between the bacteria and the fibre itself are not known. Methods used to distinguish between adhered and non adhered bacteria are fairly uncomplicated. The general principle is to wash the surface of the textiles, following bacterial contact and incubation, in order to remove any non adhered bacteria. To observe the attachment of the adhered bacteria however more complex techniques need to be employed. Optical microscopy is used together with ImageJ computer software to determine the amount of bacteria attached and scanning electron microscopy

is used to examine the way in which the bacteria are attached (Abrigo, Kingshott and McArthur, 2015; Hsieh, et al., 1987; Hsieh and Merry, 1986; Yu, et al., 2013).

2.1.2 Body odour

Many people are concerned, particularly those living in developed countries, about the development of body odour. The human body naturally produces many different odours at different anatomical sites. The presence of these odours can lead people to believe one is unclean or has unhealthy skin. The most pungent odour, and most repulsive to those who are most concerned by the presence of odour, is that which arises from the axillary regions of the body (Labows, et al., 1982).

The axillary regions secrete a milky fluid from the apocrine and sebaceous glands on the surface of the skin. This fluid is sterile and non odiferous, and it is metabolism and degradation by the bacterial flora in these regions that results in an identifiable odour (Leyden, McGinley, Holzle, Labows and Kligman, 1981; McQueen, et al., 2007b). The bacteria present in the axillary regions are primarily *Staphylococcus, Corynebacterium, Propionibacterium* and *Micrococcus* species. Members of the *Corynebacterium* species have been found to release volatile organic compounds associated with body odour (Wongchoosuk, Lutz and Kerdcharoen, 2009). Bacterial populations vary in composition and quantity depending on body site and can vary vastly among individuals. The need to control microbial species in order to prevent offensive odour is a major contributor to commercial development of fabrics with antimicrobial treatments. The general idea of 'good personal hygiene' has lead to an increasing demand for antimicrobial textiles, shown by worldwide consumption increasing 15% every year between 2001 and 2005 (Gao and Cranston, 2008; McArthur, Tuckfield and Baker-Austin, 2012).

2.2 Antimicrobial treatments on fibres/yarns/fabrics

Antimicrobial treatments function to kill or prevent proliferation of microbes. Antimicrobial substances have been a focus for research in many different industries, including textiles in which clothing and next-to-skin fabrics provide an ideal substrate for the accumulation, multiplication and proliferation of microorganisms. Due to clothing lying against the skin, there is an assured nutrient base for the microbes in terms of ideal temperature and humidity, soiling, skin cells, and sweat and oil secretions (Dastjerdi and Montazer, 2010). Additionally, the textile fibre itself or a finish on the textile surface may provide a source of nutrition or an ideal environment for proliferation (Dastjerdi and Montazer, 2010; Gao and Cranston, 2008). Treating textiles with antimicrobial substances can prevent or minimise the unfavourable effects microorganisms have on the body and the fabric. Many different antimicrobial treatments for textiles have been developed,

with research continuing into formulation of new methods, products and finishes, as well as the effect of these on the skin and the external environment (Dastjerdi and Montazer, 2010).

Antimicrobial finishes for textiles can be categorised by chemical structure as synthetic organic compounds, metals, and naturally derived agents (Windler, et al., 2013). Reportedly, in 2013 those dominating the market were quaternary ammonium compounds and triclosan (synthetic organic compounds), silver (metallic), and zinc pyrithione (metal-organic complex) (Windler, et al., 2013). At the end of 2015 the dominant agents used in textiles appeared not to have changed, however investigations of silver were most common. Other agents that have garnered attention because of their naturally-derived origin are chitosan and natural dyes from plant and animal pigments (Kasiri and Safapour, 2013; Simoncic and Tomsic, 2010). Each of these treatments is processed in a different way contributing to differences in their effect on microbes. Table 2.2 outlines the most common antimicrobial agents used in textiles, the way they are applied to textiles, their effect on microbes and known limitations of their use.

2.2.1 Antimicrobial functionalisation

Development of an antimicrobial agent depends on the end-use of the product. Typically, antimicrobial agents in textiles are for one of two reasons: preservation of the product and protection of the end-user, such as infection control or purposeful administration of drug delivery (Windler, et al., 2013). In using textiles as a nutrient source, microorganisms can severely damage the fibre, cause staining and discolouration of the fabric, and reducing mechanical strength (Gao and Cranston, 2008). In this case, antimicrobial agents are applied to textiles in order to prolong their lifespan, this includes curtains, carpets, tents, awnings and upholstery (Windler, et al., 2013). These antimicrobial agents are designed to be bound in the textile structure, preventing microbes from residing in the fabric (Simoncic and Tomsic, 2010). Antimicrobial agents may also be incorporated into textiles to protect the wearer from the damages of pathogenic microorganisms (Windler, et al., 2013). Clothing and next-to-skin textile products may have antimicrobial agents applied that are designed to leach from the surface and disrupt or kill pathogenic microbes on the surface of the skin (Simoncic and Tomsic, 2010). This aims to control infections in compromised skin and also reduces the formation of unpleasant odours (Windler, et al., 2013).

Table 2.2

Common antimicrobial treatments applied to fibres, yarns, and fabrics, their process of application, effect and known issues

Treatment	Process	Effect	Known Issues	Reference
Quaternary Ammonium Compounds	Typically applied as a finish to the textile substrate, via 'dehydration bonding'. Dipped into a QAC solution and dried allowing the bond to form with the substrate.	A biocide therefore disrupts proliferation, and cell membrane and structure causing death. Designed not to leach onto the skin surface.	Not durable. Known to not withstand laundering.	• (Gao and Cranston, 2008) • (Tran, Hamood, de Souza, Schultz, Liesenfeld, Mehta and Reid, 2015)
Triclosan	Either by incorporation into the resin, applied to the textile at the dye stage or incorporating the compound into the synthetic polymer melt prior to spinning.	Biostat therefore inhibits growth and proliferation. Designed to leach slowly from the surface providing sustained release.	Bacterial resistance has been seen over long periods of exposure to the substance.	• (Gao and Cranston, 2008) • (Yazdankhah, Scheie, Høiby, Lunestad, Heir, Fotland, Naterstad and Kruse, 2006)
Silver	Incorporated into textiles via inclusion in the spinning melt or applied as a resin or finish.	Biocide: inhibits proliferation and respiration through biologically active Ag^{-} ions binding to the cell. Designed to be released from the surface as a leaching type.	An ongoing concern with silver is the potential for antibacterial resistance, particularly with the development of nanosilver particles and their penetrative ability.	• (Lansdown, 2006) • (Filipowska, Rybicki, Walawska and Matyjas-Zgondek, 2011) • (Gao and Cranston, 2008)
Zinc Pyrithione	Applied to fabrics as a finish through immersion of the textile in zinc pyrithione solution.	Biocide: enters the cell and disrupts solute transportation causing cell death in both bacterial and fungal colonies.	Not an overly durable finish as it is applied to the surface of fabrics as opposed to binding in the fibre structure therefore can be laundered off.	• (Morris and Welch, 1983) • (Windler, et al., 2013)
Chitosan	Can be incorporated into the textile either in the synthetic melt prior to spinning or applied as a textile finish (most common with cellulosic textiles).	Biocide: disrupts the cell causing leakage of intercellular products resulting in cell death.	Chitosan has low durability when applied as a resin therefore leaches from the textile surface during laundering. Another concern is the high molecular weight of chitosan causing detrimental effects on the hand of the textile.	• (Simoncic and Tomsic, 2010) • (Kong, Chen, Xing and Park, 2010)
Natural Colorants	Dyes and pigments extracted from natural sources e.g. plants, animals etc, and are incorporated into textiles through immersion of the fabric in dye baths.	Most are biostats which inhibit the growth of microbes on the textile surface. In this way they are not designed to be leached from the textile surface.	Tend to have low durability in laundering and rubbing of the surface, diminishing antimicrobial properties of the textile over time.	• (Singh, Jain, Panwar, Gupta and Khare, 2005) • (Gupta, Khare and Laha, 2004) • (Kasiri and Safapour, 2013)

Antimicrobial substances can be grouped into two classifications: biocides or biostats. Biocides include any agents that kill the microbe; biostats inhibit either metabolic activity and/or proliferation (Simoncic and Tomsic, 2010). Most commonly, antimicrobial agents function as biocides through interrupting the cell wall or enzymic activity resulting in the microbe dying (Nayak and Padhye, 2014). The actual function of the agent depends on the concentration on the substrate and the method of application/ adherence to said substrate. The finish on the textile can either be bound in the fibre/fabric structure, or applied to the textile with the intention of "leaching" the compound into the external environment (Simoncic and Tomsic, 2010).

Most antimicrobial agents are considered biocides and therefore cause fatal damage to bacteria. The efficacy of the agent is dictated by the method of application on the textile, the spectrum of activity on various species of bacteria and fungi, the durability of the process on the textile and the molecular weight of that agent. The action of a biocide on the microbe can be considered a six step process: adsorption on the microbial cell surface, diffusion through the cell wall, adsorption on the cell membrane, disruption of the structure of the membrane, leakage of the cytoplasmic constituents, and cell lysis (Kenawy, Worley and Broughton, 2007).

Different methods for adding an antimicrobial agent to a textile exist. For example, the agent may be applied to the surface of a textile as a finish, typically a method used for textiles manufactured from natural fibres (e.g. cotton, wool). If the fibre is man-made (e.g. polyester, polyamide, viscose) the agent may be added to the polymer melt before spinning or blended with the fibre during processing. Agents that are added to the fibre prior to or during spinning are often more durable than those added as a finish, since the agent is bound to the fibre polymer and thus less likely to be removed through use or cleaning (Gao and Cranston, 2008).

Antimicrobial agents applied to the surface of a textile tend to be "leaching-type." They are designed to be released from the surface in order to maximise the efficacy and ensure wide distribution of the agent. The disadvantage of this process however, is that the agent is exhausted from the textile faster than those bound within the textile. Also, through laundering and wear, leaching types have the potential to be removed from the surface more quickly than a consumer would desire (Nayak and Padhye, 2014). Another issue arises in use of these textiles in clothing and healthcare based settings. The release of the agent from the surface may cause it to enter through any break in skin integrity, such as open cuts and wounds, or be absorbed through the epidermis in which case it may be toxic for the wearer. It is reported that the majority of antimicrobial agents in the textiles industry are leaching-type in order to provide a consistent and controlled release of the agent onto the skin for the most efficient biocidal effect (Simoncic and

Tomsic, 2010). The safer and more desirable alternative in textiles designed for human wear is "non-leaching type" antimicrobials. These agents are bound within the textile structure and exhibit antimicrobial action when a bacterium or fungi comes in direct contact with the agent. These agents are more durable to cleaning processes and have a longer lifespan within the textile (Nayak and Padhye, 2014). Their durability also results in a much lower risk for human toxicity and are considered safer for the environment as they do not leach into waterways during cleaning processes (Simoncic and Tomsic, 2010). However, these agents are likely to have a lower efficacy, as they rely on direct contact between the agent in the textile and the microbe itself (Nayak and Padhye, 2014).

The method by which an antimicrobial finish or process is applied to the textile directly affects the mode by which the textile exhibits antimicrobial action. The general mode involves the structural attributes of the bacteria cell membrane. Bacteria exhibit a net negatively-charged outer membrane (Timofeeva and Kleshcheva, 2011). The cell membrane is comprised of a phospholipid bilayer housing essential enzymes for cell function and proliferation (Campbell and Reece, 2002). The function of the cell membrane is to regulate the transfer of solutes in and out of the cell for purposes of cell continuity and communication (Campbell and Reece, 2002; Timofeeva and Kleshcheva, 2011). Therefore, antimicrobial processes are designed to disrupt the cell membrane and its functionality. Antimicrobial structures tend to have high binding affinity to the bacteria cell which enhances structural damage, resulting in cell membrane failure and in many cases cell lysis (Kenawy, et al., 2007).

The overall effect an antimicrobial textile has on a bacteria or fungi is dictated by the microbial species themselves. Antimicrobial textiles are more effective on particular strains due to the way their outer membrane is structured and therefore the ease of which an agent can bind to the microbe (Timofeeva and Kleshcheva, 2011). Bacteria can be classified as being either gram negative or gram positive which inherently relates to their gram cell staining. Gram positive bacteria have relatively simple cell walls with high concentrations of peptidoglycan, whereas gram negative bacteria have more structurally complex cell walls with less peptidoglycan present (Campbell and Reece, 2002). Gram negative bacteria exhibit higher resistance to antimicrobial agents than gram positive due to the greater complexity of the cell wall, reducing the ability of the agent to bind and pass through the cell membrane (Timofeeva and Kleshcheva, 2011). The ability for the agent to pass through the cell membrane is also directly related to the molecular weight of said agent. The molecular weight must be within a particular "ideal" range in order to possess optimal antimicrobial activity. A molecular weight range between 5×10^4 and 1.2×10^5 Da (Dalton) is considered the optimal range (Kenawy, et al., 2007). Agents with a molecular weight lower than 5 ×

10^4 Da, exhibited increasing antimicrobial efficiency with increasing molecular weight. Antibacterial efficiency decreased rapidly with increasing molecular weight over 1.2×10^5 Da. The molecular weight directly relates to the agent's ability to successfully and efficiently pass through the bacteria cell membrane (Kenawy, et al., 2007).

An updated comprehensive comparative review of commercially available antimicrobial agents and products would be helpful. Windler, et al. (2013) has demonstrated a good foundation for the comparative review, however with the ever-increasing use and development of antimicrobial textiles, there is need for an updated and all inclusive review. A comparative risk assessment of agents used in clothing and healthcare settings would provide consumers and manufacturers with an improved understanding of the relative merits of the different treatments. Which agents are safest for human use, which have the greatest efficacy, and which are least harmful on the environment need to be identified and consolidated for ease of reference. These factors are important when considering using an antimicrobial agent in different commercial contexts. For example, an antimicrobial agent designed to be released onto the skin, is likely to be loosely bound to the textile structure and therefore maybe harmful on the environment due to leaching into waterways. A comparative assessment would allow one to decide whether the pros of using a particular antimicrobial product outweighed the cons. However, with the continuous development of treatments and antimicrobial textile products and applications, a review of this type would need to be carried out annually to account for new developments.

2.3 Scope of industry

Antimicrobial textiles have a wide range of applications, with varied purposes (Windler, et al., 2013). As mentioned in section 2.2.1, the antimicrobial agent may function as a protectant for the textile product itself from microbial attack and degradation. Microbes can cause odour formation, discolouration and loss of functionality in textiles including a decrease in tenacity and elasticity (Heine, Knops, Schaefer, Vangeyte and Moeller, 2007). By incorporating antimicrobial finishes in textiles, particularly in geotextiles and those designed for outdoor use, the end user can expect the product to have increased durability for a longer lifetime (Windler, et al., 2013). On the other hand, antimicrobial agents may be incorporated for purposes of protecting the consumer. Antimicrobially-treated textile products are often used in a clinical setting in order to maintain hygiene and protect patients from bacterial and fungal infections (Windler, et al., 2013). In broader applications, antimicrobial agents have been incorporated in applications such as sportswear, lingerie, outdoor textiles, air filters, automotive textiles, domestic home furnishings and medical textiles (Gao and Cranston, 2008). The incorporation of antimicrobial agents in textiles may also lessen the frequency required for laundering clothing. This may therefore lead to lowered water

and energy consumption, and less chemicals and detergents associated with laundering leaching into waterways (Windler, et al., 2013).

Textile products with antimicrobial agents incorporated into them, must meet certain requirements in order to be considered safe and functional (Windler, et al., 2013). The requirements are subject to the end-use of the product; however of most relevance are those for next-to-skin applications. The agent must exhibit a broad spectrum of activity, targeting multiple species of microbial flora, whilst maintaining a low toxicity profile. Also, the agent must not cause/result in limited skin irritation, allergy and sensitization. If the agent is incorporated into reusable products it must be fairly durable to laundering and wear, in order to ensure a long lifetime and limit the leaching of the agent into the waterways and environment. Lastly the agent must not negatively affect the textile mechanical and aesthetic functionality i.e. must not reduce durability, handle or aesthetic appearance (Gao and Cranston, 2008).

The impact antimicrobial textiles are having on the textile industry is largely unknown; there is little information available regarding the consumption of antimicrobial textiles. In the year 2000 the Western European consumption of antimicrobials was estimated at 30,000 tonnes, with the worldwide estimate reaching 100,000 tonnes (Gao and Cranston, 2008). Between the years 2001 and 2005 it was estimated the Western European antimicrobial textile consumption increased approximately 15% annually (Gao and Cranston, 2008). Research needs to be conducted in order to truly understand the scope of the antimicrobial textile industry, in terms of amount of textile consumed per type of antimicrobial agent, and which products in particular are these agents being applied to. This will enable manufacturers and consumers to truly grasp the effect these textiles are having on the industry and the environment alike.

2.4 Antimicrobials in healthcare

Infection control in health care management is an important application of antimicrobial agents. The growth of infection in wounds, particularly those nosocomially acquired, has undergone extensive research for decades. There has been continuous development of products aimed at prevention and reduction of bacterial and fungal infection in patients and consumers. The use of antimicrobial agents is the major focus for infection control in wound management.

For the healthcare sector, antimicrobial agents may be incorporated in uniforms, curtains, bed linen, surgical drapes, bandages and wound care dressings, and implantable devices (Rajendran and Anand, 2006). The use of antimicrobial products could help reduce the spread and proliferation of microbes in the healthcare environment, therefore limiting the contraction of nosocomial infections Nosocomial infections are thought to affect 5% of all patients hospitalised, with this number being

higher in developing countries. Due to the high rate of contraction, research has been conducted to limit this, with textiles used in healthcare being a target (Borkow and Gabbay, 2008). Many studies have been conducted on the effectiveness of using antimicrobial products in healthcare settings, with varying results; for example, the use of antimicrobial agents on surgical masks or curtain drapes, both showing a marked decrease in the prevalence of bacteria (Li, Leung, Yao, Song and Newton, 2006; Schweizer, Graham, Ohl, Heilmann, Boyken and Diekema, 2012). Lazary, Weinberg, Vatine, Jefidoff, Bardenstein, Borkow and Ohana (2014), found that the inclusion of copper oxide in the bed sheets of brain injury patients, decreased the amount of bacteria present and therefore the amount of infections developed. However, Boutin, Thom, Zhan and Johnson (2014) showed the inclusion of antimicrobial had nil effect on the prevention of bacteria on healthcare workers scrubs, a common pathway for nosocomial infection transmission. Another trial on healthcare workers uniforms mirrored the results from Boutin, et al. (2014), with no decrease in bacterial counts found (Burden, Keniston, Frank, Brown, Zoucha, Cervantes, Weed, Boyle, Price and Albert, 2013). With conflicting results on the effectiveness of antimicrobial textiles in the prevention of nosocomial infections, there is insufficient research available to conclude whether the inclusion of antimicrobials is of benefit to the healthcare sector.

The most common form of antimicrobial agent used in healthcare is silver metal and its products (Simončič and Klemenčič, 2016). This may be due to the recent development of nanotechnology and the ability of silver to be processed into nanoparticles for inclusion in textiles. Using silver nanoparticles as an antimicrobial agent in textiles is advantageous, as the antimicrobial effect increases with the decreasing size of the silver particles. The smaller the silver particle is, the larger the surface area, therefore the greater potential for silver ions to be released from the surface to impart bactericidal effect (Stefaniak, et al., 2014). It has also been found that silver particles smaller than 10nm are able to penetrate the cell membrane of the bacteria and bind to thiol groups within the cell, causing inhibition of the bacteria's physiological processes (Simončič and Klemenčič, 2016). Elemental silver (Ag^{O}) itself is not inherently antimicrobial; however its cation (Ag^{+}) is highly reactive and toxic to bacteria. Ionic silver can disrupt the cell wall, cause structural changes within the cell, as well as bind to ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), inhibiting transcription and proliferation (Leaper, 2006). Although, silver in its metallic, elemental form is known to not risk human health, a major concern with silver nanotextiles is the infiltration of the particles across the epidermis, into the body in the form of cations, causing detrimental effects to the user (Simončič and Klemenčič, 2016; Stefaniak, et al., 2014). This is of particular concern with the incorporation of this agent in wound dressings, where the skin is likely to be open and vulnerable (Simončič and Klemenčič, 2016). Research is limited on the effect of nanoparticles on the

physiological skin and body, requiring further investigation into the potential evidence of nanoparticles entering the skin barrier and affecting internal physiology.

