Challenge testing microbial loads on treated and untreated soft medical fabrics

George Blackburn[1]

Author for correspondence: Dr Cath Rees Tel: 0115 9516161, Fax: 0115 9516142.
E-mail: cath.rees@nottingham.ac.uk

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Abstract:

Background. It is recognised that traditional linen curtains are fomites for infectious disease in healthcare environments, to which it is likely that these curtains contribute to the astronomical cost of healthcare associated infections (HAIs) every year. Therefore, to address this issue antimicrobial textiles have been developed. These textiles come embedded with a variety of different antimicrobial polymers. The way in which these antimicrobial polymers are incorporated into the textile differs depending upon the type of textile used. The antimicrobial textiles have to pass industry tests, these comparative tests are designed to show significant reductions in microbial loads compared to standard fabrics.

This article aims to review the evidence of the effectiveness of antimicrobial textiles in reducing disease transmission in a clinical setting and how this is achieved.

Methodology. Google Scholar, Pubmed, National Centre for Biotechnology Information (NCBI) and Springer Link were searched for publications concerning the survival and transmission of pathogenic microorganisms on antimicrobial textiles compared to linen or disposable alternatives.

Results. Many studies specify that there is a significant reduction in the survival of microbes on antimicrobial textiles. This reduction in survival was very dependent on the type and combination of antimicrobial polymers being used. It has been concluded that when in combination the reduction of microbial survival was significant for use in a clinical setting, versus insignificant when a single antimicrobial polymer is used. Studies investigating the use of various metal nanoparticles in combination with antimicrobial polymers concluded that depending on their shape and size they will have a dramatic effect on microbial survival and increase the time to first contamination. Emerging studies have illustrated that the industry tests require additional parameters to ensure that the antimicrobial curtains are to be effective in a clinical setting.

Other studies demonstrate unsuccessful reductions of microbes via industrial laundering of linen curtains, resulting in rehanging of contaminated curtains.

Conclusions. There is a significant need to further test the effectiveness of antimicrobial textiles to see whether they successfully reduce microbial carriage. This will be achieved by focusing on the modes of transmission. In addition, healthcare protocols and studies for laundering linen curtains highlights that it is an

outdated method for infection control. bThis is because of inadequate and varying changing times. Investigations have highlighted that implementing hospital curtains can alleviate costs additional associated with changing and laundering curtains whilst also reducing disease transmission.

Introduction

It is not a new concept that soft medical fabrics have huge potential to transmit disease throughout hospitals (Pinon *et al.,* 2013; Fijan *et al*., 2007; Kramer *et al.,* 2006; Trillis *et al*., 2008; Ohl *et al.,* 2012). One of the many soft fabrics used in hospitals that carry pathogenic microbes are privacy curtains. Privacy curtains were first installed into UK hospitals in the 1960s and they were designed to give patients more privacy and to help prevent the spread of disease (Accord Curtains, 2015). However, it is now known that they are a potential fomite for the spread of pathogenic hospital acquired infections (HAIs). Considering 39.5% of HAIs are multi-drug resistance organisms (MDROs), such as methicillin resistant *Staphylococcus aureus (*MRSA), vancomycin resistant *Enterococci* (VRE) and *Clostridium difficile* spores, this poses a major problem for infection management in a clinical setting (Cornejo-Juárez *et al.,* 2017; Ohl *et al.,* 2012*;* Woodland *et al.,* 2010; Fijan *et al.,* 2007; Pinon *et al.,* 2013).

Therefore, the solution is to replace traditional curtains with antimicrobial-embedded curtains. These antimicrobial curtains can be either coated, embedded with, or have woven into the textile, a variety of antimicrobial polymers. This can consist of metals such as silver, copper, and zinc, which can be in combination with substances such as quaternary ammonium compounds (QACs).
For these curtains to be deemed viable in a clinical setting they are required to pass ‘specified industry standards’ through testing. These comparative tests follow defined protocols to show the antimicrobial curtains show a significant reduction in microbial count on the curtains compared to traditional curtains. However, these antimicrobial textiles often fall into a grey area when tested, as it requires multiple different test methods to give conclusive results. These approaches include the 2011 update to the ‘International Standard’ 22196 (ISO) on “the measurement of antibacterial activity on plastics and other non-porous surfaces” (ISO, 2011) which outlines a comparative

method for testing antimicrobial surfaces to ensure their antimicrobial efficacy against an untreated sample of the same material.
Another comparative test used is ISO20743 which is titled the “determination of antibacterial activity of textile products” and is used to show the difference in bacterial levels which survive the test versus the initial concentration applied (ISO, 2013).
Finally, the American Association of Textile Chemists and Colourist (AATCC) TM100 test which is the “Test Method for Antibacterial Finishes on Textile Materials: Assessment of,” which is a quantitative procedure to evaluate the degree of antibacterial activity (AATCC, 2021).

Hence the significance of any given study comes in the form of resolvable issues. Firstly, the ‘industry standard’ tests may show significant reductions in the level of microbes present, however, this is not representative of the environmental conditions that the textiles will encounter in a clinical setting and therefore the textile may not be effective enough in reducing microbial load under these conditions. Another issue is the combination of polymers and the antimicrobials used, as some combinations may lack efficacy in real world settings, and therefore finding the most effective combination is essential.

These issues will be analysed and explained throughout the four chapters of the review. The first chapter consists of an outline of different methodologies used for challenge testing microbial loads on soft medical fabrics. Secondly there is analysis of the current successful polymers which are embedded into the antimicrobial curtains. Thirdly a review of data on the efficacy of using treated medical fabrics to show a reduction of disease transmission, with the addition of a cost-benefit analysis for using antimicrobial curtains. Finally, suggestions are made on a novel approach for challenge testing treated and untreated medical fabrics to produce a replicable methodology for testing the efficacy of current and future antimicrobial soft fabrics.

The aims of the study will all be achieved via meta-analysis of the literature, allowing for reflection and conclusion on aspects such as whether using antimicrobial textiles are beneficial in reducing disease transmission in hospitals and if they are cost effective for hospitals. In addition, the identification of the effectiveness of

antimicrobials to control microbial load, irrespective of the way the microbes get onto the curtain. Conclusions will be drawn regarding the polymer combination which most likely to have the highest efficacy in reducing microbial loads on the antimicrobial textiles. Another aspect will focus on the industry standard tests to show which are most effective in testing the antimicrobial textiles compared to a clinical setting and if these tests require additions to increase their efficacy. Furthermore, to conclude whether challenge testing antimicrobial surfaces against different concentrations of microbes may highlight an increased need for antimicrobial fabrics and therefore instigate a change in hygiene policy for certain hospitals/organisations.

Chapter 1: Critical analysis of methodologies for challenge testing soft medical fabrics

1.1 Overview

The healthcare sector is constantly evolving and due to the emphasis placed on infection control by organisations such as the NHS and NICE, strategies to reduce cross contamination from soft fabrics have been successfully implemented. An example of such a strategy is ‘bare below the elbow’. This approach, including shortening the sleeves of healthcare workers, emphasises rigorous maintenance of hand hygiene and recognises the likelihood of uniform contamination as a cause of infection transmission between healthcare workers and patients (NHS, 2021). This policy is yet to be backed with supporting evidence, however, prior investigations supporting ‘hot spots’ of bacteria around jewellery and the cuff of long sleeves concludes that by removing these there will be a reduction bacterial contamination (Trick *et al.,* 2003; Treakle *et al.,* 2009). Other investigations have concluded that when doctors ‘white coats’ are challenge tested with varying pathogenic microbes, they are a reservoir for disease transmission between healthcare workers and patients (Hamid *et al.,* 2016). This difference between specifically white coats and other medical clothing is due to the current lack of laundering the white coats, however textiles such as scrubs are more routinely laundered (NHS, 2021).

There is a key feature of many clinical settings that have been neglected with respect to infection control, despite the wealth of evidence to support the risk of contamination: privacy curtains (Woodland *et al.,* 2010; Ohl *et al.,* 2012; Owen *and* Laird., 2020; Carraro *et al.,* 2020). This contamination includes identification of curtains as a source of MRDOs (Cornejo-Juárez *et al*., 2017). This issue requires further scrutiny, as HAIs, represent a significant expenditure for organisations such as the NHS, which spent an estimated £2.1 billion in 2016/2017 due to their deleterious effect on patient outcomes (Guest *et al.,* 2020). This cost could be alleviated or decreased by implementing antimicrobial textiles into the clinical setting, illustrated in Tables 1 and 2. However it remains to be determined if antimicrobial curtains are a viable solution to reduce disease transmission.

