Development of a silver-based dual-function antimicrobial laundry additive and textile coating for the decontamination of healthcare laundry

L. Owen and K. Laird

Infectious Disease Research Group, The Leicester School of Pharmacy, De Montfort University, Leicester, UK

Keywords
antimicrobial textile, cross contamination, decontamination, Escherichia coli, healthcare laundry, laundry additive, Staphylococcus aureus.

Abstract
Aims: To repurpose a silver-based antimicrobial textile coating product (Micro-Fresh 1911) as a dual-function antimicrobial laundry additive and textile coating.

Methods and Results: Survival of Escherichia coli or Staphylococcus aureus type and clinical isolates in a domestic 40°C wash was assessed with and without soiling and biological detergent. Washing with 2% w/v silver additive (wash phase) reduced E. coli and S. aureus by 7.14–8.08 log_{10} and no cross-contamination was observed. Under dirty conditions, 0.5% silver additive in the rinse phase of a wash with biological detergent reduced E. coli and S. aureus by 7.98–8.40 log_{10} (0.00–1.42 log_{10} cross contamination). BS EN ISO 20645:2004 and BS EN ISO 20743:2013 methods were used to assess the antimicrobial activity of polycotton washed with 2% w/v silver additive against S. aureus and E. coli. The treated polycotton was antimicrobial against E. coli and S. aureus type and clinical isolates and remains active after at least one further wash cycle at 40 or 73°C.

Conclusions: The silver additive exhibits antimicrobial activity in a 40°C domestic wash, preventing cross contamination onto clean textiles and depositing an antimicrobial coating onto polycotton.

Significance and Impact of the Study: The survival of micro-organisms on healthcare uniforms during domestic laundering presents a potential risk of contaminating the home, cross-contamination of other clothing within the wash and transmitting potential pathogens back into healthcare settings via contaminated uniforms. Silver may be useful as an antimicrobial laundry additive to decontaminate healthcare laundry washed at low temperatures in domestic and industrial settings, to therefore reduce the potential risk of transmitting micro-organisms within the domestic and clinical environments.

Introduction
Reusable textile products are readily employed in the healthcare environment and include patient gowns, bed linen, towels, curtains and staff uniforms (Department of Health 2016). Textiles in the clinical environment rapidly become contaminated with bacteria, including those associated with healthcare associated infections. Perry et al. (2001) determined that 54% of healthcare worker uniforms were contaminated with one or more of methicillin resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus sp. and Clostridioides difficile at levels of one to over 100 colony forming units (CFU) upon finishing their shift. Tarrant et al. (2018) also reported that soiled linens from patients with C. difficile infections were contaminated with on average 51 CFU per 25 cm² C. difficile spores. Micro-organisms can survive on textiles for significant periods of time depending on environmental conditions, for example, faecal coliforms persisted on cotton for up to 120 days (Colclasure et al. 2015). Previous
studies have demonstrated that micro-organisms can transfer from contaminated textiles, for example 2.85 log_{10} CFU MRSA transmitted onto pig skin after 24 h incubation on the cotton (Desai et al. 2011). *Clostridioideae difficile* spores reportedly transfer from non-porous surfaces to hospital gowns, with 10^7–10^9 spores being recovered from the gowns after 10 s to 10 min contact (Dyer et al. 2019). Transmission is lower from textiles than non-porous surfaces (Lopez et al. 2013) indicating that the risk from contaminated textiles may be lower than non-porous surfaces. This suggests that contaminated textile items might serve as a source of potential pathogens, which would be of particular concern if pathogens are not removed by laundering and are brought back into the clinical setting, or for items that are infrequently changed such as privacy curtains.

There are few studies in the published literature that support the association between contaminated textiles and infection; textiles are generally considered unlikely to cause infection in the general patient population (Loveday et al. 2007). However, there have been multiple reported outbreaks in hospitals related to contaminated textiles. An outbreak of zygomycosis in immunosuppressed patients was linked with contaminated linens (Cheng et al. 2016) and Schmithausen et al. (2019) reported on an outbreak of *Klebsiella oxytoca* in a paediatric ward, which was traced to knitted clothes contaminated by a washing machine colonised with *K. oxytoca*. Further studies are needed to evaluate the level of risk of infection associated with textiles (Bloomfield et al. 2015) yet the occurrence of outbreaks demonstrates that disinfection of healthcare textiles and prevention of inadvertent environmental contamination is necessary to maintain a reduced risk of infection in vulnerable hospital patients.

Laundering processes reduce the microbial load on textiles with an aim to remove soiling and potential pathogens to minimise the risk of infection, rather than completely sterilise the textiles (Bockmühl 2017; Owen and Laird 2020). Healthcare linens including bed sheets and hospital gowns are laundered industrially, employing thermal disinfection (60–71°C) or chemo-thermal disinfection (Department of Health 2016). Linen processed in line with these policies are considered to pose little risk to infection control (Loveday et al. 2007). Some micro-organisms can survive decontamination conditions; Tarrant et al. (2018) reported that a thermal industrial washer-extractor laundering cycle (>71°C for three min) with industrial bleach detergent reduced *C. difficile* spores on naturally contaminated cotton bedsheets by only 0.45 log_{10} CFU per 25 cm², suggesting that the industrial laundering process failed to meet the UK guidelines for a ≥5 log_{10} CFU reduction. The source of up to 82% of non-hypervirulent *C. difficile* infections in hospital patients is unknown and cannot be attributed to healthcare-associated transmission from other symptomatic cases, suggesting that environmental reservoirs could potentially be a source of sporadic *C. difficile* outbreaks; it has been speculated that linen could be one such potential reservoir (Tarrant et al. 2018).