2.5 Test methods used in antimicrobial research

As antimicrobial textiles have been a focus of research for many years, standard test methods and agreed procedures have been developed to ensure homogeneity across the field. Testing for the ability of an antimicrobial treatment to be effective against bacteria is a well employed technique. Development of new antimicrobials, and any changes made to said textiles, requires a test to determine whether the desired effect is exhibited. Table 2.3 shows the test methods employed when testing the changes of antimicrobial efficacy following laundering. The table shows that regardless of the materials tested, the AATCC (American Association of Textile Chemists and Colorists) Standard Test Method 100: Antibacterial Finishes on Textile Materials: Assessment of, is most commonly used to determine the effect the textile has on bacteria. AATCC 100 is a quantitative test method that gives a numerical estimation of the bactericidal and bacteriostatic activity of a fabric. Although this is helpful in comparing levels of antimicrobial activity among fabrics, it can be difficult if the fabric is hydrophobic (in order to efficiently inoculate) and does require the researcher to decide on "success criteria" which does not allow for inter-institution comparisons. More often than not, a fabric's antimicrobial activity must be determined for commercial sale, and therefore a yes/no criterion is more efficient. AATCC 147 – Antibacterial Assessment of Textile Materials: The Parallel Streak Method, is a qualitative method that allows for a yes/no criterion and is a quick and easy way of determining whether a fabric is antimicrobial. Other test methods used to test antimicrobial efficacy for commercial use are ISO 20743:2013 Textiles – Determination of Antibacterial Activity of Textile Products, and the EUCAST Disk Diffusion Method (International Organization for Standardization, 2013; Matuschek, Brown and Kahlmeter, 2014). ISO 20743 outlines three different methods that can be used depending on resources available and which test most suits the fabric and its' intended use. All the methods are quantitative and involve directly inoculating the test fabric with bacteria, incubating for 24 hours at 37°C, placing the sample in solution and shaking to retrieve any bacteria left. The EUCAST disk diffusion method is similar to AATCC147 by the way of plating a fabric on top of bacterial culture. The EUCAST disk diffusion method is a more recent review of testing for antimicrobial efficacy, paralleling methods like AATCC147 however further standardising inoculums and nutrient agar towards typical microbiological research standards (Matuschek, et al., 2014). Pinho, Magalhães, Henriques and Oliveira (2010) provided a reasonable comparison among some common test methods used, both quantitative and qualitative. They concluded that AATCC 147 is very effective as a simple test determining whether a not a textile is antimicrobial, regardless of its ability to

diffuse, whereas ISO 20645 (the previous revision of ISO 20743) may only be effective with diffusing antimicrobials, limiting its scope.

The most common bacterial strains used to test the antimicrobial effect are the gram negative bacteria *Escherichia coli* and the gram positive bacteria *Staphylococcus aureus* (however not all research uses this). There are also cases of the use of *Klebsiella pneumoniae*, however these are not as common (Yiqi, Corcoran, Vorlicek and Shiqi, 2000). The issue with these bacteria is they are all pathogenic to the human skin; therefore do not provide an indication of the effect antimicrobial textiles has on the natural flora. In fact any research testing the effect the textiles have on natural flora strains is extremely scarce.

It also appears there is no general consensus on the contact times (the length of time the textile is exposed to the bacterial culture) and the incubation periods (the length of time the textile is incubated on to allow bacteria any bacteria to grow on the textile). Contact times vary from 5 minutes to 24 hours, with no explanation as to reasoning behind this decision, and incubation periods may vary from 15 hours to 24 hours. All papers incubate the bacteria at 37°C as this is the healthy internal temperature of the human body and the temperature at which these strains of bacteria thrive, however this may not give an indication of how the bacteria would grow on textiles adjacent to the skin which tends to have a lower mean temperature (Wilson, 2009).

In the formulation of new textiles, and while looking at textiles that are designed for repeated use, it is important to determine the laundering durability. With antimicrobial finishes on textiles, there is a possibility these finishes could be washed off during normal household laundering. This has the potential to lower the antimicrobial efficacy of the textile; therefore it is necessary to test for any antimicrobial changes. Along with AATCC Standard Test Method 100, to test the antimicrobial effectiveness before and after laundering, standard test methods are employed which provide guidelines for the laundering methods used. Table 2.4 shows the laundering procedures employed when testing the changes of antimicrobial efficacy following laundering. There are two standard test methods that are typically used; AATCC Standard Test Method 124: Smoothness Appearance of Fabrics after Repeated Home Laundering and AATCC Standard Test Method 61: Colorfastness to Laundering: Accelerated. Both of these standards are used on a variety of materials and the number of washes is tailored to each different piece of research, however all use a detergent without optical brighteners (WOB). The majority of articles tend to test the antimicrobial efficacy before washing (T_0) then every 5 (T_5) or 10 (T_{10}) washes up to 50 (T_{50}) washes. .

Table 2.3

Comparison of antimicrobial efficacy methods used in literature investigating antimicrobial durability

Materials	Standard Test Method	Organisms	Contact Time and Incubation	Reference
Various halamine compounds treated on cotton and cotton/polyester blends	AATCC Standard Test Method 100: Assessment of Antibacterial Finishes on Textiles	*Escherichia coli*	Contact times of 5, 15 and 30 minutes	Qian and Sun (2004)
Standard wool fabric dyed with pure curcumin	AATCC Standard Test Method 100-1999	*Escherichia coli* *Staphylococcus aureus*	Not explicitly stated	Han and Yang (2005)
Acid dyed nylon 6 and nylon 66 fabrics treated with quaternary ammonium salts	AATCC 100-1993	AATCC 2666 *Escherichia coli*	Contact period of 18 hours Incubated for 18 hours at 37°C	Young Hee Kim and Sun (2000)
Unbleached worsted wool, finished with a quaternary ammonium salt compound	AATCC Standard Test Method 100	*Escherichia coli*	90 minute contact time Incubated for 15 hours at 37°C	Zhu and Sun (2004)
Cotton/ polyester blend treated with either triclosan or chitosan	AATCC 147-Antibacterial Assessment of Textile Materials: The Parallel Streak Method, as well as AATCC 100	*Escherichia coli* *Staphylococcus aureus*	All incubations for 18-24 hours at 37°C	Ranganath and Sarkar (2014)
Cotton treated with silver nanoparticles	AATCC 100-2004	*Escherichia coli* *Staphylococcus aureus*	Contact time of 24 hours at 37°C Also 24 hours incubation at 37°C	Liu, Lv, Deng, Li, Yu, Huang and Fan (2014)
Cotton, polyester and acrylic fabrics treated with halogenated phenols, amine compounds and cationic dyes	AATCC Test Method 100	*Escherichia coli* *Klebsiella pneumoniae* *Staphylococcus aureus*	Not explicitly stated	Yiqi, et al. (2000)

When testing commercial textiles for their antimicrobial efficacy and durability to laundering, it would be advantageous to test before washing, giving an indication of the effectiveness at the time of purchase, then after a variety of washes in order to test how the textile wears over time. It is possible that most pieces of research test the antimicrobial efficacy after 50 washes as it is likely this is the number of washes a piece of clothing may subjected to over the course of a year i.e. approximately once a week. By testing the antimicrobial efficacy before washing and again after, the bacterial inhibition can be compared giving a quantifiable difference in effectiveness

Although bacterial adherence on textiles has been well researched, observation of bacterial attachment on textiles with an antimicrobial finish is rare. Changing the surface topography of textiles changes the way bacteria interact, therefore it would be advantageous to examine the way in which bacteria adhere to antimicrobial textiles, if at all. If bacteria are able to attach to the surface of an antimicrobial textile initially, there is a possibility the bacteria die on the surface and remain attached; therefore live/dead assays should be consulted to determine the extent to which he antimicrobial is effective (Abrigo, et al., 2015; Yu, et al., 2013). If dead bacteria were to remain attached to the surface, this would create a "biofilm"; a three dimensional matrix of adhered bacteria surrounded by a secreted extracellular polymeric substance which protects the bacteria from antibiotic action (Poulter, Vasilev, Griesser and Griesser, 2013; Wirth, et al., 2016). A biofilm would provide a surface for live bacteria to proliferate on without coming in contact with the antimicrobial surface, and in turn affecting the efficacy of the antimicrobial textile (Yu, et al., 2013).

2.6 Detrimental effects for consumers and environment

2.6.1 Concern for the individual

Although research on antimicrobial textiles is extensive, especially in regards to the use of silver in healthcare, information on the detrimental effects these agents are having on the consumer and environment alike, are extremely limited. Cause for concern with using antimicrobial substances on the skin is the potential negative effect of the substance on the skin's resident microflora. There is insufficient evidence available to determine whether topical antimicrobial textiles are resulting in an unhealthy skin state. Evidence has suggested that the use of antibiotics internally results in a reduction in diversity of healthy gut bacteria and reduces gut microflora in the long term (Jakobsson, Jernberg, Andersson, Sjolund-Karlsson, Jansson and Engstrand, 2010; Jernberg, Löfmark, Edlund and Jansson, 2007).

Table 2.4

Comparison of wash processes and methods in literature investigating antimicrobial durability

Materials	Standard Procedure	Number of Standard Wash Cycles Tested	Reference
Various halamine compounds treated on cotton and cotton/polyester blends	AATCC 124-1999 AATCC 1993 WOB* standard detergent Tumble dried	0, 10, 20, 30, 40, 50	Qian and Sun (2004)
Cotton, polyester and acrylic fabrics treated with halogenated phenols, amine compounds and cationic dyes	AATCC 124 AATCC WOB standard detergent	0, 1, 5, 10, 25, 50	Yiqi, et al. (2000)
Standard wool fabric dyed with pure curcumin	AATCC 124-2001 test for colour fastness Tumble dried AATCC 1993 Standard detergent WOB	0, 1, 5, 10, 20, 30	Han and Yang (2005)
Acid dyed nylon 6 and nylon 66 fabrics treated with quaternary ammonium salts	AATCC 61-1994 for washing durability.	0, 40	Young Hee Kim and Sun (2000)
Unbleached worsted wool, finished with various quaternary ammonium salt compounds	AATCC 61 AATCC Standard detergent WOB	0, 5, 25, 35, 50	Zhu and Sun (2004)
A cotton/ polyester blend treated with either triclosan or chitosan	AATCC 61 (condition 3A-industrial) AATCC Standard detergent WOB	0, 50	Ranganath and Sarkar (2014)
Cotton treated with silver nanoparticles	AATCC 61-2006 (condition 2A) WOB detergent (using accelerated laundering to efficiently achieve high numbers of wash cycles)	0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250	Liu, et al. (2014)

* = Without optical brightener

Hoefer and Hammer (2011) researched the effect of silver antimicrobial fabrics on the human skin flora and physiological skin parameters after long term wear. The study examined the microbial flora population counts as well as skin capacitance, transepidermal water loss (TEWL) and pH of 60 participants following 6 weeks of wear. It was found there was no reduction in the microbial flora populations or alterations in the skin's physiological parameters. However, it must be noted that this study only tested one antimicrobial agent and on one area of the skin's surface (a notable dry
This finding is concerning if the case is true for skin microflora also. Long-term use of antimicrobial clothing may therefore have negative effects on skin health and therefore overall physiological health.

If there is a reduction in diversity and population of skin microflora then there is a potential for this to have an effect on other indicators of skin health, for example a rise in skin pH causing the skin to become too alkaline. The biggest concern would be inhabitance of pathogenic microbes. Following the removal of the antimicrobial textile, if the skin microflora has become reduced, this could allow pathogenic bacteria to reside on the skin due to a lack of competition. For example, if *Staphylococcus epidermidis* population were to reduce, this would allow the inhabitance of *Staphylococcus aureus* which has been linked to the development of atopic dermatitis (Hannigan and Grice, 2013). Unfortunately the effect of antimicrobial agents on the healthy skin parameters and on the resident microbial flora is rarely researched.

area skin, therefore varied in populations (Grice and Segre, 2011)). The area of skin measured was the scapula, with the opposite scapula used as a control. It is possible that some motile bacterial strains may have migrated from one scapula to the other during wear therefore producing no difference between the antimicrobial and the control. The garments used were also not form fitting therefore may not be a representative indication of next-to-skin antimicrobial textiles such as sportswear and wound dressings and as such, the study was limited to a particular end user case.

A more recent study by Walter, McQueen and Keelan (2014) examined the effects of a range of antimicrobial textiles on the skin's resident microbial flora. This study is the only one of its kind in studying the effect of various antimicrobial textiles on resident skin microflora. The researchers examined the effect of three antimicrobial textiles, a triclosan, a zinc pyrithione and a silver chloride, after 24 hours of wear on 19 participant's forearm skin microflora. The results emulated those of Hoefer and Hammer (2011), showing no change in the skin microflora population numbers. However, it must be noted that this study was highly restricted in its testing parameters. The skin tested was that of the forearm only which is, again, a very dry and therefore varied area of the skin. The skin was then occluded by plastic during the 24 hours in order to increase the

moisture and therefore the bacterial growth, which is unlikely to be a representation of normal wear. The antimicrobial textile was only worn for 24 hours, which is an unlikely period of time to be wearing an antimicrobial product in real life situations. An antimicrobial product is more likely to either be worn for short periods of a few hours over long periods of time i.e. in an item of clothing, or for continuous wear of up to 7 days, i.e. in a wound dressing (Swanson, 2014). Finally, the study examined the population counts of *Staphylococcus aureus* (a pathogenic, non-resident bacterium) and *Klebsiella pneumoniae*. Testing only these bacterial strains in particular, as well as not including fungal species, is a misrepresentation of the typical residential flora on healthy, uncompromised skin. It would be advantageous to explore the effects of antimicrobial agents on resident microbial species further with a variety of antimicrobial agents, on a variety of skin areas and resident microbial populations.

Another concern with the use of next-to-skin antimicrobial textiles is the potential for the antimicrobial agent to be absorbed through the skin into the bloodstream. This has become particularly concerning with the development of nanosilver particles, with their small dimensions allowing them to pass through the skin barrier (Rovira, et al., 2016; Stefaniak, et al., 2014). An issue of physiological toxicity then becomes apparent. As with negative effects of the skin, research of the absorption of antimicrobial agents from textiles is limited. The majority of research surrounding the absorption of antimicrobial agents and negative effects of antimicrobials in the body is attributed to the use of silver. Research into the detrimental effects of silver has shown cases of silver toxicity. Trop, et al. (2006), report of a case where a patient with burn wounds was treated with nanosilver containing dressings. After prolonged use of the dressings, the patient developed hepatotoxicity and argyria, which is indicated by a greyish discolouration of the face and blueing of the lips. The toxic effects ceased with removal of the nanosilver dressings. Silver deposition in tissues, including the liver, kidney and cornea, has been reported following the use of silver as a topical antimicrobial treatment in other cases also (Hollinger, 1996). Iwasaki, Yoshimura, Ideura, Koshikawa and Sudo (1997) report of a case of neuro-accumulation of silver from absorption over the blood brain barrier. The kidneys are a major elimination factor of silver from the body. The patient reported was in end-stage renal failure, with compromised kidney elimination processes when he presented with burns to 30% of his body. When silver was applied to these burns, the patient progressively became comatose. On cessation of the silver treatment, the patient regained consciousness and lucidity however the burns evidently became infected. Silver treatment was reinstated and the patient's mental status deteriorated until he could no longer be resuscitated. As silver is eliminated from the body via the kidneys, when the kidneys are compromised the silver decreases kidney function further and accumulates in the body (Iwasaki, et

al., 1997). Evidence of toxicological effects following antimicrobial treatment with other agents however, is extremely limited.

Triclosan has been reported to be found in the plasma, urine and tissues of consumers using the agent topically, therefore dermal absorption of triclosan is evident (Fang, Stingley, Beland, Harrouk, Lumpkins and Howard, 2010). However no detrimental physiological effects have been noted due to the rapid metabolism and excretion of the agent (Leaper, Assadian, Hubner, McBain, Barbolt, Rothenburger and Wilson, 2011). It has been concluded that triclosan is not inherently toxic to humans regardless of its ability to be absorbed into the bodily tissues (Dann and Hontela, 2011).

Chitosan has been widely tested for its potential toxicity or for any negative physiological effects. All studies have concluded that chitosan has low to no toxicity in its naturally occurring state (Kean and Thanou, 2010). Limited research could be found on any negative dermal effects to prolonged use of chitosan for example, loss of resident microflora.

Research on the toxicity and dermal absorption of quaternary ammonium compounds, or zinc pyrithione alike, is extremely limited. There is limited evidence to suggest the use of zinc pyrithione may cause neurotoxicity and developmental issues, however this is not well documented (Windler, et al., 2013). It is quite unclear whether there is any evidence of detrimental effects following the use of antimicrobial agents and this would require further study in order to produce a toxicity profile. It is important that the antimicrobial is included in textiles above the antimicrobial minimum inhibitory concentration (MIC; dependent on the agent) but below the concentration that the agent becomes cytotoxic (Poulter, et al., 2013). This information is not readily available to manufacturers and consumers alike.

2.6.2 Concern for the environment

The effect these antimicrobial agents have on the environment has been widely researched. A large concern with the use of antimicrobial agents in everyday textiles is the risk of contamination of water ways following laundering. With antimicrobials included in clothing and reusable textile items, there is a large possibility for the agents to be washed into the waterways causing widespread harm to the environment. With the large increase in consumption of antimicrobial textiles internationally, the effect on the environment becomes a very real issue. The need for antimicrobial agents to be durable in wash and wear is key to ensure safety of the environment (Windler, et al., 2013).

Due to its recent popularity, antimicrobial silver has been extensively examined for its release into water during washing, and in turn into waterways. In order for silver to exhibit antimicrobial activity, it must be released from the textile in cationic form (Leaper, 2006). It is therefore reasonable to assume, if an antimicrobial silver textile releases Ag^+ ions from its surface, then these ions are being releases into wastewater during laundering. Lorenz, Windler, von Goetz, Lehmann, Schuppler, Hungerbühler, Heuberger and Nowack (2012) examined the release of silver from a range of commercially available silver textile products into wastewater. More than half the textiles tested released detectable amounts of silver into the wastewater, with one textile releasing nearly 20% of its silver composition.

Reed, et al. (2016) examined the potential toxicity of nanoparticulate silver in wastewaters using zebra fish embryos. The amount of nano silver released into the wastewater was determined by the loading of the silver onto the textile; with increasing silver concentration on the textile there was increased silver released into the wastewater. The mortality rate of zebra fish embryos was then examined having been exposed to the wastewater. It was observed that textiles containing silver nanoparticles that were tethered to the fabric (covalently bonded) displayed no toxicity to the zebra fish; however those that were untethered (easily released from the surface) resulted in a mortality rate over 30%. The mortality rates of the embryos were drastically increased by the inclusion of a detergent, with the Ag^+ salt coated textile displaying an almost 100% mortality rate. Although Reed, et al. (2016) explains the figures obtained are a worst case scenario, this is regardless a significantly poor toxicity profile for silver incorporated textiles.

The end of life toxicity potential of antimicrobials is hard to quantify due to the limited information regarding annual consumption of these textiles. It is has been recorded that nanosilver, once released into the environment from daily washing, accumulates in the sludge from wastewater treatment plants. Silver ions are also documented to inhibit the nitrification process of bacteria in waste systems, displayed by a decrease in the biological degradation rate (Limpiteeprakan and Babel, 2016). These cases give an indication on the effect silver may have, not only on bacteria found in landfills and waste treatments but once leached into the environment, the ripple effect it may have on the essential nitrogen cycle in the natural environment.

2.6.3 Marketing false claims

The perceived benefits that the addition of antimicrobial finishes gives to textiles are desirable for companies when it comes to their marketing strategy. Antimicrobial textiles can reduce or eliminate the bacteria that the product comes in contact with and can prevent bacteria from proliferating within the fabric itself. This enables companies to promise its clients that their

products will provide these perceived benefits increasing their health and wellbeing. Common marketing strategies take advantage of western society's need to be regarded as clean and healthy which often comes with the elimination of odour causing bacteria and infection. For products to be deemed antimicrobial they must meet certain minimum requirements (Gao and Cranston, 2008). There are recognised international standards that companies can use to ensure their products meet these requirements however there is no specification as to what part of the product or when in the manufacturing process these tests should be carried out. It is very likely a company may test a fibre, yarn or fabric for its antimicrobial properties however when these components are incorporated into the final product they no longer meet the standards due to finishing processes and manufacturing. However, as the product has been tested according to an international standard, with results to quote, the company can market their product as having all the perceived benefits of an antimicrobial.

With the incorporation of antimicrobials in clothing and textile products increasing, research has been completed to see whether these companies' claims are truly validated. There have been many cases where researchers have studied silver textiles, and through their antimicrobial validation have found that many commercially available products exhibit minimal to no antimicrobial activity and in some cases, no silver can even be detected (Kulthong, Srisung, Boonpavanitchakul, Kangwansupamonkon and Maniratanachote, 2010). McQueen, Keelan and Kannayiram (2010) reviewed the antimicrobial efficacy of a range of commercially available textile products which are claimed to be antimicrobial. The study found that only half those fabrics tested exhibited antimicrobial activity despite the marketing claims (McQueen, et al., 2010).

Regardless of antimicrobial testing being completed in vitro on a textile product, this efficacy does not always indicate the way a fabric will perform on the body in wear conditions. Many antimicrobial efficacy tests are designed in a way to emulate typical wear conditions however they are only an indication. The results obtained from these tests are often used by marketers of antimicrobial product as solid evidence for product behaviour. McQueen, Keelan, Xu and Mah (2013) examined the antimicrobial efficacy of silver chloride fabrics in vitro and compared this with in vivo results. It was found that after washing the fabrics, they displayed a reduction in antimicrobial behaviour, indicating use of these products over time affects their efficacy. It was also examined that there was a poor correlation between the antimicrobial efficacy of the fabrics in vitro and the way they behaved in wear. The in vivo results showed poor antimicrobial efficacy proving that despite a company testing their products for antimicrobial activity in a laboratory, this is not necessarily indicative of the benefits of the product to the consumer in end use (McQueen, et al., 2013).