Table 1: Table illustrating the difference in cost of two antimicrobial curtains versus a standard curtain

		Antimicrobial Curtain A	Antimicrobial Curtain B	Standard Curtain[b]
Direct costs of 10 curtains (6 short & 4 long)		3,380	4,810	354
Indirect costs				
Routine frequency of curtain change in 6 months[a]		Once	Once	Every 2 weeks or 13 times
Curtain change post-discharges[a]		No	No	Yes
Time to replace Curtains, min	Routine (10 curtains)[a]	6.9 × 10 = 69	2.4 × 10 = 24	4.95 × 13 × 10 = 643.5
	After 200 discharges (2 curtains)	0	0	4.95 × 200 × 2 = 1,980
Staff cost (average, $1.05/min)		72.45	25.20	2,754.68
Lost revenue while curtains were replaced as routine & postdischarge[c] [(average 24-h bed charge = $5,210, ie, $3.62/min) × time to replace (min)]		3.62 × 69 = 249.65	3.62 × 24 = $86.83	3.62 × (643.5 + 1,980) = 9,491.97
Laundering cost (average, $7.24 per piece)		0	0	7.24 × (10 × 13 + 200 × 2) = 3,837.20
Total		3,702.10	4,922.03	16,437.85

Note. Data are $HK, unless otherwise indicated.
[a]Antimicrobial curtains are replaced every 6 months; standard curtains are replaced every 2 weeks (ie, 13 times within 6 months) and upon discharge of patients (2 curtains alongside a patient's bed would be replaced).
[b]The normal lifespan of the standard curtain is 60 months, therefore the direct cost of standard curtains = direct cost of new purchase/10.
[c]Because patients could not be admitted to a bed while curtains were being replaced, loss of avenue = bed charges × time to replace curtains.

Comparison of costs ($) associated with the use of antimicrobial curtains and standard curtains in an 8-Bed cohort cubicle of an acute-care medical ward over a 6-month period. (Table reproduced from Luk *et al*., 2019)

Table 2: Table illustrating cost benefit of antimicrobial curtain versus a standard curtain

	Standard ($AUD)	Sporicidal ($AUD)
Curtains laundered and changed at 3 months and 6 months (n = 14)	421.40	0
Curtains laundered after discharge of patient with significant pathogen (n = 147)*	2,212.00	0
Time to replace sporicidal curtains every 6 months (2.3 hours)	0	92.00
Initial cost to purchase and install 14 pairs of sporicidal curtains every 6 months	0	1,100.00
Total	2,633.40	1,202.00

NOTE. There is no capital outlay shown for standard curtains because these were purchased when the hospital was established. At time of submission $AUD 1 = $USD 0.93.
*MRSA, VRE, *C difficile*, or CRE.

Cost associated with changing ICU curtains over a 6-month period. (Table reproduced from Kotsanas *et al*., 2014)

1.2 Hand/touch transmission

There is a significant body of work outlining different standards for hand hygiene within hospitals (World Health Organization, 2009). Hand transmission between healthcare workers, patients, and visitors may represent the largest deposit of pathogens onto fabrics, in terms of number of cells/virions, particularly in high “touch traffic” areas.

For example, on a curtain healthcare professionals, patients and visitors will likely touch the same part of the curtain to draw it back each time, increasing the probability of contamination (Neely *and* Maley., 2000; Woodland *et al.,* 2010; Sridhar *et al*., 2016; Owen *and* Laird., 2020). This concentrated location is repeatedly observed in many clinical studies investigating microbial loads on hospital curtains which all report taking samples from specific heights of the curtain (Woodland *et al.,* 2010; Ohl *et al.,* 2012; Schweizer *et al*., 2012).

1.3 Aerosol transmission

Pathogens are able to spread as particles or droplets in the air (Flügge, 1897). This can come in a variety of human secretions including, exhalation via the nose or mouth, vomiting and diarrhoea (Eames *et al.,* 2009). These particles/droplets are capable of carrying bacteria and viruses acting as a medium to enable transmission of infectious disease. The size of the droplet and the humidity of the environment have been shown to affect the distance in which droplets can travel. It was concluded that the medium-sized droplets (60 μm) are the most affected by humidity,

compared to the small and large droplet sizes. This is because small droplets begin to evaporate upon release, and larger droplets fall to the floor, therefore the medium droplets are able to move further with increased humidity (Lui *et al.,* 2017). Precautions to prevent droplet infection transmission can be made such as wearing full personal protective equipment (PPE) and regular sanitation of all surfaces (NHS, 2021). However, typical linen privacy curtains are a limiting factor with their replacement time varying and evidence to show survival of pathogens after laundering, which will be discussed later in the review (Riley *et al.,* 2017; NHS, 2021). Therefore, there is a significance in simulating aerosol transmission in this study. By observing aerosol transmission, the study will create a variety of factors replicable to a clinical setting, which will give conclusive data on the efficacy of the antimicrobial textiles.

Aerosol transmission in a laboratory setting can be achieved by using a nebuliser (May, 1973). One advantage of using a nebuliser is that known concentrations of inoculum can be prepared and placed inside the nebuliser to give an even and replicable coverage of organism over the fabric, recreating a clinical setting (Turgeon *et al.,* 2014). Variability between brands of nebuliser has been discussed by Turgeon *et al*. (2014) using four different brands of nebuliser to ensure supporting data was viable.

Chapter 2: Incorporation of polymers into antimicrobial textiles and potential resistance to used antimicrobials

2.1 Overview

Incorporating polymers into textiles is a relatively new concept, with products utilising quaternary compounds and silver nanoparticles as the antimicrobial agents dominating the market. The leading manufacturers of these products are Marlux Medical, Endurocide, Hygenica and Interweave. All these companies produce antimicrobial curtains for use in a clinical setting, with each brand containing varying concentrations of antimicrobial metals in combinations with other patented antimicrobials. This chapter will review the antimicrobial mechanisms and applications of these commonly used antimicrobials via analysis of the literature.

This will highlight the link between structure and function of these antimicrobial polymers. In addition, this chapter of the review will include analysis of the reported sporicidal properties of these novel medical textiles.

2.2 Silver, a microbial additive for coatings textiles:

Due to its low toxicity to humans (Clement and Jarrett, 1994) silver can safely be used as an additive to give textiles a long-lasting efficacy against pathogenic activity, making silver a popular additive to medical textiles (Modjarrad and Ednesajjad, 2013). Silver is an established antimicrobial with activity against bacteria, fungi and yeast (Moyer *et al.,* 1965; Fox and Modak, 1974). The mechanisms by which silver targets microbes are diverse and very dependent on which form silver is being utilised (Rai *et al.,* 2009).

One of these forms includes metallic silver, which reacts with the fluid from wounds. The silver becomes ionised, facilitating binding to bacterial cell walls and nuclear membranes resulting in membrane and cell wall disruption, lysis and cell death. In this metallic form silver can also bind to bacterial DNA and RNA denaturing the polynucleotides and therefore inhibiting replication and protein synthesis (Lansdown, 2002; Castellano *et al.,* 2007). However, metallic silver has weak antimicrobial activity and deteriorates rapidly. This is because silver is very easily adsorbed by proteins which will reduce its effectiveness before it can interact with bacterial cell walls, membranes, or polynucleotides (Castellano *et al.,* 2007; Rai *et al.,* 2009).

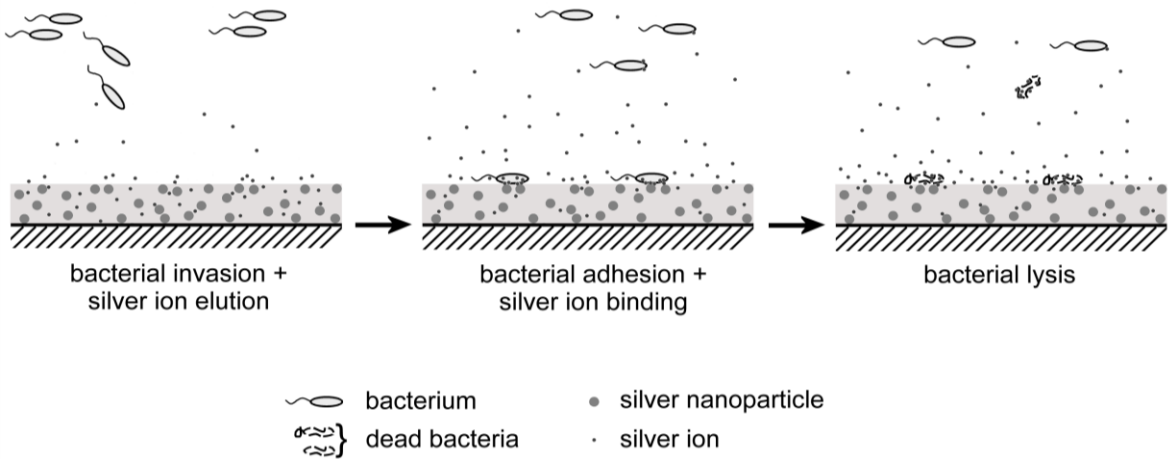
Another antimicrobial preparation of silver is silver sulfadiazine (AgSD). With a chemical formula of $C_{10}H_9AgN_4O_2S$, this complex organic structure is a sulphonamide-based topical agent with both antibacterial and antifungal activity.

The mechanism of action involves interactions with sodium chloride-contained in body fluids, which induces the slow release of silver ions into the wound area. The now ionised silver atoms catalyse the disruption of disulphide bonds, causing changes in protein structure, and can also disrupt the active sites of thiol-containing enzymes. Another antimicrobial mechanism of AgSD involves binding to the DNA, specifically to all the base pairs in the double helix, therefore inhibiting transcription

(Rosenkranz and Rosenkranz, 1972; McDonnell and Russell, 1999). AgSD is also a competitive inhibitor of para-aminobenzoic acid (PABA), which promotes the degradation of serotonin, which can result in fibrotic changes, meaning there will be no formation of scar tissue (PubChem, 2021). The mechanism of AgSD and PABA is via inhibition of bacterial dihydropteroate synthase which will result in the disruption of folic acid metabolism and therefore DNA synthesis (Sandmann *et al.,* 1974; Bult and Plug, 1984). AgSD does not affect human cells is because of the inability to penetrate the surface of the epidermal layer, meaning AgSD will only specifically bind to the DNA of the microbe to perform its mechanism of action (Gallagher *et al.,* 2007). AgSD is used primarily used in the treatment of burn wounds (Fox and Modak, 1974; Atiyeh *et al*., 2007) but is associated with a number of side effects that may make it unappealing for clinical use, including severe allergic reactions, which are uncommon but well characterised (Fuller, 2009).