For many antimicrobial agents, the agent effect is environment dependent. Due to the antimicrobial nature of silver requiring the molecule to exist in cationic form to be functional, this ion can interact with anionic species. Physiological secretions mostly contain NaCl as well as macromolecules such as proteins like albumin. In aqueous solutions, NaCl and elemental silver dissociate into ionic form and can further interact, including the binding of AgCl resulting in an inhibition of Ag^+ antimicrobial activity (Schierholz, Beuth, Pulverer and König, 1999). It is likely that due to secretions in wounds as well as the high NaCl content in sweat, an antimicrobial silver textile may drop below the minimum inhibitory concentration (McQueen, et al., 2013). The antimicrobial efficacy of textile could be tested and verified in the lab, but due to environmental implications of the end use, for example sweat in sportswear applications, the antimicrobial effect could be diminished.

Although there are standard test methods for antimicrobial efficacy, there are no regulations regarding minimum or maximum amounts of antimicrobial required on a product, nor are there regulations as to how the antimicrobial is incorporated. Lorenz, et al. (2012) evaluated the efficacy and silver release from varying commercially available antimicrobial textile products. Of the seven products tested there was broad variability with the amount of silver detected, ranging from 1.5 to 2925 mg Ag/kg with one of the products not exhibiting any detectable silver despite being marketed as such. Not surprisingly this meant there were inconsistencies in antimicrobial activity, with three of the products displaying no antibacterial activity. One of the textile products in particular had a high content of silver (700 mg kg^{-1}) however displayed no antimicrobial activity as the silver was incorporated as silver metal wires. As silver needs to be in cationic form to be antimicrobial, this textile is unable to exhibit biocidal behaviour. In this case it can be assumed the marketers relied on the common knowledge that silver can be antimicrobial rather than tests from the fabric itself. It is uncertain whether companies that market their products as antimicrobial when no biocidal behaviour can be detected do so out of scientific ignorance or whether they neglect their findings in order to dupe consumers. More transparency in the supply chain of antimicrobial textiles, and international guidelines/standards around antimicrobial efficacy of a final product would be advantageous in order to avoid marketers making false claims regarding an antimicrobial textile product.

2.6.4 Antimicrobial resistance

Microbial organisms, in particular bacteria, have very short lifespans and therefore exhibit a high population turnover. This ability to overturn populations at a rapid rate enables microbes to adapt to extreme and compromising environments. Therefore with development and application of antimicrobial agents, there is evidence of microbes becoming resistant to these agents as they adapt

to the hostile environments they provide (Campbell and Reece, 2002). Antimicrobial resistance is a critical issue, as more strains of microbes become resistant to antimicrobial agents, the less defence the human population has against the lethal microbe populations. Antimicrobial resistance is estimated to cause 700,000 deaths annually and without initiatives this is thought to increase to 10 million deaths by 2050 (Hoffman, et al., 2015). Microbial organisms can be resistant to antimicrobial agents in a variety of ways. The strains may be inherently resistant to certain agents or they may become resistant to the agents over time. It is those populations that become resistant that are of most concern as it suggests they have a great ability for adaptation (Tenover, 2006).

Antimicrobial agents can have varying degrees of potential for strains to develop a resistance towards them. If an agent has one mechanism of action on microbes then it is likely to have a high risk factor for resistance. This is due to providing a fairly minimal level of hostility to the microbe allowing easy adaptation. An agent may also have high risk if it has similar mechanisms of action to other antimicrobial agents as this provides a fairly consistent environment giving microbes opportunity to adapt. Of course, in contrast to this, if an agent has multiple pathways of microbial inhibition, then it will exhibit a low risk factor for developing resistance (Windler, et al., 2013).

With the growing industry and application of antimicrobial agents, particularly in clothing and textiles, the question arises as to whether we are overusing antimicrobial agents. With the antimicrobial sector as large and far reaching as it is, the ability for microbes to become resistant to these agents increases due to the level of exposure. In healthcare, the use of antibiotics to treat infections and ailments is closely monitored, and systems are in place to ensure over-prescription is limited (Shallcross and Davies, 2014). However, this is not the case in the textile sector. Antimicrobial agents are added to many different clothing and textile applications in order to produce consumer desired effects, for example reduction of body odour and limiting infections (Windler, et al., 2013). Without regulation of the incorporation of these agents, there is a concern that the industry is overprescribing and overusing antimicrobials. With laundering of these products, the agents enter our waterways and therefore drinking water, raising concern that there is a potential case for mass prescription of antimicrobial agents and in turn, mass antimicrobial resistance. A lack of scientific knowledge, or potentially a lack of consideration for health driven by the need for sales, may result in the textile industry largely contributing to a worldwide antimicrobial resistance crisis.

2.7 Conclusion

With extensive development of antimicrobial textiles and increasing global use, further research needs to be conducted into the potentially more damaging characteristics of these textiles. With the

variety of agents available and their differing application methods and effects on microbes, updated comparative assessments on the commercially available antimicrobial agents and the common products they are applied to need to be conducted. Comparative risk assessments of the available antimicrobial agents will provide clarity to manufacturers and end users about the potential risks and benefits of using certain antimicrobial textiles. Assessments of this kind should include antimicrobial efficacy, environmental impact, human toxicity and economic contribution. Consumption data is lacking, therefore it is difficult to quantify the true impact antimicrobial textile production and use is having on the economy of the textile industry as well as the global effect on the environment. Life cycle analysis of the various antimicrobial agents may help to provide insight.

The effects of laundering antimicrobial textiles has had limited attention. It would be advantageous to examine the effect of washing reusable antimicrobial clothing and textile products on their antimicrobial ability. Also testing the durability of the antimicrobial agent on the textile would provide indication of the amount of agent being washed into the waterways and therefore the potential environmental impact.

Research regarding the detrimental effects of antimicrobial agents on the physiological health of the user is also currently scarce. Limited research has been conducted on the effects of antimicrobial textiles on the resident microbial flora on the skin. It would be valuable to examine the effects of a range of commercially available antimicrobial textile products, in various skin environments on the most predominant microbial species. In conjunction with testing the effect on resident microbial flora populations, other skin physiological parameters could be tested in order to better understand the effects on healthy human skin.

There is little to no research available on the evidence of infiltration of topical antimicrobial agents into the skin. Particularly with the development of nanoparticles, it is necessary to investigate whether these antimicrobial agents are being absorbed into the skin, and if so, is there evidence of this in internal physiology (such as potential concentration levels observed in the blood and urine), and does this have any negative repercussions for physiological processes. By researching all of the potential damaging effects of antimicrobial textiles on the human physiology, a concise human toxicity profile could be produced. This would enable manufacturers and consumers to analyse the potential effects of using an antimicrobial textile and decide whether the benefit of doing so is worth the risk. This may also provide more transparency for corporations using these antimicrobial textiles, potentially decreasing unnecessary use and limiting over-prescription. Encouraging

manufacturers and end users to use antimicrobial agents responsibly would allow the textile industry to contribute to slowing the effects of antimicrobial resistance.

To gauge how potentially damaging antimicrobial textiles can be to the consumer and environment alike, further research must be undertaken on the effect of laundering antimicrobial textiles as well as the effects they have on healthy human skin. Testing should be undertaken on the amount of treatment that is washed into waterways through laundering, as well as how this may affect the antimicrobial efficacy of the textiles. It is also necessary to test the potential detriments to healthy human skin caused by wearing antimicrobial textiles. To do so it would be beneficial to test for topical effects, such as changes in resident microbial flora, effects on skin health parameters (skin capacitance, pH and transepidermal water loss), as well as internal effects like evidence of absorption into the skin and potentially the bloodstream.

Chapter 3

Methods

3.1 Fabric characterisation

3.1.1 Fabrics

Three fabrics were examined. A silver-treated, 100% polyester woven textile was sourced by a New Zealand apparel company from an anonymous manufacturer (name of manufacturer confidential to the New Zealand supplier)[1]. The second fabric was a 100% polyester woven textile with similar structural properties to the antimicrobial fabric but without a silver treatment. The second fabric was matched as closely as possible to the antimicrobial, was purchased as a treatment control. In order to represent fabrics typically worn against the skin and to control whether fabric structure altered bacterial growth and attachment, the third fabric was a 100% polyester knit textile which was also matched to the physical properties of the antimicrobial fabric (Table 3.1).

3.1.2 Sampling of fabrics

Specimens were cut from the fabrics to ensure that each represented separate warp and weft yarns or wale and course yarns. All were sampled in accordance with BS EN 12751:1999 Textiles- Sampling of fibres, yarns and fabrics for testing (European Committee for Standardization, 1999).

3.1.3 Environmental conditions

Specimen preparation, conditioning and testing was carried out under standard atmospheric conditions (20±2°C, 65±4% RH) in accordance with ISO 139:2005 Textiles – Standard atmospheres for conditioning and testing, unless otherwise specified (International Organization for Standardization, 2005).

All specimens were laid flat in a relaxed state and conditioned for a minimum of 24 hours under these conditions prior to testing. Specimens were also tested under these conditions unless stated otherwise.

3.1.4 Mass per unit area

Mass per unit area of each fabric (n=5) was measured using 100 x 100 mm specimens, in accordance with BS EN 12127:1998 Textiles – Fabrics – Determination of mass per unit area using small samples (European Committee for Standardization, 1998).[2]

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[1] Five metres of a silver treated fabric supplied by a New Zealand apparel company. The fabric is silver treated by the yarn. The type of silver and the method of treatment are unknown.

[2] Mettler Toledo AT400 balance; Mettler-Toledo GmBh, Medic, Lower Hutt, New Zealand

Table 3.1

Fabric coding

Fabric	Code	Details
Woven Control	WCW	100% polyester woven washed
Knit Control	KCW	100% polyester knit washed
Non-washed Antimicrobial	WAN	100% polyester woven silver treated non-washed
Washed Antimicrobial	WAW	100% polyester Silver treated washed

Mass per unit area was determined as follows:

$$M = \frac{m \times 10000}{A}$$

(Equation 1)

Where: M = mass per unit area (g/m^2)

m = conditioned weight (g)

A = area (cm^2)

(European Committee for Standardization, 1998)

3.1.5 Thickness

The thickness of each fabric was measured in the centre of each specimen (n=5), in accordance with ISO 5084:1996 Textiles – Determination of thickness of textiles and textile products (International Organization for Standardization, 1996). The thickness gauge[3] has a circular presser foot with an area of 2000 ± 20 mm^2, which was lowered onto the specimens at a pressure of 1 ± 0.01 kPa.

3.1.6 Sett/stitch density

The number of warp/wale or weft/course yarns per 100mm^2 was counted at five random positions on each fabric using a counting glass in accordance with ISO 7211-2:1984 Textiles - Woven fabrics – Construction - Methods of analysis - Part 2: Determination of number of threads per unit length and BS 5441: 1988 British Standard methods of test for knitted fabrics (British Standards Institution, 1988; International Organization for Standardization, 1984).

3.2 Antibacterial efficacy

3.2.1 Fabrics

Fabrics were prewashed prior to testing in accordance with ISO 6330:2012 Textiles – Domestic washing and drying procedures for textile testing (International Organization for Standardization, 2012). Silver treated fabric was washed separately from the non-treated fabrics with a new set of baffles in order to ensure cross contamination of treatment did not occur. Washing separately ensured that, should the silver treatment leach from the fabric during wash, the non-treated fabrics were not exposed to the antimicrobial finish.

Sixty specimens were cut randomly from each of the prewashed fabrics as well as from the non-washed antimicrobial silver fabric (20 x 20 mm, n=240). By testing the new, non-washed fabric, the antimicrobial efficacy of the fabric as sold by the manufacturer could be tested and results can be

[3] SDL Atlas MO34 digital thickness gauge; SDL Atlas Textile Testing Solutions, Stockport, England

compared to the washed fabric as a means of evaluating the durability of the finish. The technical face and rear of the specimens were marked using an ISO 301 lockstitch with contrasting bobbin and needle threads (International Organization for Standardization, 1991). Fabric specimens were stored flat under standard atmospheric conditions (20±2°C, 65±4% RH) until required for testing.

3.2.2 Sterilisation

To ensure the fabrics were free of bacteria prior to being inoculated, the samples were sterilised. ISO 20743:2013 Textiles - Determination of antibacterial activity of textile products, suggests autoclaving as the method of sterilisation most suited to antibacterial testing (International Organization for Standardization, 2013). An autoclave decontaminates materials by subjecting them to a moist heat of 121°C. However, polyester has a glass transition temperature, i.e. the temperature at which the fabric will soften and changes become irreversible, of 60°C therefore there is potential the autoclave may alter the structure of the fabric samples (Hearle and Morton, 2008).

In order to evaluate whether the autoclave might damage the fabric or whether it is an effective form of sterilisation for the samples, a fabric specimen of each fabric (n=3) were first trialled. To evaluate the sterility of the fabrics, specimens of each fabric were placed into individual autoclavable bags and run through a sterilisation cycle. The fabric specimens were allowed to dry in these bags, under ambient conditions. The specimens were then removed with flame sterilised tweezers, placed into tryptic soy broth (TSB) and incubated at 37°C for 24 hours. The sterility of the fabrics was determined by examining the broth for bacterial colonies indicated by the presence of cloudiness in the vial. Subsequently, on inspection of the broth appeared clear therefore it can be concluded the autoclave sterilisation method effectively sterilised all of the fabric samples examined. The fabrics were then examined to assess absence or presence of fibre damage. A sample of each fabric was folded in half, placed in individual autoclavable bags and run through a sterilisation cycle in the autoclave as described above. The samples were removed, allowed to dry in the bags over night, and then checked for any structure damage. If the moist heat had damaged the fabric in any way this would be evident by a permanent deformation in the fabric structure. The fabric samples displayed a clear crease down the fold line, indicative of structural damage. It must be noted that although the yarns did not appear damaged to the naked eye, at the molecular level the crease indicated that the high heat of the autoclave softened the intermolecular bonds, altering the arrangement of the molecules within the fibre (Hearle and Morton, 2008). It was concluded that while the structural damage may have an effect on bacterial attachment, however as all specimens will be autoclaved, damage will be consistent among all fabric specimens. Differences between fabrics will thus be a result of the differences in fabric types and bacterial strains rather than due to differences in treatment.

Other potential decontamination methods were also considered. ISO 20743:2013 Textiles - Determination of antibacterial activity of textile products suggests if autoclaving is not a viable option then ethylene oxide gas or γ rays are possible alternatives (International Organization for Standardization, 2013). For this work, the alternatives were not viable options therefore subsequent forms of sterilisation had to be considered i.e. ultraviolet (UV) sterilisation. UV sterilisation requires specimens be exposed to a dose UVC for a specific period of time. UV sterilisation is a common method of decontamination for water and food products however research documenting the use of this technique on fabrics is limited (Guerrero-Beltran and Barbosa-Canovas, 2004). There are many confounding factors that need to be considered when using UV as a form of sterilisation for fabric i.e. the distance between the fabric and the UV source, penetration through the fabric, and length of exposure. Whether the level of UV for sterilisation results in marked damages to the fibres, or whether it is an effective method of sterilisation for fabrics cannot be determined. Autoclaving was subsequently adopted as the most appropriate sterilisation technique for this research.

Fabric specimens were all laid flat in autoclavable bags, ensuring they did not overlap, and run through a glass sterilisation cycle in an autoclave. The specimens were sterilised as needed and a sample was placed in TSB following every sterilisation cycle as a sterility control. The specimens were left in the autoclavable bags lying flat over night to dry.

3.2.3 Bacterial organisms

For testing of microbiological effects, cultures of bacterial strains known to exist naturally on the skin were purchased[4]. A combination of both gram negative and gram positive, and aerobic and anaerobic strains were selected due to the difference in their cell structures. The strains used were *Staphylococcus epidermidis* (*S. epidermidis*), *Propionibacterium acnes* (*P. acnes*) and *Pseudomonas aeruginosa* (*Ps. aeruginosa*). The bacterial strains were inoculated onto the appropriate agar type and incubated at 37 ± 2°C for 24 hours in order to ensure a sufficient amount of bacteria was obtained i.e. 10^9 CFU/ml (Table 3.2). Stock plates of each bacterial strain were streaked weekly by lifting a line of bacterial colonies using a flamed inoculating loop and streaking this onto a fresh agar plate, to ensure the strain remained fresh (Appendix A). Due to the anaerobic nature of *Propionibacterium acnes,* all periods of incubation had to be conducted in an anaerobic jar to ensure optimal growth. The anaerobic jar, a rectangular plastic container with a sealed lid, contains 2-5 anaerobic packs (depending on the size of the jar), which eliminates oxygen from the container and must be

[4] Bacterial strains purchased from the Institute of Environmental Science and Research Limited, New Zealand Reference Culture Collection

Table 3.2

The bacterial organisms used including their natural growth characteristics and specific strain information

Organism	Relationship to Host	Gram Staining	NZRM Strain	Source of Strain	Growth Media	Incubation
Staphylococcus epidermidis	Commensal	Positive	2205 (ATCC 14990)	Nose	Trypticase Soy Agar	Aerobically 37 ± 2°C 24 hours
Propionibacterium acnes	Commensal, can be pathogenic	Positive	1078 (ATCC 6919)	Facial acne	Anaerobic Blood Agar	Anaerobically 37 ± 2°C 5 days
Pseudomonas aeruginosa	Commensal, can be pathogenic	Negative	2576 (ATCC 9027)	Outer ear infection	Trypticase Soy Agar	Aerobically 37 ± 2°C 24 hours

replaced every time the jar is opened and closed. The American Type Culture Collection (ATCC) recommendation for *Propionibacterium acnes* incubation is anaerobically for 72 hours, however after monitoring the growth of the culture, the ideal colony number (10^9) was more reliably reached after five days (American Type Culture Collection).

3.2.4 Antibacterial activity

The bacteriostatic activity of the fabrics was determined in accordance with a modified version of the EUCAST disk diffusion method (Matuschek, et al., 2014). An inoculum suspension was prepared for each of the bacterial strains. Following incubation for 24 hours, several morphologically similar colonies were suspended in tryptic soy broth (TSB) and 0.1 ml of each organism's inoculum was pipetted onto Petri dishes laid with appropriate nutrient agar. The inoculum was spread over the plate with a sterilised glass spreader to produce an even 'lawn' of bacteria. Two fabric specimens were then placed firmly onto each plate over the spread inoculum: one autoclaved and one non autoclaved to account for any differences that may have occurred during sterilisation. The Petri dish was then incubated within 15 minutes of inoculation for 24 hours at 37 ± 2°C. At the end of the incubation period, antimicrobial activity was determined by the measurement of any halo that may have formed around the edges of the fabric samples. The halo method is typically used for diffusible antimicrobials, but because the fabrics can be lifted, inspection underneath the specimens was possible showing bacterial growth in direct contact with the fabrics.

The fabric specimens were also tested for antimicrobial activity against dilute inoculums to examine whether the germicidal action is concentration dependent. A dilution series of each bacterium was produced and 0.1 ml of 10^{-4} and 10^{-6} (approximately 10^5 and 10^3 CFU/ml) dilutions were spread onto appropriate agar to form plated dilutions of 10^{-6} and 10^{-8} (approximately 10^3 and 10^1 CFU/ml). The Petri dishes were incubated within 15 minutes of inoculation for 24 hours at 37 ± 2°C and antimicrobial activity was determined by the presence of a halo around the fabric specimens.

3.2.5 Bacterial activity on fabrics in simulated wear

As the antimicrobial fabric is sold for commercial use in clothing, it is necessary to test the fabric's antimicrobial properties in simulated wear conditions to establish whether environment has an effect on the bactericidal activity. In order to test the bacterial growth in as close to wear conditions as possible, an incubated humidity chamber was developed that enabled the fabrics to be inoculated with bacteria, and suspended in a controlled humidity and temperature environment (Figure 3.1, 3.2). The chamber was comprised of an autoclavable plastic jar with a screw-on lid. In the chamber, three metal rods were suspended above 100 ml of distilled water. Fabric specimens

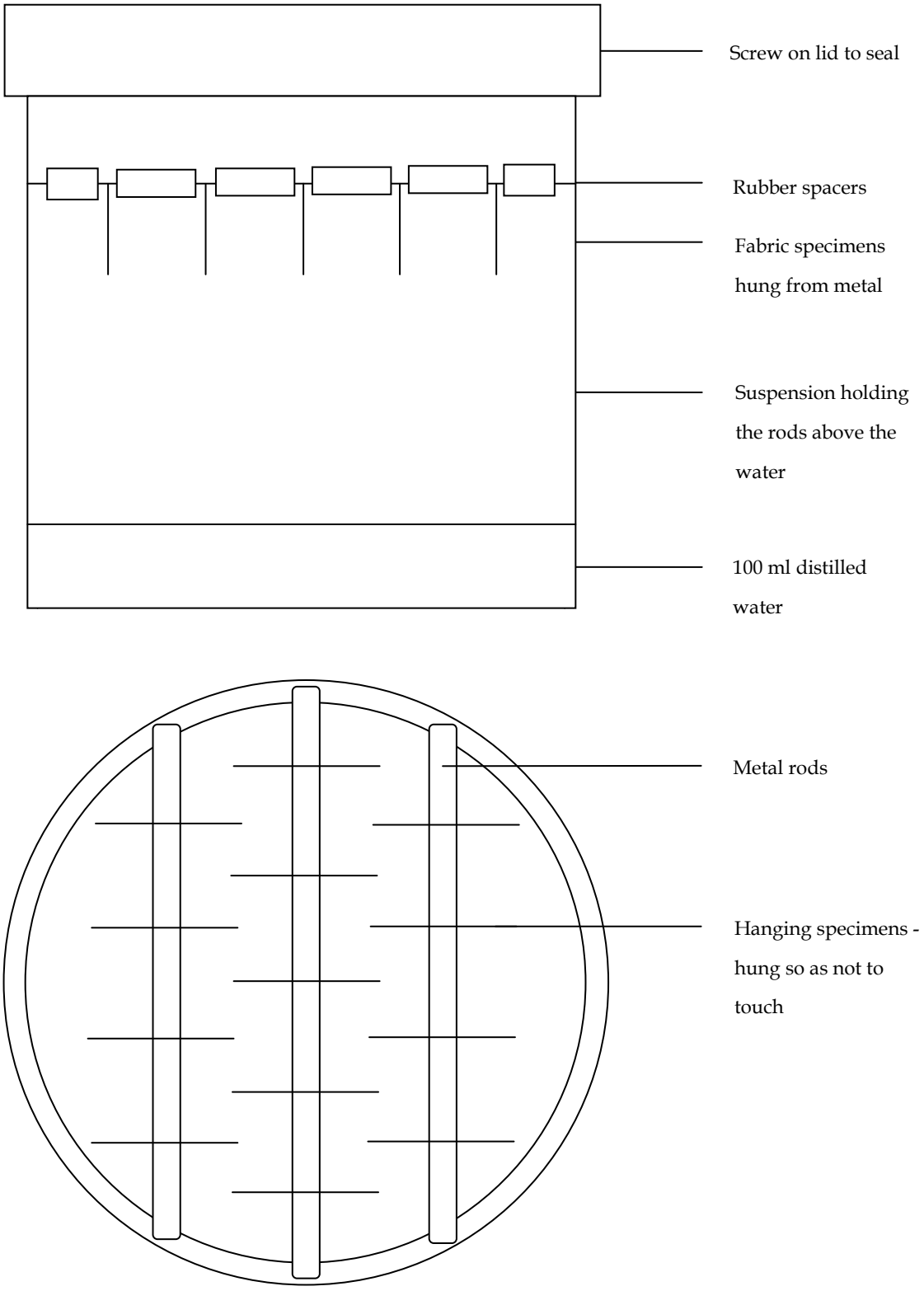


Figure 3.1 Schematic of a humidity chamber in which inoculated fabric specimens can be suspended under controlled ambient humidity and temperature

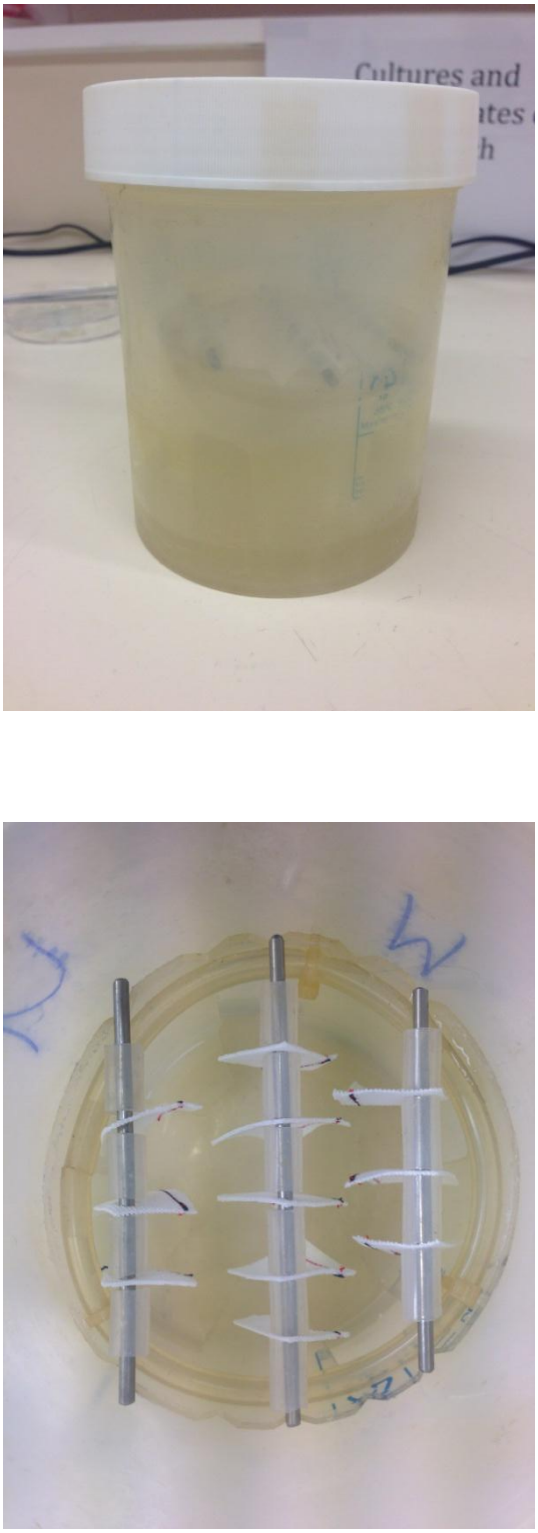


Figure 3.2 Photographs of a humidity chamber in which inoculated fabric specimens can be suspended under controlled ambient humidity and temperature

(20 x 20 mm) had a small hole punched into the corner, and were threaded onto the rods. In between each specimen, a sterile rubber spacer prevented the specimens from touching. The three rods were then inserted so that the fabric specimens were offset from each other to avoid contamination, and the lid screwed on tightly. An iButton® (Maxim Integrated)[5] device was also suspended from the lid which monitored the internal temperature and humidity of the chamber. The chamber maintained a consistent 90% relative humidity (RH) and 25°C. All chambers were autoclaved prior to use.

Culture was incubated for 24 hours in TSB for each of the bacterial strains (and 5 days in an anaerobic jar for *Propionibacterium acnes*). The broth was then centrifuged for 15 minutes at 3000 rpm and 20°C. The supernatant was discarded and the pellet resuspended in 10mls of 0.1% peptone, purifying the bacterial culture. As the overnight culture was expected to have approximately 10^9 CFU/ml, a dilution series was set up; 1 ml of culture was pipetted into 9 ml of 0.1% peptone giving a 10^{-1} dilution (approximately 10^8 CFU/ml), then 1ml of this dilution was further pipetted into 9 ml of 0.1% peptone giving a 10^{-2} dilution (approximately 10^7 CFU/ml). This is repeated, pipetting 1 ml of each further dilution into 9mls of 0.1% peptone until a dilution of 10^{-6} (approximately 10^3 CFU/ml) is reached (Figure 3.3).

A drop plate was produced for each bacterial strain, in order to enumerate the cells per ml the inoculum contained. An agar plate of the appropriate nutrient agar was divided into quarters, with each quarter representing a higher dilution. Five 0.01 ml aliquots of the dilutions 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were plated in separate quarters giving plated dilutions of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} (Figure 3.3).

The inoculated plate was then incubated inverted for 24 hours at 37°C (and for 5 days in an anaerobic jar for *Propionibacterium acnes*). Following incubation, a dilution was chosen that displayed 2 to 20 visible, defined colonies in each drop (Appendix B). The colonies for each replicate were counted and a mean number of cells determined (Figure 3.3). From this mean colony count, the number of cells per ml of the original inoculum was determined by scaling up the count in accordance with the factor of dilution used:

$$Bacterial\ Culture = Mean\ count\ \times\ dilution^{-1}\ \times 100$$

(Equation 2)

Where: Bacterial culture = CFU/ml

[5] iButton ®, Maxim Integrated, San Jose, California, United States

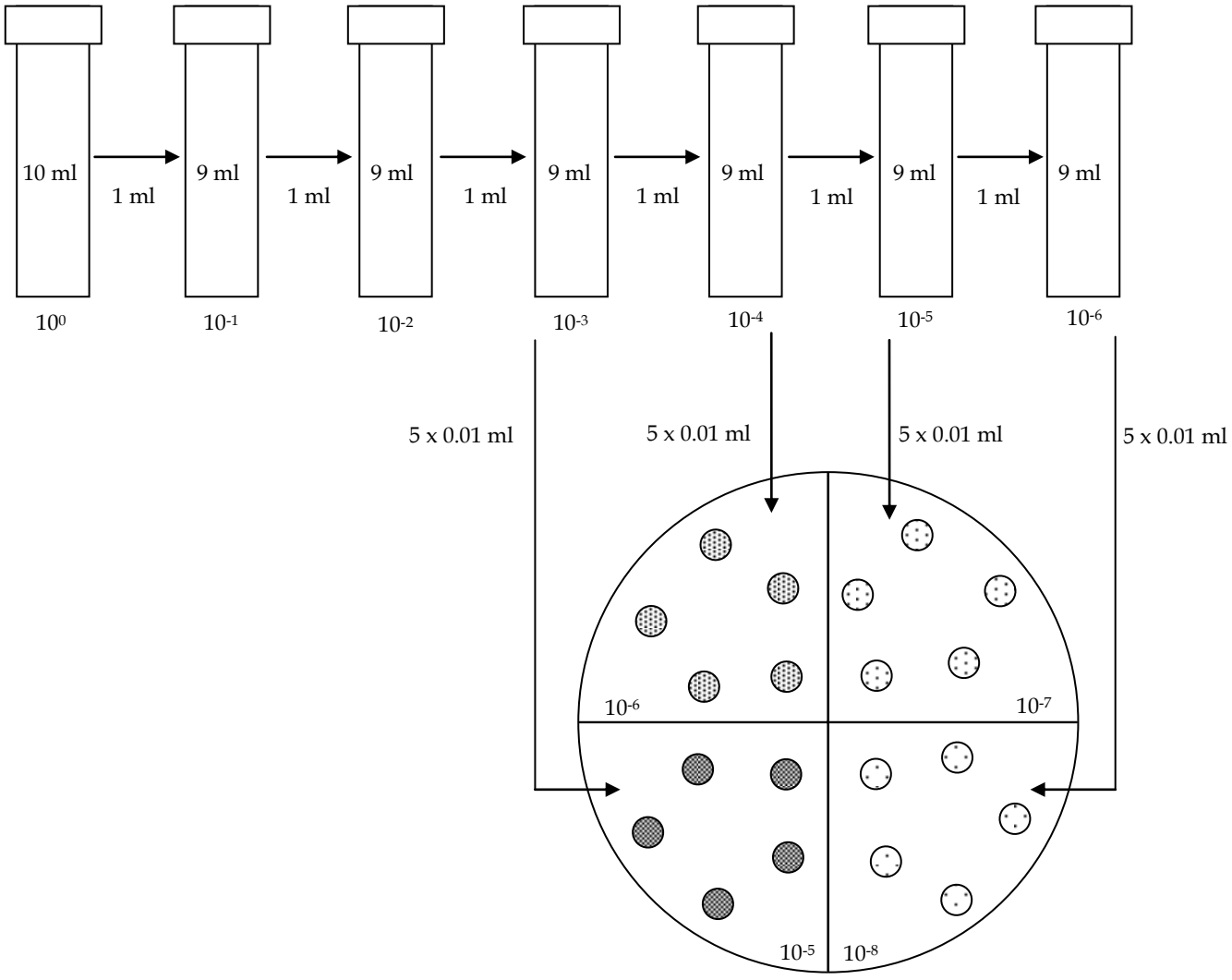


Figure 3.3 Schematic of a dilution series and resultant inoculated drop plate

For the following, all bacterial experimental work was completed under a PC2 hood, in a sterile environment. All tools were sterilised prior to use, including being flamed after touching different specimens and chamber components i.e. when threading the fabric and spacers onto the rods and inserting them into and removing them from the chamber.

Three specimens of each fabric were inoculated with one strain of bacteria by pipetting 0.1 ml of dilute bacterial culture at 10^{-3} dilution (approximately 10^{6} CFU/ml). The specimens of each fabric were then threaded onto the rods with spacers in between to prevent the specimens touching. The rods were then suspended in the humidity chamber and kept at high humidity (approximately 90% RH) and at 25°C, as these are the closest conditions to the most populated areas of human skin in daily wear (Wilson, 2009). The chamber was kept in an incubator (to maintain temperature) and removed after certain time periods (T_0, T_1, T_6 hours) (chamber kept in an anaerobic jar for *P. acnes*). Once the chambers were removed from the incubator, the fabric specimens were unthreaded with sterilised tweezers and immediately immersed in separate vials containing 10 ml 0.1% peptone and 0.2% Tween20® (a brand of the surfactant polysorbate 20 that aids the removal of bacteria), and three glass beads. The vials containing the specimens were hand shaken continuously for 2 minutes each. A dilution series of each specimen's solution was created to give the dilutions 10^{-6}, 10^{-7}, 10^{-8}, enabling the enumeration of the bacteria retrieved from the fabric. Drop plates were inoculated for each specimen with plated dilutions of 10^{-7} and 10^{-8}. Due to the difficulty of counting a dilution of 10^{-9} in small drops, this dilution was plated on a spread plate which allowed a larger aliquot over a greater surface area; 0.1 ml of the 10^{-8} dilution were pipetted across nutrient agar and spread using a sterilised glass spreader. All plates were then inverted and incubated for 24 hours at 37°C (5 days in an anaerobic jar for *P. acnes*). Once removed, bacterial colonies were counted and scaled up according to the dilution factor to estimate the number of bacteria removed from the fabric specimens per cm^2.

3.3 Statistical analysis

Descriptive statistics were calculated for all fabric properties i.e. mean, standard deviation and coefficient of variation, enabling ease of comparison between the fabrics. Unpaired, two sample t-tests were consulted in order to validate the control fabric and knit fabric similarity to the silver fabric. Another unpaired, two sample t-test was used to examine whether washing the silver antimicrobial had any effect on the fabric's dimensional properties, with significance determined at the $p \leq 0.05$ level.

In order to determine the bacteriostatic activity of the antimicrobial fabrics, qualitative analysis was performed by examining the presence or absence of a halo around both the non washed and

washed antimicrobial fabric specimens, as well as the woven control, on the agar plates. In the event no halo formed around the specimens, specimens were lifted to examine presence or absence of bacteria growth underneath the fabric i.e. in direct contact with the fabric and agar.

To examine whether the bacterial counts retrieved from the fabrics after suspension in the humidity chamber were different across fabrics, among bacterial strains, and over time, a univariate analysis of variance (ANOVA) was conducted. To determine the equality of the variance across the factors, a Levene's test of equality of error variances was performed. As the bacterial means exhibited unequal variances, a logarithmic transformation ($\log_{10}$) was applied to the data to improve normality. Where significant differences were identified among fabric types, bacterial strains, and time periods, a Tukey's HSD multiple comparison procedure was performed to determine which variables were significant.

Bacterial attachment was determined by calculating the percentage differences between the bacterial count means and the drop plate count i.e. the difference between what was inoculated onto the fabric and that which was removed. A positive bacterial attachment percentage indicates more bacteria was removed from the fabric than had been inoculated initially, indicating growth of bacteria on the fabric with nil to minimal bacterial attachment; the more positive the percentage, the more bacteria grew. A bacterial attachment of zero indicates that nearly all bacterial cells inoculated onto the fabric were removed, or limited numbers grew and remained attached. A negative bacterial attachment percentage indicates fewer bacteria were removed from the fabric then were inoculated. The more negative the percentage, the fewer bacteria were removed. The conclusions that can be drawn from this are two-fold: either the bacteria deficit is due to those bacterial colonies dying or they remain attached to the fabric.

Removal efficacy is determined by the bacterial percentages at T_0. At T_0, all bacteria inoculated on the woven and knit controls should be removed giving a percentage of 0%, as theoretically this is not enough time for the bacteria to become attached to the fibres. A negative percentage indicates error in the removal method as not all bacteria have been removed. A positive percentage indicates human error as no bacteria should have grown on the fabric.

A univariate ANOVA was then conducted to determine differences among fabrics, among bacterial strains and over time. Levene's test of equality of error variances were non significant, indicating equal variances. Where significant factors were identified among fabric types, bacterial strains, and time periods, a Tukey's HSD multiple comparison procedure was performed to determine which variable levels were significantly different from others.

Chapter 4

Results

4.1 Fabric properties

The mass, thickness and sett of each fabric are described in Table 4.1a. The woven control fabric (WCW) and the non-washed antimicrobial (WAN) differed in mass, thickness and sett (warp/weft) ($t_{4,10}$=32.54, $p \leq 0.001$; $t_{4,10}$=32.17, $p \leq 0.001$; $t_{4,10}$=60.08, $p \leq 0.001$; and $t_{4,10}$=17.21, $p \leq 0.001$ respectively; Table 4.1b). The antimicrobial fabric was overall lighter, thinner, and had finer yarns than the control, and this needs to be considered when comparing bacterial attachment values.

The woven and knitted fabrics (WCW, KCW) selected to examine the effect on fabric structure on bacterial growth and attachment, were confirmed to be similar in sett density (warp/wales identical values; and $t_{4,10}$=1.265, p = NS for weft/courses), however they differed in mass and thickness' ($t_{4,10}$=40.21, $p \leq 0.001$; and $t_{4,10}$=55.67, $p \leq 0.001$ respectively; Table 4.1b). The differences in mass and thickness of the controls need to be considered when comparing bacterial growth in terms of the fabric structure.

4.2 Antimicrobial activity

Firstly, the agar plates were examined to determine whether or not a halo was present in the agar surrounding the fabrics. No halo was observed in association with any of the fabric specimens: the control, the non-washed and the washed antimicrobial fabrics. In addition, bacteria were observed growing underneath all specimens, and in direct contact with the fabric (Figure 4.1). Bacterial growth in direct contact with the antimicrobial fabric suggests that there was no bactericidal effect.

There is a possibility that the concentration of bacteria inoculated on the plates was too high and pure for the antimicrobial activity to be effective. In order to explore this, diluted bacterial cultures of 10^{-6} and 10^{-8} were used. Again, the bacteria grew under the fabrics in both the non-washed and washed antimicrobial fabrics for the 10^{-6} dilution (Figure 4.1). The 10^{-8} dilution did not contain sufficient bacterial cells to give conclusive results.

4.3 Antimicrobial attachment in simulated wear

4.3.1 Bacterial growth on fabrics

Bacterial counts for all fabrics are provided in Table 4.2, and the mean bacterial counts removed from the fabrics following 0, 1 and 6 hours in the humidity chamber are described in Table 4.3. Figure 4.2 and 4.3 depict the relationship among bacterial strains and fabric type for each time period in terms of bacterial counts.