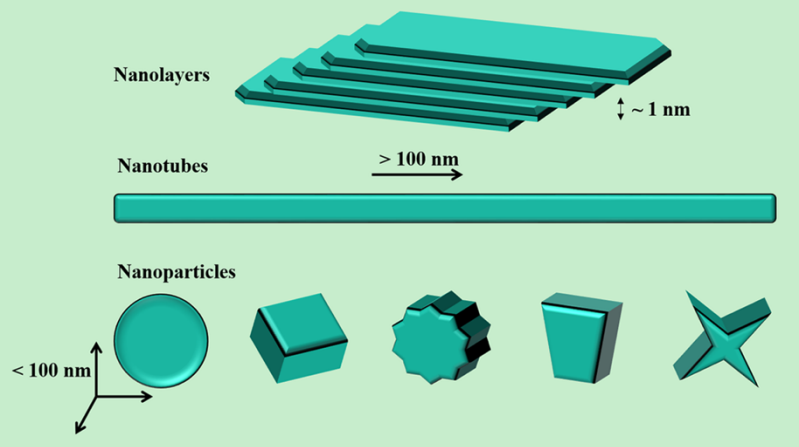
Silver nanoparticles are a much more modern approach to using silver in the medical industry. The nanoparticles are extremely effective antimicrobial agents due to their large surface area, facilitating better contact with microorganisms (Durán *et al.,* 2016; Liao *et al*., 2019). This allows the nanoparticles to attach to the cell membrane, and hence penetrate inside the bacterial cell. The silver then attacks the respiratory enzymes, reducing ATP availability, therefore inhibiting cell division, resulting in cell death (Kazachenko *et al.,* 2000) (Fig. 1).
Size and shape of silver nanoparticles has a drastic effect on their antimicrobial efficacy, with supporting evidence showing that a triangular shaped nanoparticle is far superior to a rod or spherical shaped nanoparticles (Morones *et al.,* 2005; Gong *et al.,* 2007) (Fig. 2).
The success of silver in the medical textile industry can also be partly attributed to its binding properties to polyester nonwoven fabrics (Yeo and Jeong, 2003).

Figure 1: Illustration of antibacterial mechanism of silver nanoparticles on a surface.



Silver nanoparticles have been seen to migrate and bind to microbes causing damage to proteins, genetic material and membranes, which leads to cell death. This is achieved via a direct transfer of silver ions from the oxidised silver nanoparticles to the microbe on the surface, causing varying damage to the microbe, resulting in lysis.(Diagram reproduced from Knetsch, and Koole, 2011).

Figure 2: Illustration of the varying shapes and forms of nanoscale materials.



Nanoscale materials come in many forms, including nanolayers, nanotubes, and nanoparticles. The nanoparticles come in a variety of shapes including: circular, square, pseudohexagonal and flower shaped. (Diagram reproduced from Sun *et al*., 2018).

2.3 Quaternary ammonium compounds:

Quaternary ammonium compounds (QACs) are at the interface of organic and inorganic chemistry (Fig. 3), due to their unique physiochemical properties (Bureš,

2019). QACs are very stable, and are relatively unaffected by changes in pH, remaining effective for long periods of time (Nayak and Padhve, 2014).
QACs display antimicrobial activity against a large range of pathogens, including vegetative bacteria, yeast, viruses, algae and fungi. However, QACs have been shown to be ineffective against bacterial spores, mycobacteria and hydrophilic viruses (Ravikumar *et al.,* 2006; Hegstad *et al.,* 2010; Fredell, 2019).

The antimicrobial activity of QACs is largely based on their cationic and surfactant characteristics (Gilbert and Moore, 2005; Bureš, 2019). In the form of a surfactant QACs contain a positively charged and hydrophilic head alongside a hydrophobic tail (Fig. 4).
The positively charged QAC will bind to the negatively charged microbe, forming a surfactant-microbe complex. This disrupts the cell membrane function and protein activity (Gilbert and Moore, 2005).
Similarly, QACs can disrupt bacterial DNA, meaning the cells can no longer replicate (Marini *et al.,* 2007).

Figure 3: Illustration of the structure of quaternary ammonium compounds

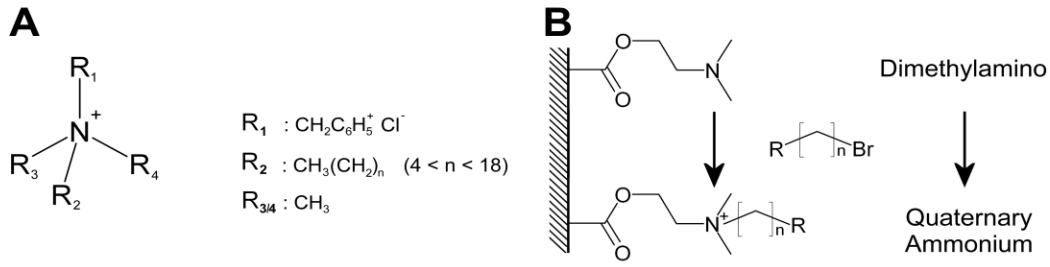


Fig 3. A: QAC only has antimicrobial properties if the akyl chain (R_2) contains between 4-18 carbon atoms B: Polymers containing tertiary amino groups, such as poly-(dimethylaminoethyl methacrylate), can be modified (e.g. quaternied) by specific functional halides. (Diagram reproduced from Knetsch, and Koole, 2011).

Figure 4: Schematic of QAC-derived cationic surfactant and overview of their structures

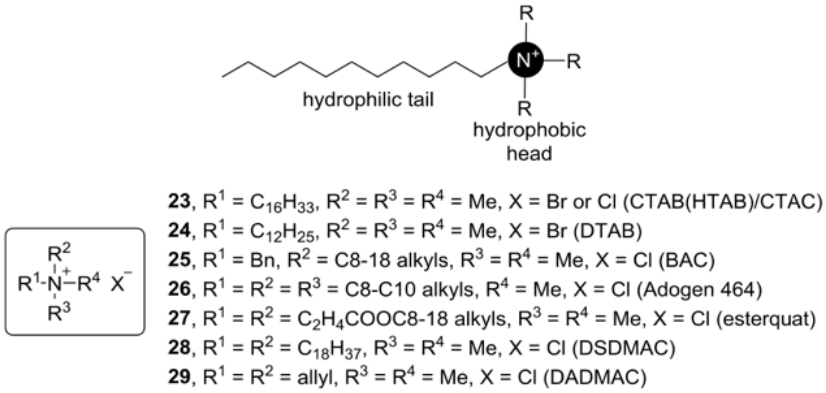


Fig. 4 Illustration highlights key aspects of the QAC-derived cationic surfactant structure showing the long tail hydrophilic tail and N^+ hydrophobic head. **23-29** are different structures of the cationic surfactant QAC. (Diagram reproduced from Bureš, 2019).

The antimicrobial activity of QACs is dependent on the length of the alkyl chain (Fig. 4), if a long hydrocarbon chain is bonded to the cationic ammonium, penetration of the hydrophobic group on the organism will allow the alkylammonium group to physically interrupt all cell functions (Simoncic and Tomsic, 2010).
These factors contribute to the widespread use of QACs, making them a very strong candidate for incorporation into antimicrobial textiles (Nohr *et al.,* 1994; Thorsteinsson *et al.,* 2003).

One of the limitations associated with QACs is that they leak from the textile to which they have been applied. This is because there are no functional groups in the structure which allow for prolonged binding to the fibres of the textile. This results in a gradual decrease in concentration, eventually falling below the minimum inhibitory concentration (MIC), meaning that QAC-incorporated textiles would lose their antimicrobial properties over time (Caillier *et al*., 2009). However, this leaking effect can be combatted with sol-gel technology, using mixtures of colloidal solutions such as tetra-alkoxysilane ($Si(OR)_4$) combined with QACs which increases their stability due to cross linking polymerisation. The sol-gel technology is also used to enhance the retention of heavy-metal ions (Zimmermann *et al.,* 2001; Wang and Wang, 2009). These factors allow for the QACs and other antimicrobial polymers to become better incorporated into the fabrics.

2.4 Sporicidal properties

Fungi are multicellular organisms that form a network of branching filaments called *hyphae*. Spores are produced from the ends of these *hyphae* as a strategy of asexual reproduction. Hyphae typically grow together across and above a surface forming a compact, macroscopically visible tuft called a mycelium. The spores at the end of the hyphae are released and spread via wind, water or animals (Madigan *et al*., 2015).

Fungal spores also have a thigmotactic response, meaning an explosive release of spores due to a stimulus or touch, which will enable spores to be released into the environment, resulting in the spores being able to adhere to surfaces, such as textiles (Jones, 1994; Lamprea *et al.,* 2008).

Fungal spores require moisture for germination/adhesion. It is therefore important to imbue medical textiles with a compound that increases their hydrophobicity, in order to render them water repellent and therefore resistant/hostile to fungal growth. Hydrophobicity is achieved by providing hierarchical micro- and nano-scale rough structures on the fabric surface (Fig 5.) (Wang *et al.,* 2019).
Zinc oxide (ZnO) nanoparticles are used as an anti-mildew additive due to their antifungal and antibacterial properties. These properties are enhanced when in combination with silver nanoparticles and other antibacterial components (Kotsanas *et al.,* 2014; Gao *et al.,* 2019).

Figure 5: Scanning electron microscope (SEM) photos of polyethylene terephthalate (PET) after γ-methacryloxypropyl trimethoxysilan (MAPS) radiation, ZnO mineralisation and annealing

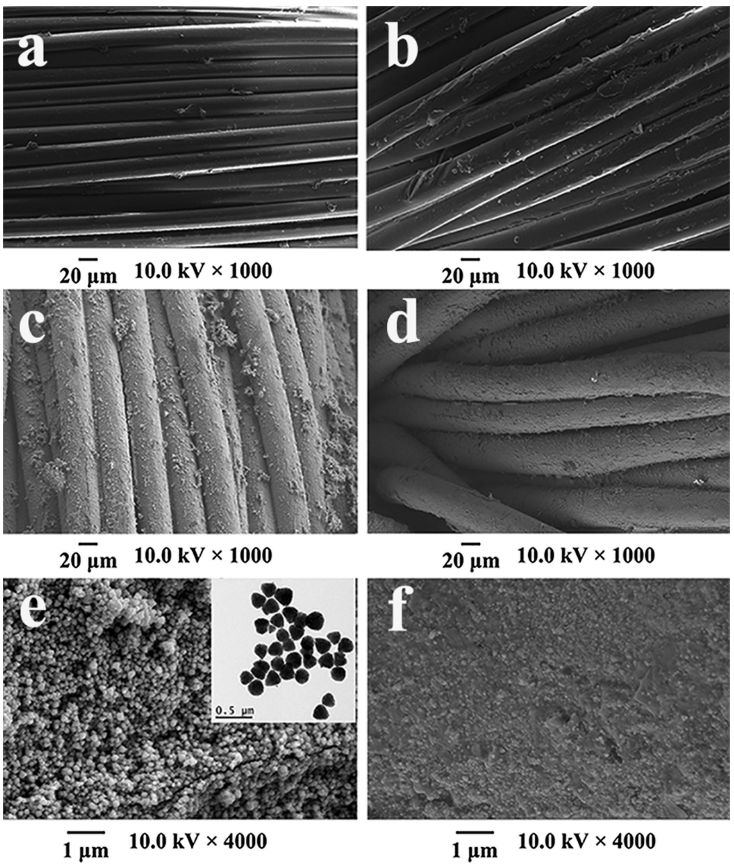


Fig 5. Surface morphologies of PET textile analysed under SEM. Fig. 5a and b show roughness due to radiation graft polymerisation chains which covered the textile resulting in the surface PET-g-PMAPS. Fig. 5c illustrates the result of in situ mineralisation treatment, forming a ZnO layer on the surface of the PET-g-PMAPS. Fig. 5d and 5f are images of the PET-g-PMAPS/ZnO surface after annealing at 122^{o}C. Fig. 5e is a transition electron microscopy image (TEM), which illustrates fig. 5c at higher microscopy, to show that the ZnO nanoparticles have uniformly covered the rugged PET-g-PMAPS surface. Fig. 5e includes the confirmation of nanoparticle size in fig. 5e of approximately 150nm, of which these nanoparticles have formed a film over the material.

ZnO nanoparticles of less than 100nm are reported to have noticeably stronger antimicrobial activities than nanoparticles larger than 100nm. In addition, the antifungal properties of ZnO are distinctively more effective when in combination with other antibacterial such as silver nanoparticles (Singh and Nanda, 2013).
ZnO is utilised primarily for its prevention of mycotoxin synthesis, which will inhibit the production of any spores. Mycotoxins are toxic chemicals present on spores and small fragments of the mould fruiting body which can be released into the air;

therefore, inhibition of mycotoxin synthesis will stop the formation of spores meaning they will never be present to transmit (Betina, 1989).

The shape of the ZnO nanoparticles also effects their efficacy, with flower shaped ZnO (Fig. 2) giving the strongest antifungal properties (Hui *et al.,* 2016; Sun *et al.,* 2018).

Chapter 3: Data linked to efficacy of using treated medical fabrics for reduction of disease transmission

3.1 Overview

Microbial growth on medical textiles is a well-known and documented mode of transmission for microbial contamination (Woodland *et al.,* 2010; Ohl *et al., 2012;* Owen and Laird, 2020). Laundering fabrics has been the general option when attempting to reduce the levels of microbial contamination on textiles (Bienert *et al.,* 2006; Hellickson and Owens, 2007; Fijan *et al.,* 2007; Nordstrom *et al.,* 2012; Honisch *et al.,* 2014). However, in recent years using disposable and antimicrobial textiles has become more prevalent to reduce disease transmission in a clinical setting (Boyken and Diekema, 2012; Luk *et al.,* 2019; Wilson *et al.,* 2020).
The increased use of antimicrobial textiles has arisen from investigations of microbial survival on untreated medical textiles, with data showing survival of a plethora of organisms, even some after laundering (Neely, 2000; Honisch *et al.,* 2014; Fijan *et al*., 2017; Riley *et al.,* 2017). This chapter will review data that both supports and opposes the use of antimicrobial textiles to try and conclude whether there is a significant grounding for their use. The supporting chapter will include a cost-benefit analysis comparing to linen laundering protocols.

3.2 Supporting use of antimicrobial curtains

Firstly, randomised investigations involving antimicrobial privacy curtains have been carried out which concluded that there were significant reductions in microbial loads

when antimicrobial curtains were used compared to that of linen curtains (Boyken and Diekema, 2012; Kotstanas *et al.,* 2014; Luk *et al.,*2019) (Table 3).

Within the studies there is emphasis on the composition of the antimicrobial textiles which are effective. To which, the curtains that contain QACs plus polyorganosiloxane were seen to be significantly more effective compared to individually built-in silver. The individual built-in silver lacked the significant efficacy against multi-drug resistant organisms (MRDOs). This included organisms such as methicillin resistant *S. aureus* (MRSA), carbapenem-resistant *Acinetobacter spp.* (CRA) and multidrug resistant *Acinetobacter spp. (*MDRA) (Luk *et al.,* 2019). However, a study investigating the activity against C. *difficile* spores using silver-lined antimicrobial curtains supported the previously mentioned opinion, that using a combination of metal nanoparticles such as silver, zinc, or copper with antimildew chemicals enables a significant reduction of microbial contamination on antimicrobial textiles compared to linen (Kotsanas *et al.,* 2014). Another study involving antimicrobial textiles outlines the use of a “complex element compound” coming in the form of a textile interwoven with silver and copper. This study concluded that there was an increased time to a first detectable level of contamination and a significant reduction of bacterial contamination compared to that of a linen curtain (Boyken and Diekema, 2012).
Another aspect to supporting the use of antimicrobial textiles is the inadequate laundering of linen in a clinical setting. NHS protocol for changing and laundering hospital curtains is a tiered system. Therefore, depending upon the ward, curtains will only be changed from every 6 months to two years, unless the curtains are visibly soiled (NHS, 2021). However, curtains can become contaminated the day they are hung without there being visible evidence of contamination. This means that potentially pathogenic microbes can be left for months and has the potential to spread from healthcare workers to patients and to visitors throughout the time in which the curtain is in place (Mitchell *et al.,* 2015).

3.3 Cost benefit analysis:

Hanging antimicrobial curtains at face value may look very expensive (Kotsanas *et al.,* 2014; Luk *et al., 2019*). However, the data presented in tables 1 and 2 both set

out the potential reduction in cost gained by using antimicrobial curtains versus a linen curtain. It is not only the mechanism by which the curtain works, but alleviation in terms labour is in terms of linen curtains being taken down, laundered and re hung that is also illustrated.

3.4 Opposing use of antimicrobial curtains

There is also evidence from studies of these antimicrobial curtains that the ISO and AATCC tests might not be a good enough representation of their effectiveness for extrapolation to a clinical setting. This is because data shows the presence of multiple microbes in-situ on supposedly antimicrobial curtains including, coagulase-negative *Staphylococci*, *Bacillus spp., Acinetobacter sp., Streptococcus spp., Pantoea spp.,* and *Enterococcus faecalis* (Sridhar *et al.,* 2016).