Table 4.1a

Fabric properties

		Mass (n=5)			Thickness (n=5)			Sett (n=5) (Warp/Weft or Wales/Courses)		
Fabric	**Code**	**Mean (g/m^2)**	**s.d.**	**C.V. (%)**	**Mean (mm)**	**s.d.**	**C.V. (%)**	**Mean (ends/cm picks/cm)**	**s.d.**	**C.V. (%)**
Woven	**WCW**	181	2.71	1.50	0.61	0.01	1.88	22/20	0.00/0.55	0.00/2.68
Knit	**KCW**	284	5.05	1.78	0.94	0.01	0.75	22/21	0.00/1.30	0.00/6.15
Non-washed Antimicrobial	**WAN**	138	1.27	0.92	0.37	0.01	3.05	60/35	1.41/1.79	2.36/5.14
Washed Antimicrobial	**WAW**	138	0.64	0.46	0.39	0.01	2.19	62/38	0.00/0.00	0.00/0.00

Table 4.1b

Significances* of differences between various fabric properties

	Mass		Thickness		Sett			
					Warp		Weft	
Between Factors	**t**	**Sig.**	**t**	**Sig.**	**t**	**Sig.**	**t**	**Sig.**
WCW x KCW	40.21	$p \leq 0.001$	55.67	$p \leq 0.001$	n.a.	NS	1.27	NS
WCW x WAN	32.54	$p \leq 0.001$	32.17	$p \leq 0.001$	60.08	$p \leq 0.001$	17.21	$p \leq 0.001$
WAN x WAW	0.63	NS	1.27	NS	3.16	$p \leq 0.05$	4.00	$p \leq 0.05$

* Students T-test

Table 4.2

Mean bacterial counts (CFU/cm²) and standard deviations for each bacterial strain in relation to fabric type after 0, 1 and 6 hours

		0 Hour		1 Hour		6 Hour	
Organism	**Fabric**	**Mean (CFU/cm²)**	**s.d.**	**Mean (CFU/cm²)**	**s.d.**	**Mean (CFU/cm²)**	**s.d.**
S. epidermidis	**WCW**	3.38×10^6	1.23×10^6	1.53×10^6	0.30×10^6	2.61×10^6	0.69×10^6
	KCW	4.68×10^6	1.04×10^6	1.94×10^6	0.86×10^6	3.01×10^6	0.73×10^6
	WAN	4.74×10^6	1.24×10^6	2.42×10^6	9.21×10^6	2.63×10^6	0.94×10^6
	WAW	3.95×10^6	0.46×10^6	1.54×10^6	0.34×10^6	1.59×10^6	0.23×10^6
Ps. aeruginosa	**WCW**	0.41×10^6	0.10×10^6	3.52×10^6	2.42×10^6	1.68×10^6	5.64×10^6
	KCW	0.59×10^6	0.30×10^6	4.27×10^6	1.16×10^6	1.57×10^6	0.31×10^6
	WAN	0.53×10^6	0.28×10^6	5.93×10^6	1.62×10^6	1.45×10^6	0.46×10^6
	WAW	0.52×10^6	0.24×10^6	7.33×10^6	1.62×10^6	1.75×10^6	0.51×10^6
P. acnes	**WCW**	0.66×10^6	0.15×10^6	0.96×10^6	0.35×10^6	0.48×10^6	0.37×10^6
	KCW	0.65×10^6	0.20×10^6	1.05×10^6	0.21×10^6	0.41×10^6	0.35×10^6
	WAN	0.76×10^6	0.26×10^6	1.17×10^6	0.22×10^6	0.29×10^6	0.13×10^6
	WAW	0.50×10^6	0.25×10^6	0.89×10^6	0.24×10^6	0.67×10^6	0.17×10^6

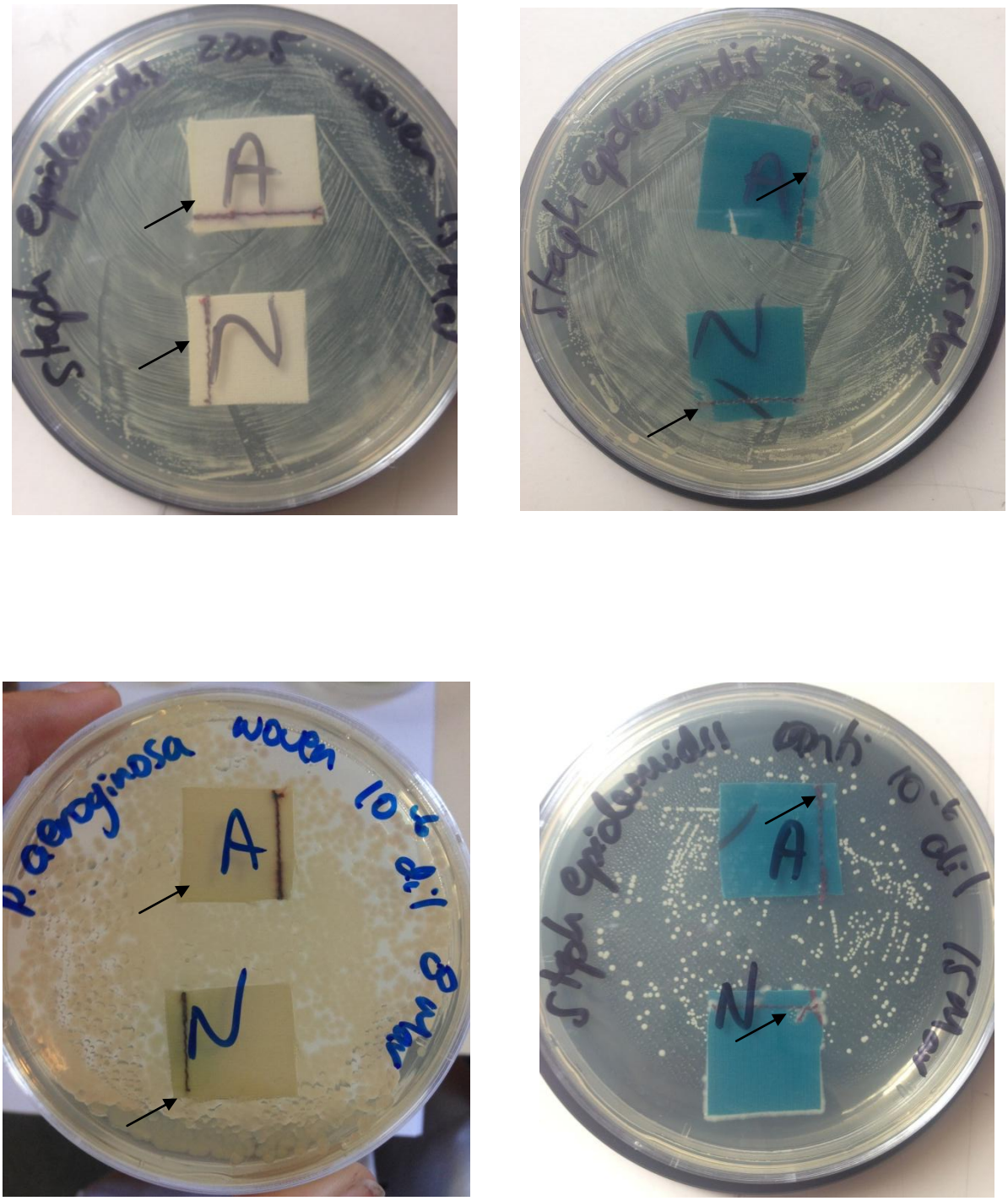


Figure 4.1 Bacterial growth around and under fabric specimens. Arrows indicate bacterial growth under the fabric

4.3.1.1 Fabric structure

Bacterial growth on the woven control fabric (WCW) and the knit fabric (KCW) did not differ irrespective of the bacterial strain or the length of time in the humidity chamber ($F_{1,36}$ = 2.14, NS; Table 4.3, Figure 4.2). Therefore the fabric structure did not affect bacterial growth. Figure 4.2 shows similar patterns of growth with overlapping standard deviations.

4.3.1.2 Bacterial strain

All bacterial strains grew differently in terms of number of colony forming units, regardless of fabric type or the length of time in the humidity chamber ($F_{2,72}$ = 165.28, $p \leq 0.001$; Table 4.3, Figure 4.3). Tukey's HSD multiple comparisons test show all bacterial strains were different from one another, with no groups of similar growth (Table 4.4). Across all fabrics, *S. epidermidis* displayed the most different growth behaviour, with the lowest bacterial counts consistently being taken at the 1 hour point, whereas *Ps. aeruginosa* and *P. acnes* appeared to have more similar patterns of growth, with the highest bacterial counts measured at the 1 hour point and lower bacterial counts measured at 0 hours and 6 hours. *Ps. aeruginosa* displayed dramatic changes in bacterial numbers, with very high bacterial counts at 1 hour and a large reduction in count at 6 hours. *P. acnes* displayed the lowest counts in bacteria overall which may be due to its anaerobic nature, resulting in a slower growth rate (Figure 4.3).

4.3.1.3 Growth over time

Bacterial growth was shown to change over time regardless of bacterial strain or fabric type ($F_{2,72}$= 38.67, $p \leq 0.001$; Table 4.3; Figure 4.2, 4.3). For *Ps. aeruginosa* and *P. acnes*, the highest bacterial counts were recorded at 1 hour whereas for *S. epidermidis*, the lowest bacterial counts were demonstrated after 1 hour. For all bacterial strains, time points 0 and 6 hours had very similar bacterial counts as shown by Tukey's groupings (Table 4.4). At time 0, this represents the amount of bacteria removed from a fabric directly after inoculation. After 1 hour, if bacteria have grown on the fabric, the bacterial counts are expected to be higher. For both *Ps. aeruginosa* and *P. acnes* both the washed and non-washed antimicrobial fabrics displayed bacterial growth after 1 hour which is not what would be expected with a functional antimicrobial fabric, which would display minimal to nil growth (Figure 4.3).

4.3.1.4 Antimicrobial treatment

The presence of an antimicrobial treatment did not reduce the bacterial growth on the fabrics. In fact, the fabric with the antimicrobial treatment was more conducive to bacterial growth than the woven and knit controls ($F_{1,72}$ = 4.77, $p \leq 0.05$; Table 4.3). The bacterial strain where this was most apparent was *Ps. aeruginosa*, where both the non-washed and washed antimicrobial fabrics (WAN, WAW) had higher bacterial counts than the woven and the knit controls (WCW, KCW), particularly after 1 hour (Table 4.2; Figure 4.2). *S. epidermidis* and *P. acnes* had the highest bacterial counts on the non-washed antimicrobial fabric (WAN) which, again, is not what would be expected from a functional antimicrobial (Figure 4.2).

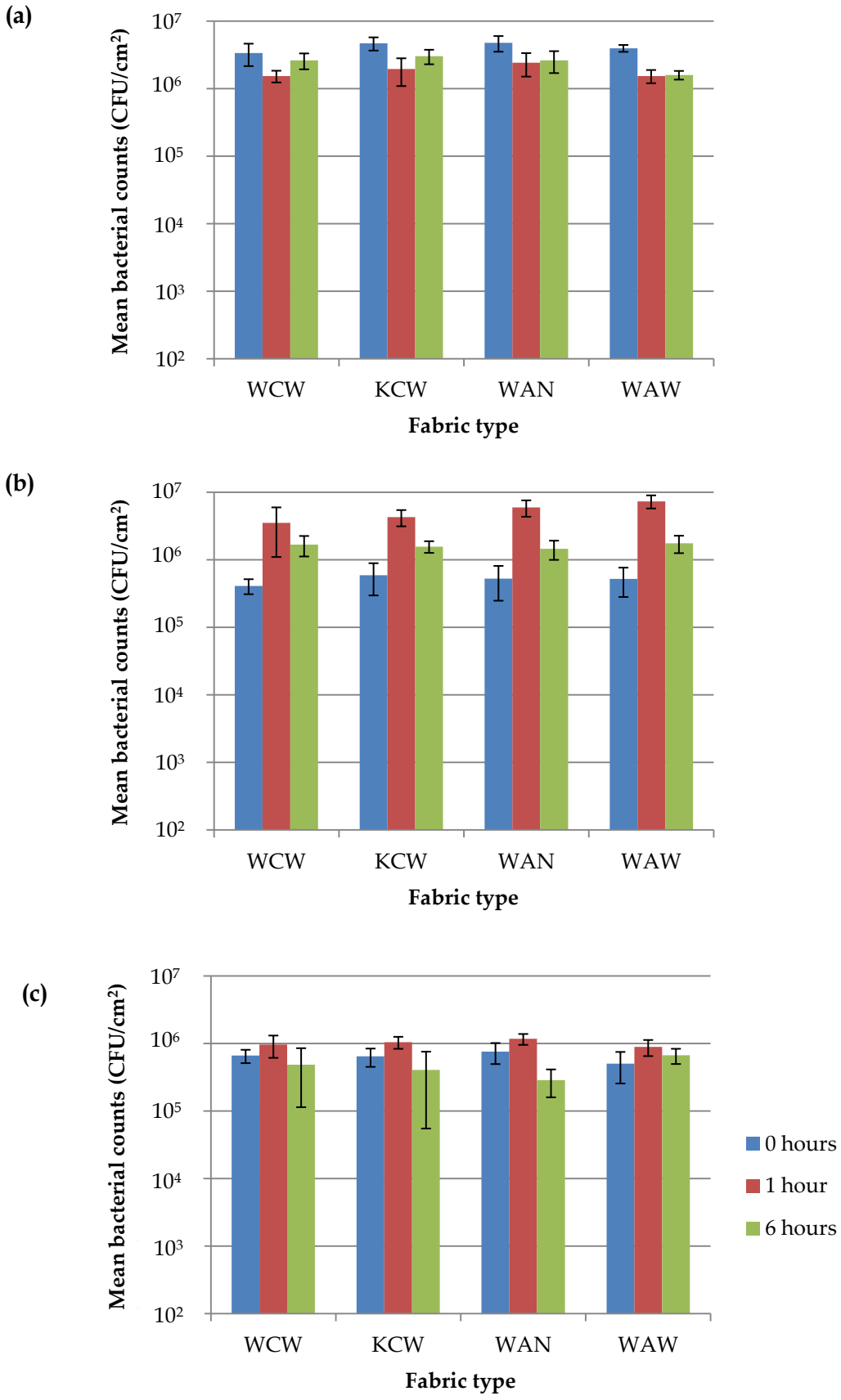


Figure 4.2 Mean bacterial counts (CFU/cm²) for each bacterial strain in relation to fabric type over 0, 1, and 6 hours:

a) ***Staphylococcus epidermidis*****, b)** ***Pseudomonas aeruginosa*****, c)** ***Propionibacterium acnes***

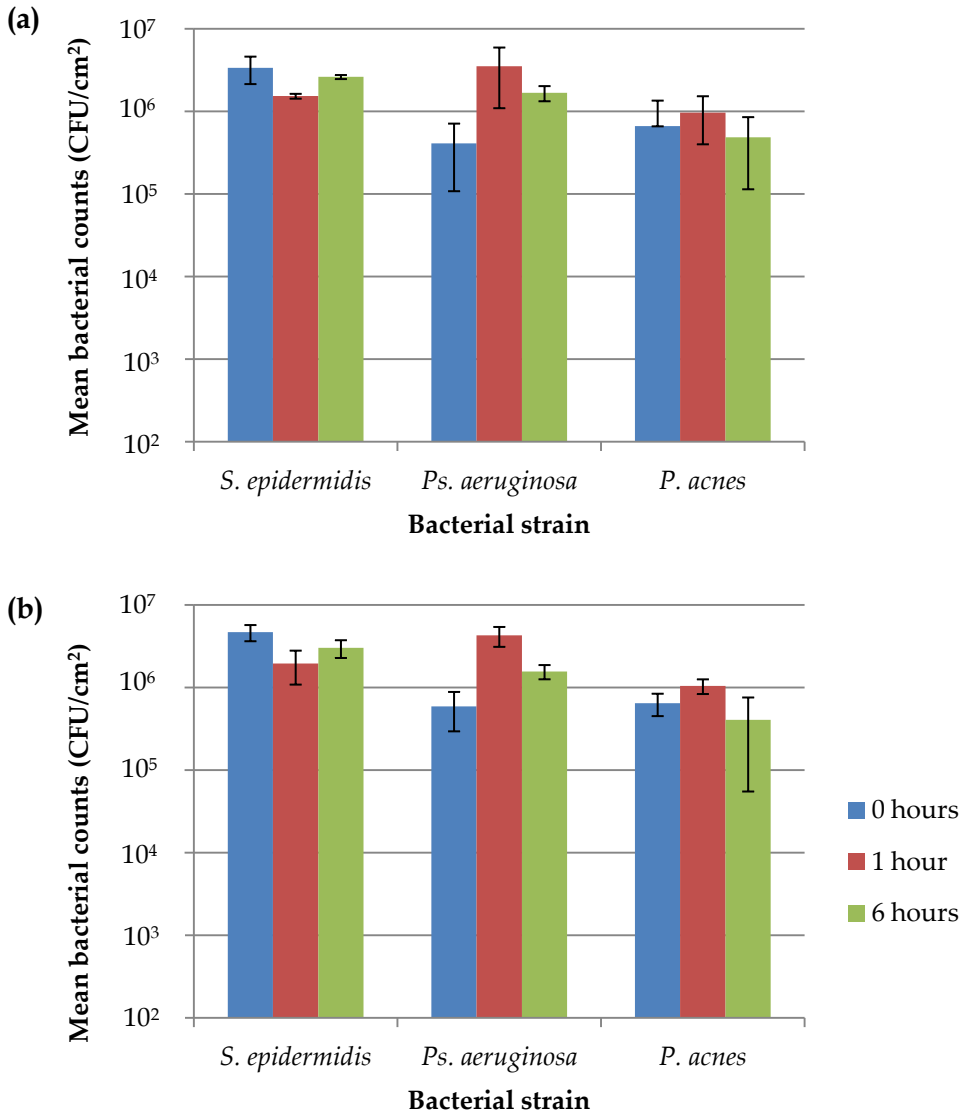


Figure 4.3 Mean bacterial counts (CFU/cm²) for each fabric type in relation to bacterial strain over 0, 1, and 6 hours:
a) Woven, b) Knit, c) Non-washed antimicrobial, d) Washed antimicrobial

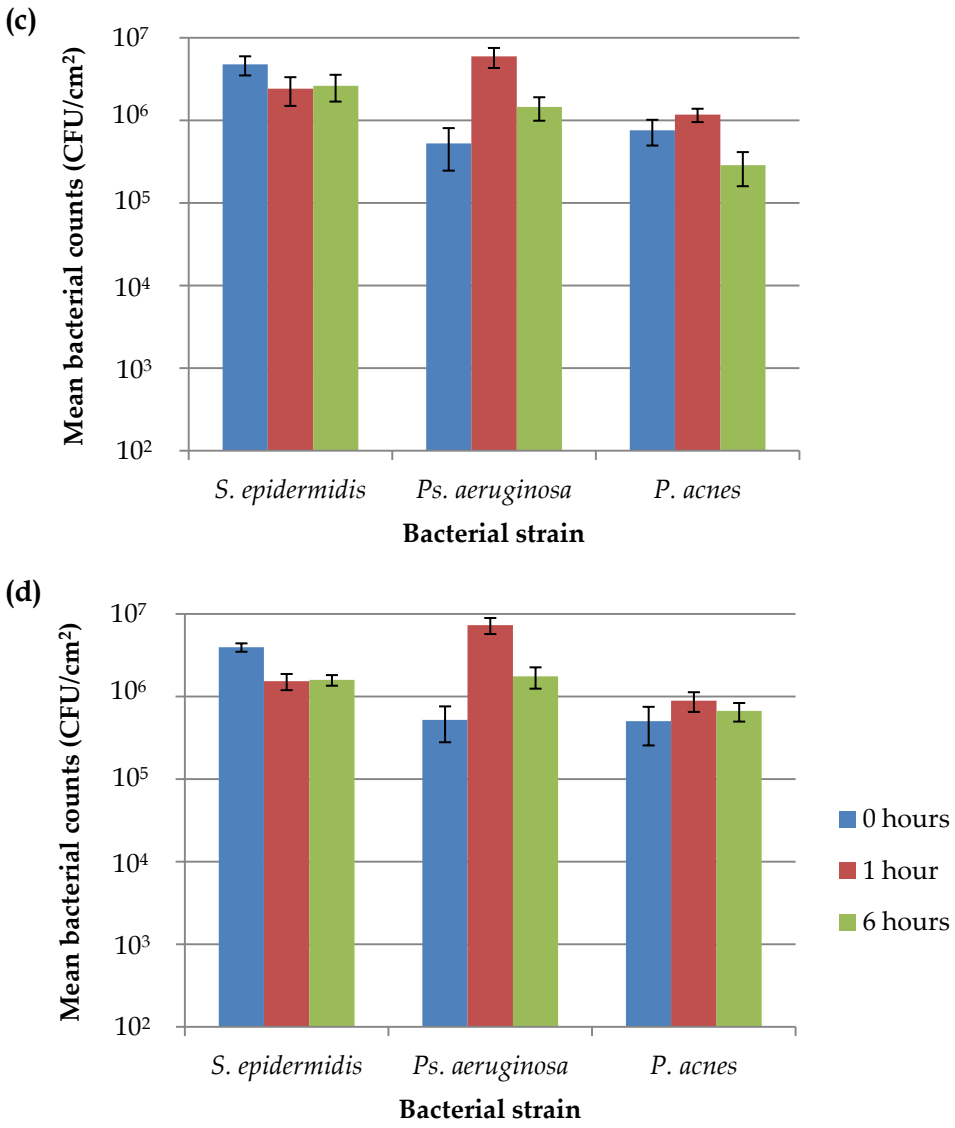


Figure 4.3 cont. Mean bacterial counts (CFU/cm²) for each fabric type in relation to bacterial strain over 0, 1, and 6 hours:

a) Woven, b) Knit, c) Non-washed antimicrobial, d) Washed antimicrobial

Table 4.3

Effects of various factors relating to mean bacterial counts*

Source of variation	Degrees of Freedom	Mean Square	F Statistic	p Value	Significance
Fabric Structure	1	0.055	2.14	0.152	NS
error	36	0.026			
Bacterial strains	2	3.017	165.28	0.000	$p \leq 0.001$
Growth over time	2	0.706	38.67	0.000	$p \leq 0.001$
Antimicrobial treatment	1	0.084	4.58	0.036	$p \leq 0.05$
error	72	0.018			
Washed antimicrobial	1	0.014	1.34	0.255	NS
error	36	0.011			

* Univariate analysis of variance (ANOVA)

Table 4.4

Groupings of similarities in a) bacterial strains and b) time periods, relative to mean bacterial counts

		Groupings (log_{10})		
b) Bacterial strains	**N**	**1**	**2**	**3**
P. acnes	36	7.80		
Ps. aeruginosa	36		8.19	
S. epidermidis	36			8.41

a) **Time**			
0 hour	36	8.03	
6 hour	36	8.07	
1 hour	36		8.30

* Tukey's HSD multiple comparisons test

4.3.1.5 Washed antimicrobial

Washing the antimicrobial fabric had no apparent effect on the mass or thickness of the fabric ($t_{4,10}$=0.63, NS and $t_{4,10}$=1.27, NS respectively; Table 4.1b). As a result of washing the antimicrobial, there was a difference in the sett. With the washed fabric, sett was tighter and count higher in both the warp and the weft ($t_{4,10}$=3.16, $p \leq 0.05$; and $t_{4,10}$=4.00, $p \leq 0.05$ respectively; Table 4.1b). However, washing the fabric did not influence bacterial growth overall, regardless of time or bacterial strain evaluated ($F_{1,36}$ = 1.34, NS; Table 4.3).

4.3.2 Bacterial attachment

The mean bacterial counts were compared to the drop plate counts (concentration of inoculated culture) and expressed as a percentage in order to evaluate the proportion of bacteria removed from the fabrics compared to that originally inoculated onto them (Table 4.5). The percentage bacteria removed gives an indication of the efficacy of the removal method when the bacterial counts at time 0 (bacteria removed from the fabric at T_0) are compared to the counts on the drop plate (amount of bacteria inoculated onto the fabric). As the removal method remained consistent, when comparing percentage differences among bacterial strains and fabric type, they give an indication of the amount of bacteria that remained on the fabric i.e. bacteria attached.

4.3.2.1 Removal efficacy

The bacterial percentages given in Table 4.5 are an indication of how many bacterial cells were removed from the fabrics, in relation to how many cells were inoculated on the specimens initially (Figure 4.4, 4.5). The bacteria percentages at time 0, gives the removal efficacy for each bacterial strain on each fabric type.

Figure 4.4 shows the bacterial attachment percentages for each bacterial strain in relation to the fabric type. In order to evaluate the efficacy of the removal method, the bacterial percentages at time 0 were considered. At time 0 it can be seen that the removal efficacy differed depending on the bacterial strain and fabric type. *Ps. aeruginosa* showed the lowest removal efficacy for both the woven and the knit fabrics, with *S. epidermidis* exhibiting the highest removal efficacy, particularly for the knit fabric which was almost 0%. These removal efficacies must be taken into account when comparing bacterial strains as, with the method of removal employed in the current work, more *Ps. aeruginosa* naturally remained on the fabric than *S. epidermidis.*

4.3.2.1 Fabric structure

Bacterial removal percentages for all fabrics are given in Table 4.5. Factors affecting the bacterial attachment percentages for each fabric type and bacterial strain following 0, 1 and 6 hours in the humidity chamber were examined (Table 4.6). Overall, bacterial attachment on the woven control

Table 4.5

Mean bacterial attachments (%) and standard deviations for each bacterial strain in relation to fabric type after 0, 1 and 6 hours

		0 Hours		1 Hour		6 Hours	
Organism	**Fabric**	**Mean difference (%)**	**s.d.**	**Mean difference (%)**	**s.d.**	**Mean difference (%)**	**s.d.**
S. epidermidis	**WCW**	-32.2	21.8	-59.4	7.88	-25.8	7.98
	KCW	-6.02	3.19	-48.5	22.2	-14.4	18.4
	WAN	-4.75	11.1	-35.8	7.47	-25.3	15.0
	WAW	-20.7	4.67	-59.2	19.2	-54.9	6.34
Ps. aeruginosa	**WCW**	-75.0	3.05	-77.2	19.7	-29.6	14.5
	KCW	-64.0	14.3	-72.3	7.50	-34.2	8.00
	WAN	-67.9	10.7	-61.5	10.5	-39.1	15.0
	WAW	-68.3	10.3	-52.4	10.5	-26.3	5.29
P. acnes	**WCW**	-44.1	9.63	-18.4	22.6	-59.0	25.9
	KCW	-45.2	10.1	-11.3	18.4	-65.5	15.9
	WAN	-35.9	15.4	-0.848	8.17	-75.7	1.76
	WAW	-57.4	15.9	-24.6	21.2	-43.5	6.85

Table 4.6

Effects of various factors relating to mean bacterial attachments

Source of variation	Degrees of Freedom	Mean Square	F Statistic	p Value	Significance
Fabric Structure	1	600.00	2.50	0.122	NS
error	36	239.67			
Bacterial strains	2	3986.63	20.85	0.000	$p \leq 0.001$
Growth over time	2	94.49	0.49	0.612	NS
Antimicrobial treatment	1	911.85	4.77	0.032	$p \leq 0.05$
error	72	191.20			
Washed antimicrobial	1	576.24	4.03	0.052	NS
error	36	142.73			

* Univariate analysis of variance (ANOVA)

fabric and the knit fabric did not differ regardless of the strain or the length of time in the humidity chamber ($F_{1,36}$ = 2.50, NS; Figure 4.4) suggesting fabric structure did not affect bacterial attachment.

4.3.2.2 Bacterial strain

Bacterial strains displayed different attachment behaviours regardless of the length of time in the humidity chamber and fabric type ($F_{2,72}$ = 20.85, $p \leq 0.001$; Table 4.6). Tukey's HSD multiple comparisons test indicated overall attachment differed among all bacterial strains (Table 4.7). Figure 4.4 shows the bacterial attachment percentages for each bacterial strain in relation to fabric type. As previously noted, *Ps. aeruginosa* displayed the lowest removal efficacy, a pattern which continued after an hour in the humidity chamber. After 1 hour, fewer bacterial cells were removed from the fabric than removed initially i.e. at time 0, possibly indicating bacterial attachment to the fabric. After 6 hours, more bacteria were removed from the fabric than at 0 and 1 hours suggesting bacterial growth may have resulted in surplus bacteria not being able to attach or to attach as efficiently as occurred at 1 hour i.e. a maximal attachment limit reached (Figure 4.4). *S. epidermidis* displayed the greatest removal efficacy at 0 hours, and exhibited greater reduction in numbers removed after 1 hour; the deficit may also be an indication of bacterial attachment on the fabric. Just as with *Ps. aeruginosa*, the increase in bacteria removed following 6 hours may be an indication of bacterial growth (Figure 4.4). *P. acnes* displayed a different pattern of attachment than the other bacterial strains. More bacteria were removed after 1 hour than at 0 hours indicating nil to minimal attachment and an increase in bacterial growth. The removal then greatly reduced after 6 hours indicating either a substantial increase in bacterial attachment, or an increase in bacterial cell death (Figure 4.4).

4.3.2.3 Growth over time

Considering generalised patterns across all bacterial strains and fabric types, overlapping standard deviations indicates the length of time in the humidity chamber did not affect bacterial attachment ($F_{2,72}$ = 0.49, NS; Table 4.6). Tukey's HSD multiple comparisons test show all time periods displayed similar patterns of bacterial removal (Table 4.7). However, different patterns can be identified when comparing each bacterial strain and fabric type separately. *S. epidermidis* and *P. aeruginosa* both show a decrease in bacterial removal at 1 hour and increase in bacterial growth at 6 hours, whereas *P. acnes* shows bacterial growth at 1 hour and evidence of bacterial attachment after 6 hours (Figure 4.4).

4.3.2.4 Antimicrobial treatment

The addition of the antimicrobial treatment alters the bacterial attachment dependent on bacterial strain and fabric type ($F_{1,72}$ = 4.77, $p \leq 0.05$; Table 4.6). As a generalised view, the antimicrobial treatment reduces the bacterial attachment percentage in most cases. Given the increase in bacterial growth after 6 hours from 1 hour in *Ps. aeruginosa* and *S. epidermidis*, there is an indication that the

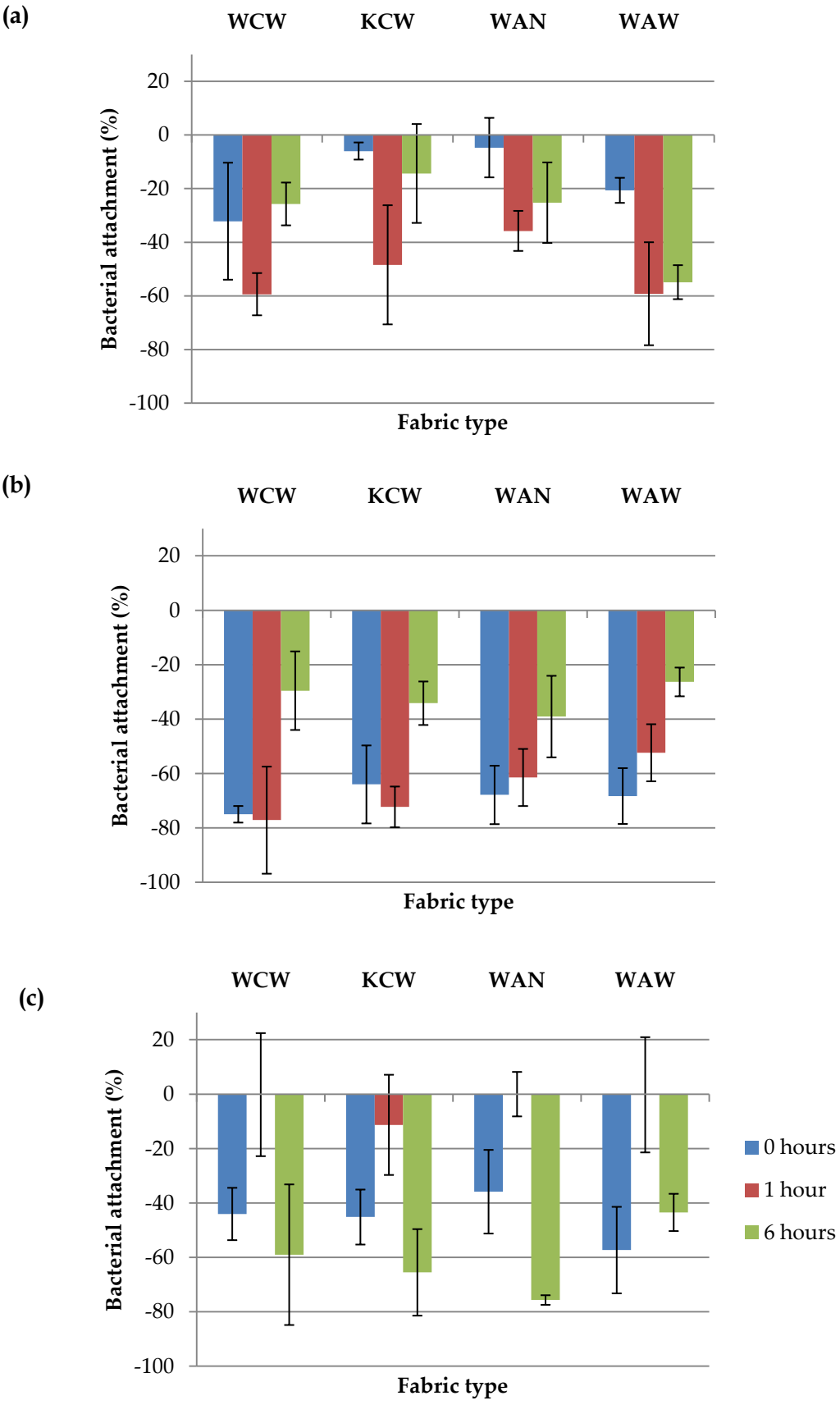


Figure 4.4 Mean bacterial attachment (%) for each bacterial strain in relation to fabric type over 0, 1, and 6 hours:

a) ***Staphylococcus epidermidis*****, b)** ***Pseudomonas aeruginosa*****, c)** ***Propionibacterium acnes***

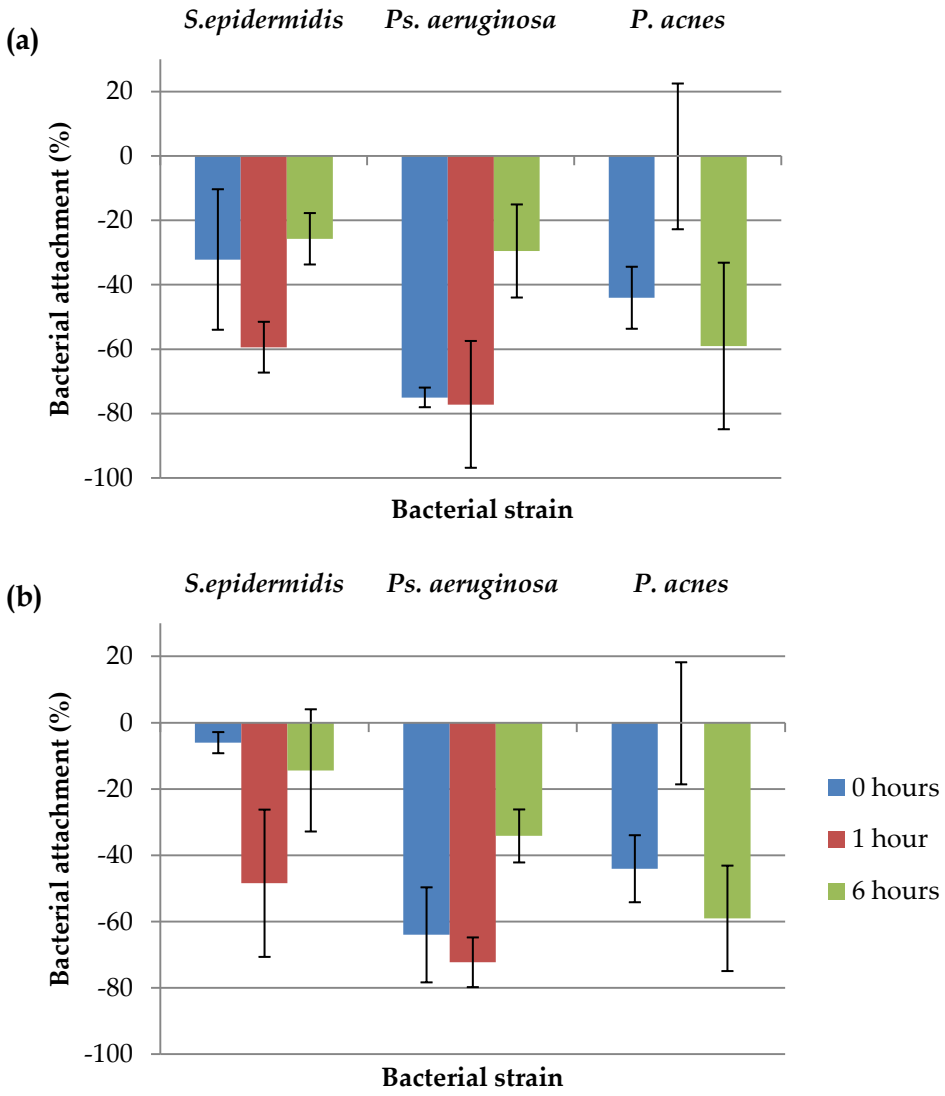


Figure 4.5 Mean bacterial attachment (%) for each fabric type in relation to bacterial strain over 0, 1, and 6 hours:

a) Woven, b) Knit, c) Non- washed antimicrobial, d) Washed antimicrobial

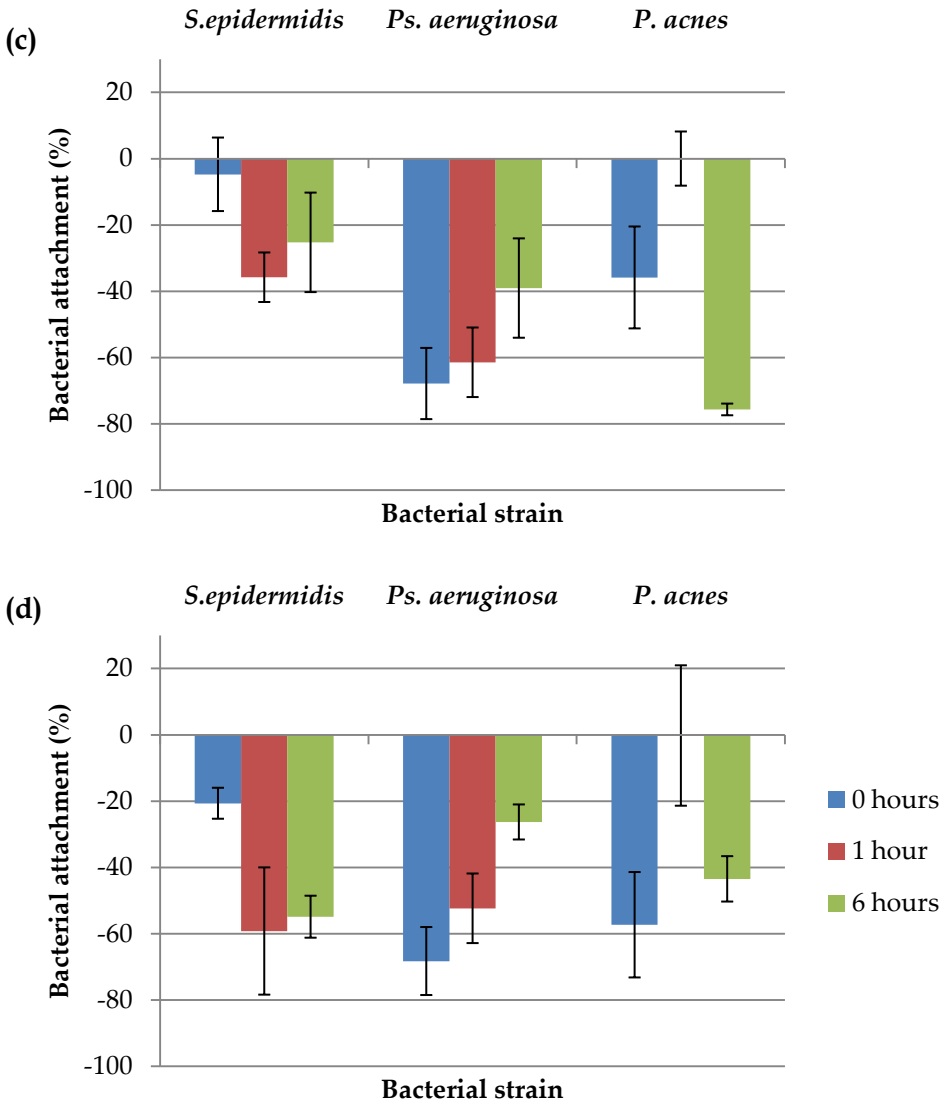


Figure 4.5 cont. Mean bacterial attachment (%) for each fabric type in relation to bacterial strain over 0, 1, and 6 hours:

a) Woven, b) Knit, c) Non- washed antimicrobial, d) Washed antimicrobial

Table 4.7

Groupings of similarities in a) bacterial strains and b) time periods, relative to mean bacterial attachments

		Groupings (%)		
a) **Bacterial strains**	**N**	**1**	**2**	**3**
P. acnes	36	-55.08		
Ps. aeruginosa	36		-39.69	
S. epidermidis	36			-31.75
b) Time				
0 hour	36	-42.94		
1 hour	36	-42.91		
6 hours	36	-40.66		

* Tukey's HSD multiple comparisons test

reduction in removal at 1 hour is related more to bacterial cell attachment than cell death (Figure 4.4). A truly antimicrobial fabric would show almost -100% bacterial attachment for all bacterial strains at each time point, indicating complete reduction in bacterial growth and total cell death.

4.3.2.5 Washed antimicrobial

In general, washing the antimicrobial fabric did not affect the bacterial attachment percentages ($F_{1,36}$ = 4.03, NS; Table 4.6). When comparing individual bacterial strains and fabric types, the washed antimicrobial (WAW) typically had larger bacterial attachment percentages in most cases, indicating a higher level of bacterial attachment than the non-washed antimicrobial (WAN) (Figure 4.5). This was most apparent with *S. epidermidis*, whereas *P. acnes* showed greater attachment on the non-washed antimicrobial (WAN) than the washed (WAW), particularly after 6 hours, however these values were more variable (Table 4.5).

Chapter 5

Discussion

5.1 Experimental methods

When an antimicrobial fabric is considered for use, it is prudent to investigate its antimicrobial efficacy. Many methods and techniques can be employed to test the effectiveness of an antimicrobial finish, the selection of which is dependent on the suitability for the antimicrobial type, and the purpose of the test i.e. what information is required (qualitative or quantitative). The antimicrobial fabric used in this work was a 100% polyester woven fabric constructed from silver treated yarn. Firstly, the information that was required from the testing was considered. The antimicrobial efficacy of the fabric needed to be confirmed under a yes/no criterion as opposed to being quantified. For this reason, qualitative tests were used. Secondly, the type of antimicrobial applied to the yarn needed to be determined. As the form of silver used was unknown prior to testing, whether the antimicrobial fabric was diffusible or a "bound-type" could not determined (Simoncic and Tomsic, 2010). The category of antimicrobial affects method selection. The EUCAST disk diffusion method is typically only used for diffusible antimicrobials; however the qualitative information obtained was suitable for the purpose of this work. The method was altered to accommodate the objectives of this study. Strain-specific agar was used to ensure optimal growth of the bacteria. Specimens were also plated in a manner that allowed them to be lifted to examine the presence of any bacterial growth underneath the fabric. Any evidence of bacteria growing under the specimens, in direct contact with the agar and the fabric, allows qualitative analysis of a bound-type antimicrobial.