Table 3: Data relating to different forms of antimicrobial curtains and their efficacy

Type of Antimicrobial textile	Result of Antimicrobial textile	Author(s)
Silver and Copper salts embedded in fibres	7 times longer for first contamination compared to standard curtain Rate of contamination was 29% lower versus normal curtains	Boyken *and* Diekema, 2012
A: Built in silver Versus B: Quaternary ammonium chlorides (QACs) plus polyorganosiloxane	Curtain B was superior to Curtain A in lowering microbial burden and MDRO contamination compared to standard curtains Curtain B vs standard curtain microbial loads: S. a*ureus*: 0.5% vs 24% CRA: 0.2% vs 22.1% MDRA: 0% vs 13.2% Including curtain B exhibiting 27.6 times longer first contamination versus a standard curtain	Luk *et al.,*2019
Combination of silver, zinc or copper nanoparticles with antimildew chemicals	Cost-effective solution for 6-monthly changing of curtains, saving $AUD 1,431.30, compared to changing and laundering standard curtains in the same time frame	Kotsanas *et al.,* 2014
Copper oxide-impregnated linens	37% reduction of HAI Clostridium difficile and MDROs	Butler, 2018
Copper-impregnated composite hard surfaces, bed linens and patient gowns	28% reduction in total Clostridium difficile and multi drug resistant organisms MDRO.	Burke and Butler, 2018
Zinc Oxide (ZnO) nanoparticles with chitosan	48% and 17% reduction of *Staphylococcus aureus* and *Escherichia coli*, respectively	Petkova *et al.,* 2014

Conclusions

Potentially pathogenic microbes have been successfully proven to survive well on linen and some antimicrobial curtains. This includes survival after industrial/domestic laundering of linens, alongside outbreak management in hospitals. Evaluation of data and hypothesises from prior investigations support of the use of antimicrobial textiles to reduce disease transmission in hospitals. This conclusion was reached by analysis of the data which indicated there was a significant reduction in infection. The investigations so far have shown that when alone and not in combination the antimicrobial polymer is not nearly effective enough in reducing microbial loads on the textile. Having investigated a variety of polymers, the best combination of antimicrobials is yet to be concluded. This is because there is a plethora of parameters which require further investigation to ensure their efficacy. Finally, it has been highlighted that the antimicrobial curtains are cost effective in a clinical setting. These tables do not highlight the cost relating to HAIs, therefore the antimicrobial curtains could have an increased cost benefit with the alleviation of medicine and patient stay time. After analysis of the ISO and AATCC testing methods for antimicrobial textiles it has been highlighted that there is a significant requirement for further testing on the antimicrobial textiles to ensure they are able to be effective in a clinical setting. This significance has been derived from supporting and opposing evidence which has been highlighted through investigations into challenge testing antimicrobial textiles. Enhancing this study to attempt to include touch and aerosol transmission is another important factor when investigating opposing data. This links to which antimicrobial polymers are being used and the way they are incorporated in/onto the textile. Reflecting on the ‘industry standards’, there is not a superior test, instead using in combination and adding ‘second-tier testing’ to include aerosol and hand transmission will increase the efficacy of these testing methods. An interesting aspect of the study would be to know the cost benefit to the NHS or if there has been a cost analysis of antimicrobial textiles done in the UK. Therefore, there is a significant motivation to move forward with the study. The data generated by this study will add to existing data to help conclude the efficacy of the antimicrobial textiles. Improving the methodologies analysed throughout the literature will give the study a stronger protocol replicable to a clinical setting.

Aims

This study will follow the ISO22196, ISO20743 and AATCC TM100, with some deviation to attempt to include environmental conditions such as durability and temperature, with the aim to create a 'realistic second-tier test' (Martínez-Abad *et al*., 2013) (ISO, 2011; ISO, 2013; AATCC, 2021). Durability is of the utmost of importance, as for use in a clinical setting in the UK, products must adhere to the Care Quality Commission (CQC) guidance. The ISO22196 and ISO20743 are the relevant methods to be following in our study to test antimicrobial surfaces to prove their antimicrobial efficacy. The ISO22196 method requires specific strains of E. *coli* and S. *aureus* to be inoculated onto the surface of treated and untreated fabrics as a comparative study (ISO, 2011). Similarly, the ISO20743 requires the use of specific strains of S. *aureus* and *Klebsiella pneumonia* (ISO, 2013). For both methods these strains can be substituted for different companies' requirements. Prior investigations have included microbes such as *MRSA, Pseudomonas aeruginosa, Salmonella typhi* and *Enterococcus faecalis* (Panea *et al*., 2014; Campos *et al.,* 2016; Aziman *et al*., 2021), which increases the efficacy of both the ISO22196/ISO20743 standard to show that the material being tested has effective antimicrobial properties against multiple organisms. Some of the limitations of the ISO22196 include a lack of environmental controls such as humidity, UV exposure and the use of release agents. All of these limitations can vary hugely in a hospital which means products may pass the ISO tests, however they may not hold up in a clinical setting.

In addition, ISO22196 and ISO20743 is based upon 24-hour exposure, whereas some of these antimicrobial products follow the Care Quality Commission guidance (CQC) (CQC, 2021), which means they can be hung for up to 6 months without requiring changing (Marlux medical, 2018). Therefore, in our investigation we intend to carry out the tests which have already been done to ensure supporting data. In addition, we can then take the process further to incorporate all necessary conditions to increase the viability of data collected.

Both hand and aerosol contamination will be simulated to ensure the same conditions are met for challenge testing the antimicrobial material. To simulate hand-

touching in the lab, a novel method will be developed to achieve reproduceable results. This method will involve preparation of known concentrations of organism, which will be used to inoculate a sterile gloved hand. The gloved hand will be pressed onto the fabric, and then, in accordance with ISO22916 and ISO20743, the fabric will be placed into the necessary agar for optimal growth conditions (including nutrient agar, temperature, pH, etc.) for propagation of the inoculating organism. This novel method simulates the scenario in which a healthcare worker is wearing contaminated gloves and touches the surface. Transmission via un-gloved skin must also be replicated, as this is a very ecologically viable mode of transmission.
The re-creation of skin touch transmission can be obtained through contaminating Gamma-irradiated pigskin substrates (Leitgeb *et al*., 2013). In order to apply this to the study, a similar protocol to Leitgeb *et al* (2013) will be used to ensure that the pigskin is completely sterile before challenge testing. Once sterile, the novel approach can be used, via inoculating the pigskin with known concentrations of organism, then press the skin down onto a treated and untreated sample of fabric. From this point the ISO22016, ISO20743 and AATCC TM100 protocol for plating and recovery of organisms will be followed.

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Challenge testing Marlux Medical silver embedded versus untreated privacy curtains

George Blackburn[1]

Author for correspondence: Mr George Blackburn Tel: 0115 9516161, Fax: 0115 9516142. Address: The School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD E-mail: stygmb@nottingham.ac.uk

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Abstract

Objective: A comparative study to determine the antibacterial activity of Marlux Medical silver embedded curtain versus the same untreated material, with the addition of simulated aerosol and touch transmission methods using a uniquely developed procedure in-line with ISO20743 and ISO22196.
Design: A unique challenge test model replicable to a clinical setting, including aerosol and touch transmission as a study with a duration of 6 weeks.
Setting: The study was performed *ex situ* in a category II laboratory at the University of Nottingham, Sutton Bonnington Campus.
Method: All equipment and solutions were prepared as described in ISO20473. Test organisms include *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa*. Cultures were confirmed prior to use by growth on selective agar. A unique methodology was developed and evaluated in comparison with the ISO20473 'transfer method' as a control. Further modifications to the ISO method were made to simulate aerosol and touch transmission as part of the unique methodology.
Results: When tested against *S. aureus* and *E. faecalis,* the curtains passed the ISO20473 standard for 'antibacterial activity', both producing <2 $\log_{10}$ reduction. However, when the curtains were tested using *P. aeruginosa,* the curtains did not pass any of the ISO standards, indicating that the treated curtain had insufficient antibacterial activity against this organism. With the unique simulated transfer methods, the highest reduction in microbial load recorded was 1.92 $\log_{10}$ and the lowest showing 0.07 $\log_{10}$, suggesting that the method of transfer can profoundly affect microbial survival.

1 Introduction

With recent catastrophic global events seen through the COVID-19 pandemic, there is a heightened awareness surrounding the spread of disease, especially in hospitals. Organisations such as the National Health Service (NHS) and the National Institute for Health and Care Excellence (NICE) have developed procedures to help reduce transmission of disease in hospitals. One example is 'bare below the elbow' whereby shortening the sleeves of healthcare workers facilitates more rigorous hand hygiene practices (NHS, 2021). Unfortunately, despite these measures, hospital acquired infections (HAIs) are estimated to have cost the NHS £2.1 billion in 2016/2017 (Guest *et al.,* 2020). In addition to this 39.5% of the HAI producing bacteria causing HAIs are multi-drug resistant organisms (MDROs), including multi/methicillin resistant *Staphylococcus aureus (*MRSA), vancomycin resistant *Enterococci* (VRE) and *Clostridium difficile*, therefore it is paramount that there are increased investigations into interventions that can reduce disease transmission in clinical settings (Cornejo-Juárez *et al.,* 2015; Ohl *et al.,* 2012).

To enable successful investigations, clinical settings must be fully examined to understand how and where disease can be transmitted. One vector for disease transmission is privacy curtains. Standard linen privacy curtains are particularly outdated, with many studies proving that they harbour nosocomial bacteria (Pinon *et al.,* 2013; Fijan *et al*., 2007; Kramer *et al.,* 2006; Trillis *et al*., 2008; Ohl *et al.,* 2012). One issue identified for linen privacy curtains is inadequate laundering practices, with evidence that certain species of Enterococci can survive laundering at 71 ^{o}C (Orr *et al.,* 2002). In addition, depending on the ward, privacy curtains can be hung for months, or even years, unless they are visibly soiled (Woodland *et al.,* 2010; Owen *and* Laird., 2020; NHS, 2020). As a result, the textile becomes a fomite for disease as healthcare workers, patients, and visitors can all potentially can touch the contaminated curtain throughout the time it is hung. The final issue with standard

privacy curtains is the cost of changing them from linen to disposable or antimicrobial textiles. Therefore, alleviation of cost must be confirmed to be able to justify such a huge investment for hospitals and there have been various studies to convey cost alleviation, but none in the UK. Therefore investigations into the factors surrounding cost of HAIs and using standard linen curtains compared to that of treated curtains in the UK must be undertaken (Kotsanas *et al.,* 2014; Luk *et al.,* 2018).