Past investigators have often used bacterial strains typically considered pathogenic or non-residential to the human skin. For purposes of dressings and wound bandages, it is appropriate to test the antimicrobial efficacy on pathogenic organisms, as the application of the textile is designed to prevent infection. However, standard test methods for testing antimicrobial efficacy and related research associated, recommend the use of gram positive and gram negative bacterial strains that are pathogenic to the skin, regardless of end use application. To test for a bactericidal effect of an antimicrobial fabric designed for clothing, it is more beneficial to examine the effect on residential human skin flora to understand how the fabric behaves in normal wear conditions. In order to give the best representation of the diversity of human skin flora, decisions were made to use both and gram positive (*Staphylococcus epidermidis*) and gram negative (*Pseudomonas aeruginosa*), and aerobic (the previous strains) and anaerobic (*Propionibacterium acnes*) strains. All sub-types of strains have differing cell membrane and molecular functions; therefore testing the efficacy on these strains provides a full functioning profile of the antimicrobial.

Qualitative analysis of the fabric specimens in this work, showed bacterial growth up to the edges of the antimicrobial fabric in the same manner as the non-treated controls, therefore giving evidence the fabric does not contain a diffusible agent. After lifting the specimens, bacterial growth

was evident under the fabric, in direct contact with the antimicrobial. An issue with using concentrated bacterial culture with silver is that the bacterial populations are present in a much greater quantity than what would be found typically against the skin. In order to rule out a concentration-dependent issue, ensuring the bacteria concentration was not exceeding a critical level of inhibition from the silver, the effect on dilute concentrations was examined. The lowest dilution (10^{-8}) had too few colonies to allow any conclusions to be drawn, however the higher concentration (10^{-6}) exhibited bacterial growth around and under the specimens just as the pure culture did. From this, conclusions were that this fabric did not display antimicrobial behaviour.

The antimicrobial fabric in this investigation is marketed for use in apparel. The fabric was therefore tested in an environment that emulated wear conditions in order to examine whether the bactericidal effect is environment dependent. Humidity and temperature can affect the interactions of bacteria on textile surfaces, therefore it is important to test these interactions in typical wear conditions (Bajpai, et al., 2011). A simulated wear condition was created with a novel humidity chamber that allowed fabric specimens to be inoculated with bacteria and suspended in a controlled environment. In past research, in vitro testing of human skin bacterial strains on fabric has not yet been completed under conditions similar to that found in regular clothing use. The novel humidity chamber used in this work, tests the fabric in an environment closer to that which would be found in next-to-skin applications. Antimicrobial fabrics/garments used in wear, such as in sporting applications, and also in storage, for example in sportswear bags following use, have high humidity environments comparable with the humidity chamber used here. The fabric is inoculated with different strains of bacteria residential to human skin and suspended for lengths of time which approximate typical duration of wear, such as a sporting application for one hour or a working day of six hours. The fabrics were examined for bacterial growth as well as quantification of attachment of the bacteria on the fabric.

A known amount of cells was inoculated on the fabric in order to quantify the attachment interactions. The bacteria were grown in tryptic soy broth, centrifuged and resuspended in 0.1% peptone to allow for a pure culture. Centrifugation is known to affect the bacterial cell membrane and, in turn, adherence of the bacteria to surfaces, therefore this remained consistent for all inoculums in order to control for this effect (Bajpai, et al., 2011; Peterson, et al., 2012). Further dilutions were also created with 0.1% peptone, due to its neutrality. A truly simulated wear condition would see the bacteria inoculums made with a physiological saline. Physiological saline contains NaCl which, when in the presence of silver cations, can bind to form AgCl, de-activating the bactericidal effect of silver (McQueen, et al., 2013). Although in normal wear there is likely to be aqueous NaCl in sweat, for this study, the focus was on the attachment effect on antimicrobials as opposed to simulating a direct representation of normal wear.

In order to facilitate the removal of bacteria from the fabric and to give an indication of the number of cells adhered to the surface; a surfactant was added to the removal peptone. The surfactant

Tween 20® (polysorbate 20), was agitated with the inoculated fabrics to remove any non-adhered bacteria, which when compared with the number of cells originally inoculated on the fabrics, gives a good estimate of those bacteria adhered. As this removal method remained consistent throughout testing, when the differences between fabrics and bacterial strains are compared, they were considered as differences in attachment.

All bacterial testing was completed with sterilised equipment and under the protection of a PC2 hood. In combination with autoclaving of the fabric specimens, this ensured all bacterial growth examined was due to that which was inoculated. Autoclaving the fabric specimens did result in some structural effects related to the thermoplasticity of the fibres, shown by creasing in the test fabrics. Thermoplastic alterations to the fabrics could result in changes to the bacterial adherence; however as this sterilisation method was used for all fabric specimens, and all fabrics were polyester (thermoplastic), this change can be controlled for.

5.2 Bacterial activity

Discrepancies in bacterial attachment at time 0 hours, which represents the efficacy of the removal method, show the method is not without error. The bacterial colony units removed at time 0 hours should be the same as that inoculated on the fabric, as no attachment or cell death could have occurred. Removal efficacy differed depending on bacterial strain, likely due to an interaction of the bacteria with the surfactant. The gram positive bacterial strains (*S. epidermidis and P. acnes*) were more efficiently removed than the gram negative strain (*Ps. aeruginosa*). A different removal method may give greater uniformity among bacterial strains, however for this work it was considered a form of error that was taken into account when comparing attachments. Also, due to the growth nature of bacterial colonies, most differences examined in this study were in the order of one log or less, which is minimal in terms of bacterial growth and reduction.

After time in the humidity chamber, differing attachment behaviours were evident among the bacterial strains. *Propionibacterium acnes* is an anaerobic strain, leading it to be slower to grow than the aerobic strains (*S. epidermidis* and *Ps. aeruginosa*; (American Type Culture Collection, 2014)). This slow rate of growth may also translate in a slow rate of attachment. After an hour in the humidity chamber, *P. acnes* displayed colony growth, coupled with minimal bacterial attachment, as opposed to an increase in attachment after an hour with *S. epidermidis* and *Ps. aeruginosa*. After 6 hours in the chamber, bacterial attachment percentages of *P. acnes* decreased, indicating either equivalent bacterial cell death across all fabrics, or an increase in attachment. Either scenario is a possibility, with *P. acnes* being an anaerobic bacteria, growth is heavily environment dependent and too long in the lowered temperature environment (as opposed to the ideal growth temperature of 37°C) may have resulted in perishing of some bacterial cells. However, due to the attachment behaviour evident in the other bacterial strains, it is unreasonable to assume *P. acnes* does not attach, particularly due to its gram positive similarity with *S. epidermidis*.

Staphylococcus epidermidis and *Pseudomonas aeruginosa* displayed very similar bacterial attachment behaviour. The attachment values of both bacterial strains show that differing cell membrane structures of the gram positive and gram negative strains did not affect the adherence to the textile surface of the bacteria. After one hour in the humidity chamber, both bacterial strains showed increased attachment demonstrated by more negative attachment percentages. *Ps. aeruginosa* also demonstrated marked increase in growth after 1 hour, giving a diminished increase in attachment when compared to a large increase in attachment of *S. epidermidis*. After 6 hours, both strains showed a decrease in the bacterial attachment percentages. Bacteria cells dissociating from the surface of the fabric once attached is very unlikely. Once a biofilm is created it is protected from penetration due to extracellular polymeric substance formation, therefore will remain on the fabric until the addition of an antibiotic agent (Wirth, et al., 2016). The increase in bacteria cell attachment percentages seen after 6 hours in *S. epidermidis* and *Ps. aeruginosa* is therefore unlikely due to dissociation from the textile surface. There is the possibility that the biofilm reaches a maximal limit, following which, no more bacteria can attach to the surface. Coupled with growth of the colonies, this may result in an increase in the bacterial attachment percentage, with the surplus of bacteria that is unable to attach being removed.

Fabric structure did not affect bacterial attachment to the textiles. Knit fabrics tend to exhibit a thicker three dimensional structure than woven's fabrics, evident in the fabrics used in this study. Hypotheses were made that the greater thickness may contribute to a greater internal surface for bacteria to adhere to. No differences in bacterial attachment were identified between the knit and the woven regardless of bacterial strain or length of time in the humidity chamber. This indicates that during wear, neither fabric structure (knit or woven) is more likely to harbour bacteria.

Among the more interesting findings of this study was the bacterial growth behaviour on the antimicrobial fabrics. Following the antimicrobial efficacy testing showing no bacterial inhibition on an agar plate, which contained nutrient media, it was prudent to examine the antimicrobial effect in simulated wear with the removal of this nutrient source. The bacteria were inoculated on the fabric in an aqueous form, which should provide the environment necessary for the silver to become cationic, and therefore bactericidal (Leaper, 2006). There was zero inhibitory effect evident, regardless of the length of time the bacteria were left in contact with the fabric. Not only was there no evidence of a bactericidal effect, but in some cases, such as with *Ps. aeruginosa*, bacterial growth was greater on the antimicrobial than on the non-treated control fabrics. An antimicrobially active fabric would show a 100% reduction in bacterial attachment. In addition to the increase in growth, bacterial attachment was also dependent on bacterial strain, and in some cases was increased on the antimicrobial. This may be an indication of the treatment facilitating bacterial growth due to a potential change in topography of the fibres altering surface area. If the antimicrobial treatment has resulted in a change in topography, this would be validated with scanning electron microscopic inspection of the fibres.

Past research has shown that, despite requirements that a reusable antimicrobial treatment must be durable, washing an antimicrobial fabric can affect the bactericidal effect, indicated by the removal of the agent following laundering (Gao and Cranston, 2008; Lorenz, et al., 2012). Unsurprisingly, washing the antimicrobial textile in this study had no effect on the bactericidal behaviour as no antimicrobial effect was evident in the non-washed fabric. Slightly higher bacterial adherence was noted in the washed fabric. An increase in adherence may be due to the statistically significant change in sett of the fabric. A tighter sett results in more yarns per square centimetre, potentially increasing surface area for bacterial attachment.

5.3 Marketing claims

Although the silver treatment on the profiled fabric did not exhibit antimicrobial behaviour, the manufacturer claims the yarn was treated with silver. The fabric was purchased as sold commercially for the purpose of clothing applications. Manufacturers of the fabric, market the product as a "well-being fiber" and having properties of "long-lasting cleanliness" and the ability to "remove sweat odour". The supplier indicated that the fabric was silver treated by the yarn and was commissioned to be antimicrobially tested at the yarn stage. Results from the manufacturer indicated the fabric was tested for efficacy in accordance with AATCC Standard Test Method 100: Antibacterial Finishes on Textile Materials: Assessment of, and was shown to be 100% antimicrobial.

Following yarn manufacture and silver treatment, there are many manufacturing processes undertaken in order to result in a finished textile. The yarns would be woven or knitted into a fabric, and that fabric could undergo sizing, bleaching, dyeing, finishing treatments and potentially commercial laundering, before being sold to the supplier (Vigo, 2013). All finishing processes have the potential to alter the textile in a way that may change the antimicrobial efficacy of the fabric and bacterial attachment (Bajpai, et al., 2011). The fabric used in this investigation has been dyed and possibly printed. Prior to dyeing, the fabric may have undergone bleaching or brightening which can use a chloride based substance to improve the dyeing ability of the fabric (Vigo, 2013). Chlorine reacts with silver to create AgCl, which deactivates the antimicrobial ability of silver cation, and may affect antimicrobial efficacy of this fabric (McQueen, et al., 2013). Following this, the dyeing and printing of the fabric exposes the silver to heat, water and chemical dye molecules. The method of dyeing is unknown, but regardless of the process used, the fabric would have been scoured to remove oils, dirt or sizing from the surface that may have been added in the weaving process. The removal of surface additives from the fabric, could remove any silver treatment that is weakly bonded. In order to fix the dye to the fabric, the dye bath is rapidly heated to a temperature often above the glass transition of polyester (Vigo, 2013). The heating of polyester above its glass transition may cause structural changes to the fibre that alter the binding of silver to its surface; altering the active state of the antimicrobial. The fabric is then washed and dried before undergoing any further treatments (Vigo, 2013). Depending on the dye molecule used, if it is anionic, it could

bind to the silver rendering it inactive and its bactericidal effects unavailable. The immersion in a dye bath and the subsequent washing of the fabric may also remove the silver treatment from the yarn into solution, diminishing the antimicrobial effect. There is a possibility the differences in the yarns antimicrobial efficacy results from the manufacturer and the testing completed on the manufactured fabric in this study may have resulted due to the finishing processes on the fabric.

Fabrics marketed as exhibiting antimicrobial properties, when in actuality they have poor efficacy when tested, is not unusual. The results in this study are consistent with past research that has endeavoured to validate the antimicrobial efficacy of commercial silver fabrics, or test their effectiveness in wear (Kulthong, et al., 2010; Lorenz, et al., 2012; McQueen, et al., 2010; McQueen, et al., 2013). Companies often use any form of evidence to make claims about a product, even if it is just the use of common consumer knowledge about that product. For example, marketers will use the common knowledge that silver has antimicrobial properties, without proof of that silver being active in the finished product (Lorenz, et al., 2012). The danger here is that regardless of the silver being in an active cationic state, its presence in the fabric may result in the washing of that silver into the waterways. This highlights the importance of transparency in trade chains to ensure consumers are aware of the actual properties of the product they are using.

Quantifying the concentration of silver present on the fabric used in this study, would be useful to give an indication of its potential cytotoxic and environmental impact. This can be achieved by elemental analysis through inductively coupled plasma mass spectrometry (Rovira, et al., 2016). Although the silver does not exist on the fabric in an antimicrobially active state, it was definitely added to the yarn during manufacturing, as indicated by the manufacturers antimicrobial efficacy testing. With the silver present, it has the potential to be washed into waterways during laundering and accumulate in the environment. Accumulation of silver in the environment contributes to toxicity in waterborne organisms and a decrease in biological degradation rates in waste systems (Limpiteeprakan and Babel, 2016; Reed, et al., 2016). Also, consistent exposure to antimicrobial agents ultimately can lead to antimicrobial resistance, which is an eminent threat to society (Hoffman, et al., 2015; Tenover, 2006). The inclusion of antimicrobials to textiles, without sufficient evidence of bactericidal behaviour, has the potential for greater detriment than perceived benefit.

Chapter 6

Summary, conclusions and recommendations

6.1 Summary

The aim of this study was to investigate the activity of resident human skin bacterial strains on an antimicrobial silver fabric. The objectives were to determine whether bacterial interactions on fabrics were influenced by: an antimicrobial finishing, washing an antimicrobial, length of exposure to the bacterial strains and fabric structure. A commercially available, silver treated, antimicrobial fabric was supplied and compared to a structurally similar 100% polyester woven fabric and a 100% polyester knit. The antimicrobial fabric was laundered and the non-washed and washed antimicrobial fabrics were tested for antimicrobial efficacy. A novel humidity chamber was designed which simulated the body's ambient humidity and temperature in a controlled environment. Fabric specimens were inoculated with human skin bacterial strains and suspended in the humidity chamber for varying lengths of time that coincide with normal wear practices. Bacterial cells were removed from the fabrics and grown over 24 hours in order to quantify bacterial growth on the fabrics. Growth was represented as a percentage of the colonies originally inoculated onto the fabric in an attempt to quantify bacterial adherence.

The antimicrobial silver fabric for commercial use was shown not to exhibit any detectable bactericidal effect. The fabric, which is sold as an antimicrobial textile under the intention of maintaining "cleanliness" and "removing sweat odour," had no detectable antimicrobial effect on natural skin bacteria (which causes this odour), regardless of the bacterial strain or concentration of bacterial populations. Testing the antimicrobial effect in a novel simulated wear condition, showed this fabric would not behave in application use, as marketing indicated. Due to the absence of antimicrobial effect, the interactions of the natural skin bacterial strains on the fabrics did not reduce with the addition of the "antimicrobial" treatment. In some cases bacterial activity was increased with the addition of the antimicrobial treatment indicating a potential alteration in fibre topography. Although washing the antimicrobial fabric resulted in a tighter sett, there was no change in the bacterial interactions with the fabrics. As there was no antimicrobial effect present initially, washing the fabric was not expected to alter this. For *Staphylococcus epidermidis*, the tighter sett did result in a greater bacterial interactions, however this difference was minimal. Anaerobic bacteria did not display fabric interactions until after 6 hours as opposed to one hour for the aerobic bacterial strains. Gram positive and gram negative cell membrane structure differences did not influence bacterial activity on the fabrics. In general the longer the fabric was exposed to the simulated wear environment, the more bacteria grew and the greater the fabric interactions. There is a possibility that a maximum level of adherence was reached for the aerobic bacterial strains. Overall, regardless of the differing fabric properties between the woven and knit fabrics, fabric structure had no affect on bacterial growth or fabric interactions. It must be noted that most differences recorded in this study were in the order of one log or less which is minimal in terms of bacterial growth.

6.2 Conclusions

A novel humidity chamber, designed to simulate wear conditions, was used to determine the bacterial interactions of known natural residential skin bacteria strains on a commercially available antimicrobial silver fabric. The bacterial interaction on the fabrics was analysed in terms of antimicrobial finish, fabric structure, length of time exposed to the controlled environment, and laundering of the fabric. The antimicrobial fabric was found to not exhibit any bactericidal effect. Bacterial interactions were influenced by the bacterial strain and the length of time the bacteria was in contact with the fabrics in the controlled environment. It is common for an antimicrobial fabric to be marketed as such, with limited proof of bactericidal effect. Whether companies do so out of scientific ignorance or whether findings are neglected or altered to appeal to consumers is uncertain, however transparency in supply chains, and standardised international requirements of antimicrobial textiles would limit these false claims. Due to the international concern of widespread antimicrobial resistance, manufacturers and consumers need to use antimicrobials responsibly by considering perceived benefits versus detriments.

6.3 Recommendations

The following recommendations have been suggested for further research to examine bacterial adherence on antimicrobial fabrics and the effect of antimicrobial fabrics on natural skin bacterial strains.

- Quantification of the silver concentration in the fabric examined in this study using inductively coupled plasma mass spectrometry, would give an indication of environmental impact.
- Examination of the bacterial adherence on a proven antimicrobial fabric using the novel humidity chamber designed in this study, enabling true evaluation of bacterial adherence of natural skin bacterial strains in simulated wear.
- Examination of bacterial adherence using the experimental design used in this study with trialling of a different removal method that exhibits greater consistency.
- Visual examination of the bacterial adherence on antimicrobial fabrics with the use of appropriate microscopy techniques to evaluate the way in which the bacteria adhere, if at all.
- Live/dead assays of bacteria on antimicrobial fabrics to examine whether they remain attached after perishing.
- Examination of the effects of antimicrobial agents against natural skin bacterial strains during normal wear in vivo.
- Examination of how finishing processes, including dyeing, affect antimicrobial efficacy.

References

Abrigo, M., Kingshott, P. and McArthur, S.L. 2015. Electrospun Polystyrene Fiber Diameter Influencing Bacterial Attachment, Proliferation, and Growth. *ACS Applied Materials and Interfaces* 7 (14): 7644-7652.

American Type Culture Collection 2010. *Useful Terms for ATCC Microbiology*. Virginia, USA: American Type Culture Collection

American Type Culture Collection 2014. *Propionibacterium acnes (ATCC 6919) Product Sheet*. Virginia, USA: American Type Culture Collection.

Bajpai, V., Bajpai, S., Jha, M., Dey, A. and Ghosh, S. 2011. Microbial adherence on textile materials: A review. *Journal of Environmental Research And Development* 5 (3): 666-672.

Baviera, G., Leoni, M.C., Capra, L., Cipriani, F., Longo, G., Maiello, N., Ricci, G. and Galli, E. 2014. Microbiota in Healthy Skin and in Atopic Eczema. *BioMed Research International* 2014: 1-6.

Borkow, G. and Gabbay, J. 2008. Biocidal textiles can help fight nosocomial infections. *Medical Hypotheses* 70 (5): 990-994.

Boutin, M.A., Thom, K.A., Zhan, M. and Johnson, J.K. 2014. A randomized crossover trial to decrease bacterial contamination on hospital scrubs. *Infection Control and Hospital Epidemiology* 35 (11): 1411-1413.

British Standards Institution 1988. *BS 5441: British Standard Methods of test for Knitted fabrics*. London, United Kingdom: British Standards Institution.

Burden, M., Keniston, A., Frank, M.G., Brown, C.A., Zoucha, J., Cervantes, L., Weed, D., Boyle, K., Price, C. and Albert, R.K. 2013. Bacterial contamination of healthcare workers' uniforms: A randomized controlled trial of antimicrobial scrubs. *Journal of Hospital Medicine* 8 (7): 380-385.

Campbell, N.A. and Reece, J.B. 2002. *Biology*. San Francisco, California: Benjamin Cummings.

Cua, A.B., Wilhelm, K.P. and Maibach, H.I. 1990. Elastic properties of human skin: relation to age, sex, and anatomical region. *Archives of Dermatological Research* 282 (5): 283-288.