To tackle contaminated linen or disposable curtains, antimicrobial curtains have been developed with a variety of antimicrobials either woven into, or embedded within, the fabrics.
The focus of this study was Marlux Medical privacy curtains which are embedded with Microbans SilverShield technology. Silver is a well-established antimicrobial, known for its broad range of activity against a diverse range of microbes (Moyer *et al.,* 1965; Fox and Modak, 1974; Rai *et al*., 2009). Applications of silver in clinical settings comes in a variety of forms, including the use of silver nanoparticles, which is a particle that ranges between 1 and 100 nanometres in size. These nanoparticles have extremely different physical and chemical properties compared to their larger particle equivalents; therefore, this is the most modern approach to using silver. In addition, a large surface area contributes to the extreme efficacy of silver nanoparticles, resulting in more successful contact with, and therefore killing of, the microorganisms (Atiyeh *et al.,* 2007; Durán *et al.,*2016).

The efficacy of antimicrobial textiles is determined by a variety of tests including methods defined by the International Organisation of Stand (ISO) (ISO, 2013; ISO, 2019) and the American Association of Textile Chemists and Colourist (AATCC) (AATCC, 2021). However, these standardised methods come with little consideration of the different types of transmission that may occur in a practical setting. Instead, the ISO20743 ‘transfer method’ is used to simulate prolonged contact between textile and organism. To make the study applicable to a clinical setting, the two most common modes of transmission of microbes onto the fabric should be addressed; namely touch and aerosol. With addition of these parameters, the textiles will be put under more advanced challenges in killing the concentration of bacteria placed onto them. One of the aims of the study is to understand how the test organisms behave when applied to the textile by these different routes.

Specifically, to understand whether different organisms are still affected by the antibacterial product on the curtain if applied this way.

There are studies which illustrate the effectiveness of the antimicrobial curtains after a significant amount of time (Luk *et al.,* 2019), however there are no studies specifically on Marlux Medical fabrics with additional 'second-tier' testing alongside the ISO standards. This procedure will be carried out via a novel methodology, based on the methods described in ISO20743 and ISO22196 (ISO, 2013; ISO, 2019). This novel methodology will allow for accurate calculations of 'antibacterial activity' as described in ISO20743 for the 'second-tier' testing (ISO, 2013). This study will indicate the suitability of the ISO standards for fabrics to be used in a clinical setting.

Hypothesis

It is suggested in previous studies that using the ISO standards will detect effective 'antibacterial-activity' against the test organisms. However, any variations of inoculation on the textile may reveal a drop in viability of >2 $\log_{10}$ as described in ISO20743 meaning the textile may not be deemed fit for use in a clinical setting.

Materials and Methods

2.1 Chemicals and Media

Specifics of solutions and media used in the study are presented Table 2.1. Suppliers of all chemical substances and media used in this thesis are listed in Table 2.1. All solutions and media used in the study were prepared to either as described by the manufacturers standards or according to the method specified in the ISO20474 standard.

Table 2.1 Chemical substances and media used during this study

Compound	Supplier
Nutrient Broth No.2 (CM0067)	Oxoid, UK Ltd
Nutrient Agar (CM0003B)	Oxoid, UK Ltd

Pseudomonas Agar (CM0559)	Oxoid, UK Ltd
Glycerol (G5516)	Sigma-Aldrich, UK Ltd
Bile Aesculin Agar (CM0888)	Oxoid, UK Ltd
Baird-Parker Agar (CM0961)	Oxoid, UK Ltd
Brain Heart Infusion Agar (CM1136)	Oxoid, UK Ltd
Egg Yolk Tellurite Emulsion (SR0054)	Oxoid, UK Ltd
Sodium Chloride (NaCl)	Fisher Scientific, UK Ltd
Tryptone (LP0042B)	Fisher Scientific, UK Ltd
Tween 80	Fisher Scientific, UK Ltd
Potassium Phosphate Monobasic (KH_2PO_4)	Fisher Scientific, UK Ltd
Sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot H_2O$)	Fisher Scientific, UK Ltd
L-alpha-Lecithin, granular from soybean oil	Acros Organics B.V.B.A
L-Histidine Hydrochloride ($C_9H_9N_3O_2.HCl.H_2O$)	Fisher Scientific, UK Ltd

2.2 Bacterial strains and growth conditions

Bacterial strains used in the study are described in table 2.2. These strains were obtained from the laboratory stocks of Microbiology, Brewing and Biotechnology Division, University of Nottingham, United Kingdom. An single colony was initially obatained and inoculated into 20ml of nutrient broth and samples were incubated overnight at 37^{o}C in a rotary shaking incubator at 150 rpm. Following overnight incubation in broth, all cultures were streaked onto nutrient agar and incubated overnight at 37^{o}C. Finally, to confirm the presence of target bacteria, each sample was streaked onto selective media. *S. aureus* onto Baird-Parker agar, *E. faecalis* onto Bile Aesculin agar and *P. aeruginosa* on Pseudomonas selective agar. Overnight incubation allowed each culture to reach a density of approximately 1 x 10^9 cfu, ml^{-1} throughout the study. To prepare fresh liquid cultures, 200µl of each culture was sub-cultured into 20ml of nutrient broth daily to ensure fresh stock was used in each separate investigation.

Table 2.2 Bacterial strains used throughout study

Bacteria	Strain Number	Selective agar used
Staphylococcus aureus	UoN SuperLab Stock	Baird-Parker agar
Enterococcus faecalis	NCTC775	Bile Aesculin agar
Pseudomonas aeruginosa	UoN SuperLab Stock	Pseudomonas selective

2.3 Transfer method of ISO20743

Following the international standard (ISO) 20743 entitled; 'Textiles- Determination of antibacterial activity of textile products', the 'transfer method' was followed as described in the sections below, with modifications of using selective agar for specified strains in Table 2.2.

2.3.1 Incubation of test strains

Once isolated cultures had been confirmed, a single colony for each organism was inoculated into 20 ml of nutrient broth and incubated overnight before being sub-cultured (see section 2.2). As specified in ISO20743, the second transfer constitutes the working culture(s).

2.3.2 Monitoring growth of bacterial cultures

After overnight incubation, culture growth was determined using Optical Density (OD_{600nm}) and the density of culture was adjusted using peptone-salt solution as a diluent to OD_{600nm} = 0.5 (approximately $1x10^8$ cfu ml^{-1}). All cultures were used immediately post dilution in experiments. The viable count (cfu ml^{-1}) was determined by performing a serial dilution and plating using the Miles and Misra Method (3 sample of 10 µl used per dilution). When bacteria were recovered from the surface of textiles, samples were plated on appropriate selective agar and incubated overnight at 37 ^oC (see table 2.2). After incubation the number of colonies were counted in the range of 3-30. When the number was less than 30 in the petri dishes, the cell number was used to calculate the average number. When the number of viable bacteria was less than 1 the average number was rounded up to 1.

2.3.3 Preparation of treated/untreated textile and inoculation of agar plates.

Working in a Class II Biological Safety Cabinet, 10 specimens of textile were cut out using a circular 3.8 cm diameter template. Of which, 5 specimens were treated and 5 were untreated textile. Then 1ml of the test inoculum was spread evenly onto the appropriate selective agar plates, using a sterile spreader and then excess liquid was removed using a pipette, and the plates left to stand for 300 s ± 30 s.

Following the standard procedure, the specimen of material was placed onto the surface of an inoculated agar plate (1 plate used per specimen) and weighed down with 200g cylinder for 60 s ± 5 s. Each specimen was then placed into a sterile petri dish with surface inoculated with bacteria uppermost using sterilised tweezers.
For the touch transmission method, a sterile glove was worn by the operator and the fingers of the gloves were placed onto an inoculated agar plate and then the bacteria transferred onto the surface of the textile by placing the fingers on the surface of the material for 60 s ± 5 s. The inoculated sample was then transferred to a sterile petri dish transferred with the inoculated surface uppermost.

For aerosol transmission, 2 ml aliquots of the inoculum were transferred into a glass bottle containing spray atomiser. Each spray was approximately 100-125 µl.
The textile was placed onto the selective agar plate, then using the atomiser the textile was sprayed until evenly covered with inoculum, (approximately 6 sprays required). After allowing approximately 60 s ± 5 s for the aerosols to settle, the textile was transferred to sterile petri dish with inoculated surface uppermost,
For incubation of all samples, a humidified chamber was recreated by soaking absorbant paper with sterile distilled water in the bottom of a sealable plastic box. The plates from the investigation were then placed into the box and incubated overnight (approx. 18h) at 37 ^{o}C.

2.3.4 Recovery of bacteria from fabric samples

Sterile stomacher bags were prepared, by aliquoting 20 ml of neutralising solution (as described in ISO20743) into each bag. The stomacher machine (Seward Stomacher 400C Lab Blender) was run using its standard setting on each side of the bag for each sample as described in ISO20743.