Dann, A.B. and Hontela, A. 2011. Triclosan: environmental exposure, toxicity and mechanisms of action. *Journal of Applied Toxicology* 31 (4): 285-311.

Dastjerdi, R. and Montazer, M. 2010. A review on the application of inorganic nano-structured materials in the modification of textiles: Focus on anti-microbial properties. *Colloids and Surfaces B: Biointerfaces* 79 (1): 5-18.

Denton, M.J. and Daniels, P.N. 2002. *Textile Terms and Definitions*. Manchester, United Kingdom: The Textile Institute.

Elsner, P., Hatch, K.L. and Wigger-Alberti, W. 2003. *Textiles and the Skin*. Basel, Switzerland: Karger.

European Committee for Standardization 1998. *BS EN 12127: Textiles - Fabrics - Determination of mass per unit area using small samples*. Brussels, Belgium: European Committee for Standardization.

European Committee for Standardization 1999. *BS EN 12751: Textiles - Sampling of fibres, yarns and fabrics for testing*. Brussels, Belgium: European Committee for Standardization.

Fang, J.-L., Stingley, R.L., Beland, F.A., Harrouk, W., Lumpkins, D.L. and Howard, P. 2010. Occurrence, Efficacy, Metabolism, and Toxicity of Triclosan. *Journal of Environmental Science and Health, Part C* 28 (3): 147-171.

Filipowska, B., Rybicki, E., Walawska, A. and Matyjas-Zgondek, E. 2011. New method for the antibacterial and antifungal modification of silver finished textiles. *Fibres & Textiles in Eastern Europe* 4 (87): 124-128.

Gao, Y. and Cranston, R. 2008. Recent Advances in Antimicrobial Treatments of Textiles. *Textile Research Journal* 78 (1): 60-72.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., Program, N.C.S., Bouffard, G.G., Blakesley, R.W., Murray, P.R., Green, E.D., Turner, M.L. and Segre, J.A. 2009. Topographical and Temporal Diversity of the Human Skin Microbiome. *Science* 324 (5931): 1190-1192.

Grice, E.A. and Segre, J.A. 2011. The Skin Microbiome. *Nature Reviews Microbiology* 9 (4): 244-253.
Guerrero-Beltran, J.A. and Barbosa-Canovas, G.V. 2004. Advantages and Limitations on Processing Foods by UV Light. *Food Science and Technology International* 10 (3): 137-147.
Gupta, D., Khare, S.K. and Laha, A. 2004. Antimicrobial properties of natural dyes against Gram-negative bacteria. *Coloration Technology* 120 (4): 167-171.
Han, S. and Yang, Y. 2005. Antimicrobial activity of wool fabric treated with curcumin. *Dyes and Pigments* 64 (2): 157-161.
Hannigan, G.D. and Grice, E.A. 2013. Microbial Ecology of the Skin in the Era of Metagenomics and Molecular Microbiology. *Cold Spring Harbor Perspectives in Medicine* 3 (12): 1-15.
Hatch, K.L., Markee, N.L. and Maibach, H.I. 1992. Skin Response To Fabric. A Review of Studies and Assessment Methods. *Clothing and Textiles Research Journal* 10 (4): 54-63.
Hatch, K.L., Markee, N.L., Maibach, H.I., Barker, R.L., Woo, S.S. and Radhakrishnaiah, P. 1990. In Vivo Cutaneous and Perceived Comfort Response to Fabric: Part III: Water Content and Blood Flow in Human Skin Under Garments Worn by Exercising Subjects in a Hot, Humid Environment. *Textile Research Journal* 60 (9): 510-519.
Hearle, J.W. and Morton, W.E. 2008. *Physical properties of textile fibres*. Cambridge, England: Woodhead Publishing Ltd.
Heine, E., Knops, H.G., Schaefer, K., Vangeyte, P. and Moeller, M. 2007. Antimicrobial Functionalisation of Textile Materials. In *Multifunctional Barriers for Flexible Structure*, edited by Duquesne, S., Magniez, C. and Camino, G., 23-38. Berlin, Germany: Springer Heidelberg.
Heritage, J., Evans, E.G.V. and Killington, R.A. 1996. *Introductory Microbiology*. Cambridge: Cambridge University Press.
Hoefer, D. and Hammer, T.R. 2011. Antimicrobial active clothes display no adverse effects on the ecological balance of the healthy human skin microflora. *ISRN dermatology* 2011: 1-8.
Hoffman, S.J., Outterson, K., Røttingen, J.-A., Cars, O., Clift, C., Rizvi, Z., Rotberg, F., Tomson, G. and Zorzet, A. 2015. An international legal framework to address antimicrobial resistance. *Bulletin of the World Health Organization* 93 (2015): 66-66.
Hollinger, M.A. 1996. Toxicological Aspects of Topical Silver Pharmaceuticals. *Critical Reviews in Toxicology* 26 (3): 255-260.
Hsieh, Y.-L., Timm, D.A. and Merry, J. 1987. Bacterial adherence on fabrics by a radioisotope labeling method. *Textile Research Journal* 57 (1): 20-28.
Hsieh, Y.L. and Merry, J. 1986. The adherence of Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli on cotton, polyester and their blends. *Journal of Applied Bacteriology* 60 (6): 535-544.
International Organization for Standardization 1984. ISO 7211-2: Textiles - Woven Fabrics - Construction - Methods of analysis - Part 2: Determination of number of threads per unit length. In Geneva: International Organization for Standardization
International Organization for Standardization 1991. *ISO4915: Textiles - Stitch Types - Classification and terminology*. Geneva: Interntional Organization for Standardization.
International Organization for Standardization 1996 *ISO 5084: Textiles - Determination of thickness of textiles and textile products*. Geneva: International Organization for Standardization
International Organization for Standardization 2005. *ISO 139: Textiles - Standard Atmospheres for Conditioning and Testing*. Geneva: International Organization for Standardization.
International Organization for Standardization 2010. *Technical specification ISO/TS 80004–1:2010(E). Nanotechnologies - Vocabulary*. Geneva: International Organization for Standardization.
International Organization for Standardization 2012. *ISO 6330: Textiles - Domestic washing and drying procedures for textile testing*. Geneva: International Organization for Standardization
International Organization for Standardization 2013. *ISO 20743: Textiles - Determination of antibacterial activity of textile products*. Geneva: International Organization for Standardization.

Iwasaki, S., Yoshimura, A., Ideura, T., Koshikawa, S. and Sudo, M. 1997. Elimination study of silver in a hemodialyzed burn patient treated with silver sulfadiazine cream. *American Journal of Kidney Diseases* 30 (2): 287-290.

Jakobsson, H.E., Jernberg, C., Andersson, A.F., Sjolund-Karlsson, M., Jansson, J.K. and Engstrand, L. 2010. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PloS One* 5 (3): 1-12.

Jernberg, C., Löfmark, S., Edlund, C. and Jansson, J.K. 2007. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *The ISME Journal* 1 (1): 56-66.

Kasiri, M. and Safapour, S. 2013. Natural Dyes and Antimicrobials for Textiles. In *Green Materials for Energy, Products and Depollution*, edited by Lichtfouse, E., Schwarzbauer, J. and Robert, D., 229-286. Germany: Springer Heidelberg.

Kean, T. and Thanou, M. 2010. Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews* 62 (1): 3-11.

Kenawy, E.-R., Worley, S.D. and Broughton, R. 2007. The Chemistry and Applications of Antimicrobial Polymers: A State-of-the-Art Review. *Biomacromolecules* 8 (5): 1359-1384.

Kong, M., Chen, X.G., Xing, K. and Park, H.J. 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology* 144 (1): 51-63.

Kownatzki, E. 2003. Hand hygiene and skin health. *Journal of Hospital Infection* 55 (4): 239-245.

Kulthong, K., Srisung, S., Boonpavanitchakul, K., Kangwansupamonkon, W. and Maniratanachote, R. 2010. Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. *Particle and Fibre Toxicology* 7 (1): 1-9.

Labows, J.N., McGinley, K.J. and Kligman, A.M. 1982. Perspectives on axillary odor. *Journal of the Society of Cosmetic Chemists* 33 (4): 193-202.

Lambers, H., Piessens, S., Bloem, A., Pronk, H. and Finkel, P. 2006. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science* 28 (5): 359-370.

Lansdown, A.B.G. 2006. Silver in health care: antimicrobial effects and safety in use. *Current problems in dermatology* 33: 17-34.

Lazary, A., Weinberg, I., Vatine, J.J., Jefidoff, A., Bardenstein, R., Borkow, G. and Ohana, N. 2014. Reduction of healthcare-associated infections in a long-term care brain injury ward by replacing regular linens with biocidal copper oxide impregnated linens. *International Journal of Infectious Diseases* 24: 23-29.

Leaper, D., Assadian, O., Hubner, N.O., McBain, A., Barbolt, T., Rothenburger, S. and Wilson, P. 2011. Antimicrobial sutures and prevention of surgical site infection: assessment of the safety of the antiseptic triclosan. *International Wound Journal* 8 (6): 556-566.

Leaper, D.J. 2006. Silver dressings: their role in wound management. *International Wound Journal* 3 (4): 282-294.

Leyden, J.J., McGinley, K.J., Holzle, E., Labows, J.N. and Kligman, A.M. 1981. The Microbiology of the Human Axilla and Its Relationship to Axillary Odor. *Journal of Investigative Dermatology* 77 (5): 413-416.

Li, Y., Leung, P., Yao, L., Song, Q.W. and Newton, E. 2006. Antimicrobial effect of surgical masks coated with nanoparticles. *Journal of Hospital Infection* 62 (1): 58-63.

Limpiteeprakan, P. and Babel, S. 2016. Leaching potential of silver from nanosilver-treated textile products. *Environmental Monitoring and Assessment* 188 (3): 1-12.

Liu, H., Lv, M., Deng, B., Li, J., Yu, M., Huang, Q. and Fan, C. 2014. Laundering durable antibacterial cotton fabrics grafted with pomegranate-shaped polymer wrapped in silver nanoparticle aggregations. *Scientific Reports* 4 (5920): 1-9.

Lorenz, C., Windler, L., von Goetz, N., Lehmann, R.P., Schuppler, M., Hungerbühler, K., Heuberger, M. and Nowack, B. 2012. Characterization of silver release from commercially available functional (nano)textiles. *Chemosphere* 89 (7): 817-824.

Matuschek, E., Brown, D. and Kahlmeter, G. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection* 20 (4): O255-O266.

McArthur, J.V., Tuckfield, R.C. and Baker-Austin, C. 2012. Antimicrobial Textiles. In *Antibiotic Resistance*, edited by Coates, A. R. M., 135-152. Berlin, Germany: Springer Heidelberg.

McQueen, R.H., Keelan, M. and Kannayiram, S. 2010. Determination of Antimicrobial Efficacy for Textile Products Against Odor-Causing Bacteria. *AATCC Review* 10 (4): 58-63.

McQueen, R.H., Keelan, M., Xu, Y. and Mah, T. 2013. In vivo assessment of odour retention in an antimicrobial silver chloride-treated polyester textile. *The Journal of The Textile Institute* 104 (1): 108-117.

McQueen, R.H., Laing, R.M., Brooks, H.J.L. and Niven, B.E. 2007a. Odor Intensity in Apparel Fabrics and the Link with Bacterial Populations. *Textile Research Journal* 77 (7): 449-456.

McQueen, R.H., Laing, R.M., Wilson, C.A., Niven, B.E. and Delahunty, C.M. 2007b. Odor Retention on Apparel Fabrics: Development of Test Methods for Sensory Detection. *Textile Research Journal* 77 (9): 645-652.

Morris, C.E. and Welch, C.M. 1983. Antimicrobial Finishing of Cotton with Zinc Pyrithione. *Textile Research Journal* 53 (12): 725-728.

Nayak, R. and Padhye, R. 2014. Antimicrobial finishes for textiles. In *Functional Finishes for Textiles: Improving Comfort, Performance and Protection*, edited by Paul, R., 361-386. Amsterdam: Elsevier Science.

Pelczar, M.J., 2014. Microbiology. Encyclopædia Britannica Online. http://www.britannica.com/science/microbiology, Last updated: 12/12/14 [Accessed: 20 April].

Peterson, B.W., Sharma, P.K., van der Mei, H.C. and Busscher, H.J. 2012. Bacterial Cell Surface Damage Due to Centrifugal Compaction. *Applied and Environmental Microbiology* 78 (1): 120-125.

Pinho, E., Magalhães, L., Henriques, M. and Oliveira, R. 2010. Antimicrobial activity assessment of textiles: standard methods comparison. *Annals of Microbiology* 61 (3): 493-498.

Poulter, N., Vasilev, K., Griesser, S.S. and Griesser, H.J. 2013. Silver Containing Biomaterials. In *Biomaterials Associated Infection: Immunological Aspects and Antimicrobial Strategies*, edited by Moriarty, F. T., Zaat, A. J. S. and Busscher, J. H., 355-378. New York, NY: Springer New York.

Qian, L. and Sun, G. 2004. Durable and regenerable antimicrobial textiles: Improving efficacy and durability of biocidal functions. *Journal of Applied Polymer Science* 91 (4): 2588-2593.

Rajendran, S. and Anand, S. 2006. Contribution of textiles to medical and healthcare products and developing innovative medical devices. *Indian Journal of Fibre and Textile Research* 31 (1): 215-229.

Ranganath, A.S. and Sarkar, A.K. 2014. Evaluation of Durability to Laundering of Triclosan and Chitosan on a Textile Substrate. *Journal of Textiles* 2014: 1-5.

Reed, R.B., Zaikova, T., Barber, A., Simonich, M., Lankone, R., Marco, M., Hristovski, K., Herckes, P., Passantino, L., Fairbrother, D.H., Tanguay, R., Ranville, J.F., Hutchison, J.E. and Westerhoff, P.K. 2016. Potential Environmental Impacts and Antimicrobial Efficacy of Silver- and Nanosilver-Containing Textiles. *Environmental Science & Technology* 50 (7): 4018-4026.

Rovira, J., Nadal, M., Schuhmacher, M. and Domingo, J.L. 2016. Trace elements in skin-contact clothes and migration to artificial sweat: Risk assessment of human dermal exposure. *Textile Research Journal* DOI: 10.1177/0040517516639816: 1-13.

Saito, S. 2015. Kaiser Permanente Rejects Antimicrobials for Infection Control. In Oakland, California: Kaiser Permanente.

Schaechter, M. 2009. *Encyclopedia of Microbiology (Third Edition)*. Oxford: Academic Press.

Schierholz, J.M., Beuth, J., Pulverer, G. and König, D.-P. 1999. Silver-Containing Polymers. *Antimicrobial Agents and Chemotherapy* 43 (11): 2819-2821.

Schmid-Wendtner, M.H. and Korting, H.C. 2006. The pH of the Skin Surface and Its Impact on the Barrier Function. *Skin Pharmacology and Physiology* 19 (6): 296-302.
Schweizer, M., Graham, M., Ohl, M., Heilmann, K., Boyken, L. and Diekema, D. 2012. Novel Hospital Curtains with Antimicrobial Properties: A Randomized, Controlled Trial. *Infection Control & Hospital Epidemiology* 33 (11): 1081-1085.
Shallcross, L.J. and Davies, D.S.C. 2014. Antibiotic overuse: a key driver of antimicrobial resistance. *British Journal of General Practice* 64 (629): 604-605.
Simončič, B. and Klemenčič, D. 2016. Preparation and performance of silver as an antimicrobial agent for textiles: A review. *Textile Research Journal* 86 (2): 210-223.
Simoncic, B. and Tomsic, B. 2010. Structures of Novel Antimicrobial Agents for Textiles - A Review. *Textile Research Journal* 80 (6): 1721-1737.
Singh, R., Jain, A., Panwar, S., Gupta, D. and Khare, S.K. 2005. Antimicrobial activity of some natural dyes. *Dyes and Pigments* 66 (2): 99-102.
Soanes, C. and Stevenson, A. 2008. *Concise Oxford English Dictionary*. Oxford, United Kingdom: Oxford University Press.
Stefaniak, A.B., Duling, M.G., Lawrence, R.B., Thomas, T.A., LeBouf, R.F., Wade, E.E. and Virji, M.A. 2014. Dermal exposure potential from textiles that contain silver nanoparticles. *International Journal of Occupational and Environmental Health* 20 (3): 220-234.
Swanson, T. 2014. *Modern Dressings and Technologies*. Wound Management for the Advanced Practitioner. Australia: IP Communications Pty Ltd.
Tannock, G.W. 1995. *Normal Microflora: An Introduction to Microbes Inhabiting the Human Body*. London, United Kingdom: Chapman & Hall.
Tenover, F.C. 2006. Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine* 119 (6): S3-S10.
Timofeeva, L. and Kleshcheva, N. 2011. Antimicrobial polymers: mechanism of action, factors of activity, and applications. *Applied Microbiology and Biotechnology* 89 (3): 475-492.
Tran, P.L., Hamood, A.N., de Souza, A., Schultz, G., Liesenfeld, B., Mehta, D. and Reid, T.W. 2015. A study on the ability of quaternary ammonium groups attached to a polyurethane foam wound dressing to inhibit bacterial attachment and biofilm formation. *Wound Repair and Regeneration* 23 (1): 74-81.
Trop, M., Novak, M., Rodl, S., Hellbom, B., Kroell, W. and Goessler, W. 2006. Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. *Journal of Trauma and Acute Care Surgery* 60 (3): 648-652.
Vigo, T.L. 2013. *Textile Processing and Properties: Preparation, Dyeing, Finishing and Performance*. Amsterdam, The Netherlands: Elsevier Science.
Walter, N., McQueen, R.H. and Keelan, M. 2014. In vivo assessment of antimicrobial-treated textiles on skin microflora. *International Journal of Clothing Science and Technology* 26 (4): 330-342.
Wilson, M. 2009. *Bacteriology of Humans: An Ecological Perspective*. Queensland, Australia: John Wiley & Sons.
Windler, L., Height, M. and Nowack, B. 2013. Comparative evaluation of antimicrobials for textile applications. *Environment International* 53 (2013): 62-73.
Wirth, S.M., Bertuccio, A.J., Cao, F., Lowry, G.V. and Tilton, R.D. 2016. Inhibition of bacterial surface colonization by immobilized silver nanoparticles depends critically on the planktonic bacterial concentration. *Journal of Colloid and Interface Science* 467 (2016): 17-27.
Wongchoosuk, C., Lutz, M. and Kerdcharoen, T. 2009. Detection and Classification of Human Body Odor Using an Electronic Nose. *Sensors* 9 (9): 7234.
Yazdankhah, S.P., Scheie, A.A., Høiby, E.A., Lunestad, B.-T., Heir, E., Fotland, T.Ø., Naterstad, K. and Kruse, H. 2006. Triclosan and Antimicrobial Resistance in Bacteria: An Overview. *Microbial Drug Resistance* 12 (2): 83-90.
Yiqi, Y., Corcoran, L., Vorlicek, K. and Shiqi, L. 2000. Durability of Some Antibacterial Treatments to Repeated Home Launderings. *Textile Chemist & Colorist & American Dyestuff Reporter* 32 (4): 44-49.

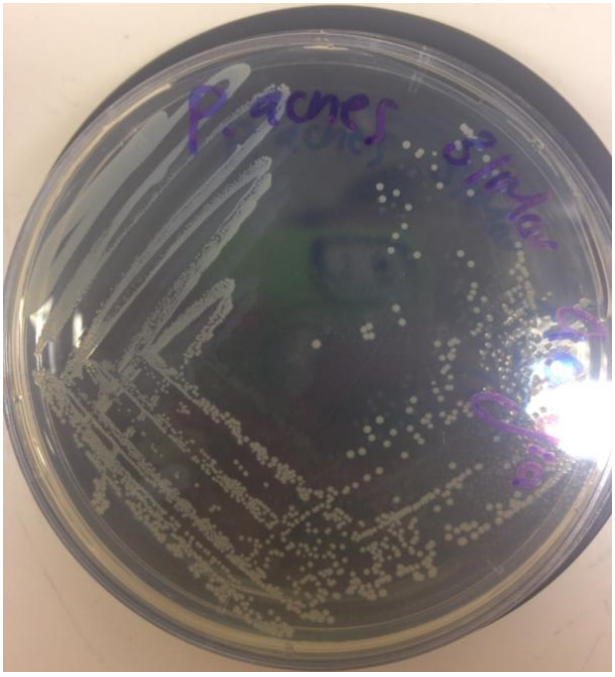
Young Hee Kim and Sun, G. 2000. Dye Molecules as Bridges for Functional Modifications of Nylon: Antimicrobial Functions. *Textile Research Journal* 70 (8): 728-733.

Yu, Q., Cho, J., Shivapooja, P., Ista, L.K. and López, G.P. 2013. Nanopatterned smart polymer surfaces for controlled attachment, killing, and release of bacteria. *ACS Applied Materials and Interfaces* 5 (19): 9295-9304.

Zhu, P. and Sun, G. 2004. Antimicrobial finishing of wool fabrics using quaternary ammonium salts. *Journal of Applied Polymer Science* 93 (3): 1037-1041.

Appendix A

Example of a stock plate for selection of individual colonies



Appendix B

Examples of drop plates for each bacterial strain