2.3.5 Calculations

Calculation of bacterial concentration on each plate derived from ISO20743 (ISO, 2013)

Bacterial concentration of each plate can be determined using the following formula:

$C_B = Z \times R$

Where:

C_B = bacteria concentration in $cfu.ml^{-1}$
Z is the average value of the two petri dishes in $cfu.ml^{-1}$
R is the dilution rate

Number of bacteria calculations
Bacteria can be enumerated using the following formula:

$M = C_B \times 20$

Where:

M = the number of bacteria per specimen
C_B = bacteria concentration obtained

Calculation of Antibacterial Activity:

Antibacterial activity can be quantified using the following formula:

$R = U_t - A_t$

Where:

R is the antibacterial activity.

U_t is the average of the common logarithm of the number of viable bacteria, in cells cm^{-2}, recovered from the untreated test specimens after 24 h.

A_t is the average of the common logarithm of the number of viable bacteria, in cells cm^{-2}, recovered from the treated test specimens after 24 h.

For the parameter R to be within acceptable limits, successful the difference in log_{10} values of cell number must be <2.

3 Results:

3.1 Experimental aim and design

The purpose of this study was to understand the limitations of the ISO standards. The limitation identified involved an unrealistic challenge model for organisms in a clinical setting. Including how bacteria would encounter the untreated or treated textile. Therefore, through analysis of bacterial transmission in hospitals, the most

prevalent modes of transmission were identified as touch and aerosol transmission. Research into differing surfaces to simulate touch transmission included pig skin and latex gloves, however, using a sterilised latex glove was deemed the most appropriate and safe way to handle the bacteria and textile in this study. For aerosol transmission, nebulisers and atomiser spray bottles were the two considerations, resulting in the use of an atomiser, due to the study requiring more precision of the exact volume of liquid being placed onto the textile. Using a nebuliser would not have been a good challenge model for the textile, as a nebuliser does not simulate a sneeze or a faecal explosion. Therefore, an atomiser was chosen for the study, to ensure that the study was simulating human-like transmission onto the textile.

Each organism was chosen for their difference in mode of transmission and location in the clinical setting in which they are found, and the concentration in which they are found. Additionally, each organism is ACDP level 2 and fast growing, meaning they are easier and safer to work with than more resistant strains which are ACDP 3.

The results shown in Table 3 show that there is a reduction in the concentration of bacteria which is retrieved from treated textiles compared to untreated after the transfer method. However, this reduction in $\log_{10}$ viable count does not satisfy ISO20473 or ISO22196, with all organisms performing >2 $\log_{10}$ when the touch and aerosol transmission methods of inoculation were used.

3.2 Challenge testing

Throughout each sequential investigation, the standard ISO technique was performed as a control.

To begin the study, each organism (Table 2.2) was used in a challenge test following the ‘transfer method’ of ISO20473 to begin the study to ensure results were in line with previous data. The standard procedure did show significant ‘antibacterial activity’ against both *E. faecalis* and *S. aureus* (Table 3A). This resulted in all plates for treated fabrics showing no growth on the plates after dilution, therefore antibacterial activity was quantified as <2 $\log_{10}$. However, *P. aeruginosa* consistently grew on both treated and untreated textiles (Appendix 2) showing insignificant

‘antibacterial activity’ against the organism. For *Pseudomonas* the highest difference in $\log_{10}$ was 0.42 and the lowest was 0.15.

3.3 Challenge testing with added parameters

3.3.1 Touch transmission

The touch method was developed with the aim of simulating touch transmission and to add a realistic second tier parameter replicable to a clinical setting in the study. The touch method was developed by firstly touching the fabric with a glove but no organism to understand how the textile would be handled by healthcare workers, patients, or visitors. Next a test was carried out alongside a standard challenge test to trial the touch transmission. The results from this investigation showed that the methodology was sufficient and therefore this methodology was used throughout the investigations. The results consistently showed that with the addition of touch method onto the textiles, there was a significant reduction in the effectiveness of the treated and untreated textile. Including a difference of 0 in antibacterial activity between the treated and untreated textile for *P. aeruginosa*.

3.3.2 Aerosol Transmission

To develop aerosol transmission using the atomiser spray bottle, distilled water was sprayed onto the fabric to ensure an even coverage as a trial run. This trial run also allowed for timings for the aerosols to settle to be determined. After this trial the results were then used to then perform the experiments with bacteria instead of water. The results agreed with those gained using the touch inoculation, indicating that with the added parameters the ‘antibacterial activity’ of the treated and untreated textile were not notably different when the bacteria were deposited onto the surface this way. One anomalous result was gained using *Enterococcus* where there was no growth seen on the treated textile. Unfortunately, despite no growth of *Enterococcus* on the treated textile, there was still not sufficient $\log_{10}$ difference between the treated and untreated textile to deem the treated textile to be effective, illustrating a reduction in microbial load of 1.2 $\log_{10}$. However, *E. faecalis* also illustrated a negative difference, meaning that the untreated textile outperformed the treated

textile indicating that the antimicrobial embedded into the textile is least effective against *E. faecalis*.

Table 3: Table of results to show antibacterial activity of specified strains including test including variations of standard method

A) Test 1:

Test Organism: *Staphylococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	13.6	272	0.434
Treated + Touch	5	100	
Untreated +Aerosol	8.83	176	0.64
Treated +Aerosol	2	40	
Untreated	36	720	2.857
Treated	0	0	

Test Organism: *Pseudomonas*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	24.6	493	0
Treated + Touch	24.6	493	
Untreated +Aerosol	70.6	1413	0.44
Treated +Aerosol	25.5	510	
Untreated	16.5	330	0.31
Treated	8	160	

Test Organism: *Enterococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})

Untreated + Touch	63.3	1266	0.027
Treated + Touch	59.5	1190	
Untreated +Aerosol	26	520	-0.07
Treated +Aerosol	30.8	616	
Untreated	14.3	286	2.46
Treated	0	0	

B) Test 2:

Test Organism: *Staphylococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	4.16	83	1.92
Treated + Touch	0	0	
Untreated +Aerosol	37.5	750	0.91
Treated +Aerosol	4.6	93	
Untreated	30	600	2.77
Treated	0	0	

Test Organism: *Pseudomonas*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	52	1040	0
Treated + Touch	28.3	566	
Untreated +Aerosol	64	1280	0.41
Treated +Aerosol	24.6	493	
Untreated	26.6	533	0.02
Treated	25.3	506	

Test Organism: *Enterococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})

Untreated + Touch	73	1460	0.19
Treated + Touch	47	940	
Untreated +Aerosol	20	400	0.23
Treated +Aerosol	11.8	236	
Untreated	28.3	566	2.75
Treated	0	0	

C) Test 3:

Test Organism: *Staphylococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	52	1040	0.25
Treated + Touch	28.6	573	
Untreated +Aerosol	44.5	890	0.42
Treated +Aerosol	17	340	
Untreated	41.3	826	2.92
Treated	0	0	

Test Organism: *Pseudomonas*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})
Untreated + Touch	68.3	1366	0.51
Treated + Touch	20.8	416	
Untreated +Aerosol	24.5	490	0.17
Treated +Aerosol	16.5	330	
Untreated	29.3	586	0.15
Treated	20.7	413	

Test Organism: *Enterococcus*			
Textile and transfer method	Bacterial Concentration ($CFU.ml^{-1}$)	Number of Bacteria per specimen	Antibacterial activity (log_{10})

Untreated + Touch	118	2360	3.37
Treated + Touch	0	0	
Untreated +Aerosol	22.7	453	0.32
Treated +Aerosol	10.8	216	
Untreated	62.6	1253	3.09
Treated	0	0	

Table 1: Results of challenge tests, illustrating the comparative study between the treated and untreated textile for each test. Test 1, 2 and 3 are replicated of the same experiment. Untreated and treated represent the type of textile used and touch or aerosol represents the mode of transmission. Bacterial concentrations and antibacterial activity were calculated using equations from 2.3.5 .

4 Discussion

4.1 Overview

Using the standard ISO20743 'transfer method' the results gained were as expected, indicating that the treated textile was successful in removing all bacteria for both *S.* aureus and *E. faecalis.* Conversely, the treated textile was unsuccessful in killing *P. aeruginosa,* however, the reasoning behind will be discussed below.
The results given in Table 2.2 all illustrated a significant lack of 'antibacterial activity' and failed to produce the reduction of >2 $\log_{10}$ for all organisms when more realistic inoculation methods were used. This indicates that despite using the same concentration of bacteria, using realistic inoculation methods reduces the efficacy of the treated textile. Therefore, via analysis of ISO20473 standard (ISO, 2013), inoculating any surface with between $1x10^8$ CFU ml^{-1} and $3x10^8$ CFU $ml^{-1}$of bacteria is not representative of a clinical setting. Consequently, with this large log drop, the expectation is that textiles which pass this test will outperform and kill the lower concentrations of bacterium found on the curtains *in situ.* The hypothesis that standard ISO testing would show that the treated materials worked for the most part was shown to be correct (Table 2.2). However, with the addition of simulated aerosol and touch transmission, all tested organisms showed significant growth, and therefore using these modes of inoculation the treated textile was found to have insignificant 'antibacterial activity'.

4.1.2 *Staphylococcus aureus*

It has been reported in previous studies that silver nanoparticles have a significant effect on the growth of S. *aureus* (Li *et al.,* 2011; Swolana *et al.,* 2020).

As a bacterium that can normally reside harmlessly on the surface of human skin, *Staphylococcus aureus* has capacity to cause infection when the skin barrier is damaged via a cut or lesion and can also be inhaled nasally (Solberg, 2000). The two most common forms of transmission for these bacteria are touch and airborne. The results of the study are showed conclusively that there was a significant reduction in antibacterial activity effect when both touch and aerosol transmission was used. Despite this, the results from the study indicated that the treated textile had the most significant effect on aerosol transmission of S. *aureus,* compared to the effect seen on the other tested organisms.

4.1.3 *Enterococcus faecalis*

Enterococcus faecalis is an opportunistic pathogen typically found in the gut and bowel, therefore in a clinical scenario, diarrhoea or faecal matter in gastro-intestinal wards is where this is found. Therefore, the concentration used for inoculation (between $1x10^8$CFU/ml and $3x10^8$CFU/ml) as specified by the ISO20743 method, is more representative of levels expected to be found in faecal matter which contains incredibly high concentrations of these bacteria (Sghir *et al.,* 2000). The primary mode of transmission for this organism is through the hands of healthcare workers (Weinstein and Hayden, 2000). Therefore, the results gained here which illustrate that *E. faecalis* has the lowest overall sensitivity to 'antibacterial activity' when the touch mode of transmission was used. Indicating that when touch transmission was used, *E. faecalis* had the most significant growth on the treated textile (Table 2.2A). However, under NHS and the Care Quality Commission (CQC) guidelines (NHS, 2021; CQC, 2021), which Marlux Medical adhere to, the curtain should be taken down when visibly soiled. Unfortunately, unless in incredibly high concentrations, bacteria are not seen and therefore the treated textile must be able to inactivate bacteria at these high concentrations, as very small amounts of faeces can contain high levels of bacteria.

4.1.4 *Pseudomonas aeruginosa*

Potential reasoning behind the standard ISO technique showing unsuccessful 'antibacterial activity' against *Pseudomonas aeruginosa* is due to this organism having natural mechanisms of silver resistance. *Pseudomonas* achieves this by producing the redox-active metabolite pyocyanin (Muller, 2018). Pyocyanin is produced by these bacteria to scavenge for iron when in the body, but the siderophore can also bind to other metal ions. A study by Muller and Merrett (2014) suggests a highly positive and significant correlation between pyocyanin production by *P. aeruginosa* and silver resistance. When stressed, *Pseudomonas* produces these green pigments and in this instance the silver ions on the fabrics appear to make *Pseudomonas* more stressed (see Appendix 2), the cells are upregulating their virulence genes and therefore become more virulent than when they were inoculated onto the untreated surface. However, there is literature supporting the effectiveness

of silver as an antimicrobial to *Pseudomonas* (Hwang *et al.,* 2007; Bjarnsholt *et al.,* 2007), therefore further investigation into how and why this was unsuccessful is required.

4.2 Conclusions

4.2.1 Marlux Medical Curtains

Using the ISO standards, the embedded curtain illustrated effective 'antibacterial activity' against both *E. faecalis* and *S. aureus* and confirm previous results that the textile will pass the ISO standard. However, this was not seen for *P. aeruginosa.* Therefore, further investigation is required to determine what antimicrobial would be more successful against *P. aeruginosa.*

4.2.2 Suggestions

To further the work described in this project, it is evident that making as more to realistic challenge model to simulate how bacteria would be typically encountering the textile is essential as the different modes of transmission used here strongly affected the results gained. Therefore, an additional type of touch transmission with skin-like material, for example sterile, irradiated pig skin, could be evaluated as a viable realistic challenge test for the textile (Leitgeb *et al.,* 2013). In addition, running tests on the fabrics for longer durations of time would be beneficial to understanding if the treated textile requires longer periods of time to kill microorganisms. For hospitals which are implementing antimicrobial curtains, the use of different material and polymer on rotation could increase their overall efficacy to avoid the development of resistant populations. Antimicrobial resistance is a constant battle, with the 'race' against bacterial resistance becoming ever more difficult. By introducing different antimicrobials on a rotational basis this would ensure any bacteria that are becoming resistant are not feeding back into the hospital's population. One concern raised in this study is that the antimicrobial activity was not equally effective against different types of bacteria typically found in different wards of hospitals. Therefore, a key aspect of the study would be to challenge test different samples of an untreated curtain from each ward of the hospitals. This test would

ensure that upon rotation, the different polymer curtain could be placed into the ward depending on which bacteria are most likely to predominate in that specific ward. Parallel to this study, polymer research is essential for different strains of bacteria. Specifically, it has been shown that the size and shape of nanoparticles has a drastic effect on their efficacy (Sun *et al.,* 2018). Therefore, understanding the size and shape of the silver nanoparticles which are being embedded into the curtains could be optimised to increase antimicrobial activity on the textile. Research into different metal ions effectiveness could result in a successful rotational curtain business. With different ions and compounds, also comes the possibility of using different concentrations. Perhaps in wards such as ICU an increased concentration of the antimicrobial may be required to help reduce the risk of transmission between severely ill patients.
Finally, research into resistant strains of bacteria is required and must be incorporated into polymer development, which is also the link behind the reasoning for a rotational requirement for polymers, to slow the resistance to the antimicrobials.

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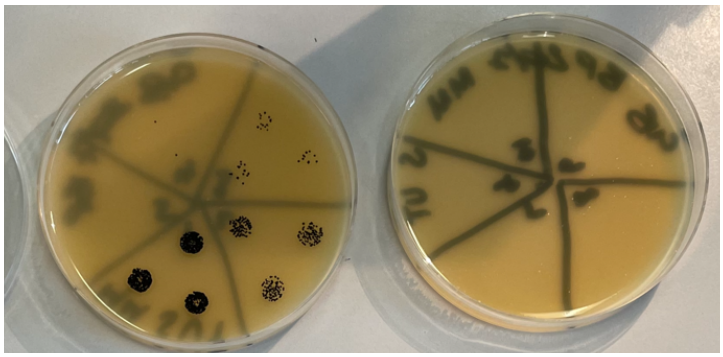
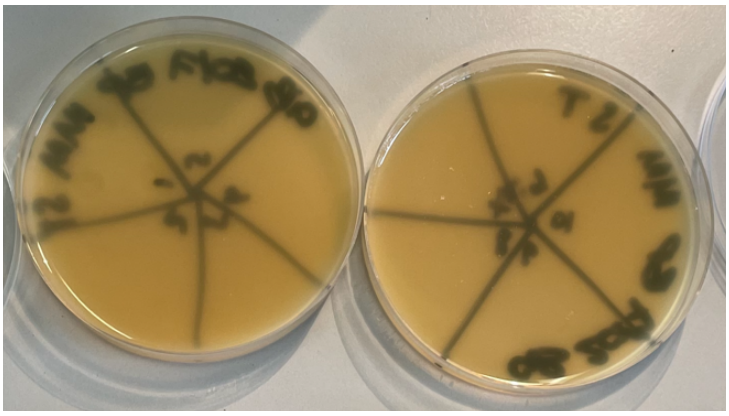
Appendices

Appendix 1.1 + 1.2 : Visual depiction of bacterial growth from ISO20473 transfer method for S. *aureus* and E. *faecalis* on treated versus untreated textile.

Appendix 1.1, Photographic display of untreated medical fabric conveying growth on the plate, with confirmative black colonies for S. *aureus.* LHS dilutions 1-5 RHS dilutions 6-10

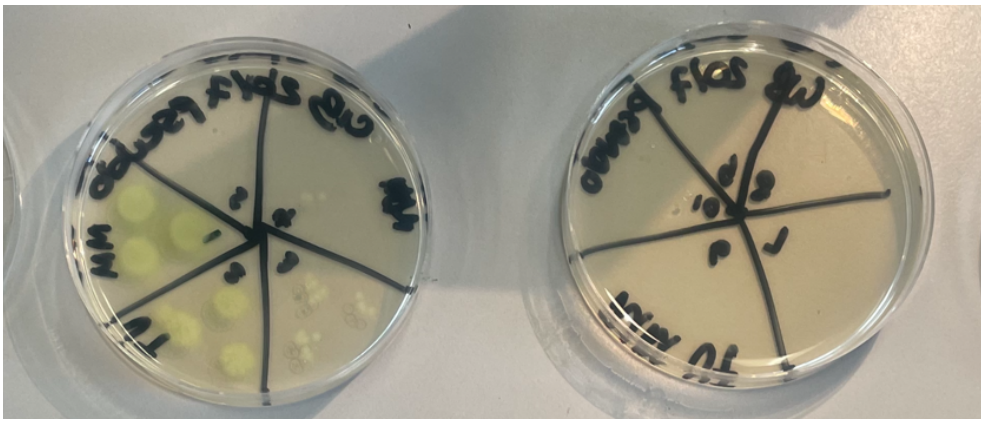


Appendix 1.2, Photographic display of treated medical fabric conveying no growth on the plate, with zero colonies shown. LHS dilutions 1-5 RHS dilutions 6-10



Appendix 2.1 + 2.2: Visual depictions of Pseudomonas after ISO transfer method 2.1 Untreated sample of Pseudomonas after transfer method, 2.2 Treated sample of Pseudomonas after transfer method

Appendix 2.1: Pseudomonas showing growth on untreated fabric following transfer method derived from ISO20473



Appendix 2.2: Pseudomonas showing growth on treated fabric following transfer method derived from ISO20743.