Healthcare worker uniforms are commonly laundered at home in the UK to reduce NHS costs (Riley et al. 2015). The UK Department of Health and National Health Service recommend that domestic washing of healthcare worker uniforms is conducted at 60°C to kill most micro-organisms (Department of Health 2010; NHS England 2020). However previous research has demonstrated that 44% of nurses launder their uniforms below 60°C and 40% wash them with other items (Riley et al. 2015), which may lead to potentially pathogenic micro-organisms surviving on domestically laundered textiles. Previous research has reported that potentially pathogenic micro-organisms may survive domestic laundering of healthcare worker uniforms, which could lead to potential cross contamination of other textiles in the home and/or microbial transmission into clinical settings (Fijan and Turk 2012). For example, three to four log_{10} CFU *Escherichia coli* and *S. aureus* survived a 40°C wash cycle with biological detergents, that is, detergents that contain enzymes and a further three log_{10} CFU cross contaminated previously sterile fabric in the wash (Riley et al. 2017). In addition, domestically laundered scrubs may harbour significantly (*P* ≤ 0.05) higher bacteria loads (143 CFU per cm²) than industrially laundered scrubs (143 vs four CFU per cm² respectively) (Nordstrom et al. 2012).

Antimicrobial laundry additives could be employed to decontaminate healthcare linen washed at low temperatures, as an alternative to thermal disinfection in domestic or industrial settings, thereby reducing the risk of cross contamination. Quaternary ammonium chlorides such as benzalkonium chloride have been employed in the rinse phase of laundering cycles for this purpose (Bockmühl 2017), however, much of this research has been conducted by laundry chemical companies and there is a paucity of studies in the published scientific literature demonstrating the efficacy of antimicrobial laundry additives.

Antimicrobial textile finishes have been extensively studied as a means to prevent or reduce microbial contamination of clothing and linen (Morais et al. 2016). Common finishing agents include metals such as zinc oxide nanoparticles, which reduced *E. coli* by one to two log_{10} CFU and *S. aureus* by less than one log_{10} CFU over 24 h (Petkova et al. 2016). Silk impregnated with silver nanoparticles reduced *E. coli* and *S. aureus* by...
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approximately 7 \log_{10} CFU over 2 h while linen, nylon and polyethylene terephthalate impregnated with silver nanoparticles reduced \textit{E. coli} and \textit{S. aureus} by less than 1 \log_{10} CFU (Emam et al. 2016). There have been concerns noted about some antimicrobial finishes in relation to human health, causing allergic reactions and other toxic effects (Alhosseini 2016; Hilgenberg et al. 2016), demonstrating that the safety of antimicrobial finishes should be confirmed prior to their implementation. The development of antimicrobial laundry additives that also deposit an antimicrobial coating on textile could prevent or reduce the contamination of textiles worn in the healthcare environment.

Micro-Fresh is an antimicrobial silver-based product first used as an antifungal treatment for preserving leather during shipping before being developed as an antibacterial textile coating. This study aimed to repurpose the silver-based antimicrobial product as a dual-function antimicrobial laundry additive and wash-in textile coating for the decontamination of healthcare laundry in domestic and industrial settings by determining its efficacy against \textit{S. aureus} and \textit{E. coli} type and clinical isolates.

Materials and methods

Micro-organisms

\textit{Escherichia coli} NCTC 8003, \textit{S. aureus} ATCC 6538 and clinical isolates of \textit{E. coli} and \textit{S. aureus} (Leicester Royal Infirmary, Leicester, UK) were cultured aerobically with nutrient broth and agar (Oxoid, Basingstoke, UK) at 37°C for 18–24 h.

Minimum inhibitory concentrations of silver additive, biological detergent and industrial bleaching agent

The antimicrobial activity of the silver additive Micro-Fresh 1911 (Micro-Fresh International, Leicester, UK), biological detergent and sodium hypochlorite-based industrial bleaching agent were assessed by measuring minimum inhibitory concentrations (MICs), to determine an appropriate starting dose of the silver additive to be used for subsequent laundering trials. A microdilution method adapted from the International Standards Office (2006) antibiotic susceptibility test was used. A series of dilutions of silver additive (16–0.031% w/v), biological powder detergent (64–0.125 g l\(^{-1}\)) or industrial bleaching agent (1–0.00125% v/v) were prepared in nutrient broth. An aliquot of each dilution (100 \(\mu\)l) was mixed with an equal volume of either \textit{S. aureus} or \textit{E. coli} type or clinical isolate (10^6 CFU per ml) in a 96-well plate. Controls of broth alone and inoculated broth were included. The optical density (595 nm) of the plates were measured before and after 24 h incubation at 37°C using a Spectramax Plus 384 microplate reader (Molecular Devices, San Jose, CA) and used to calculate MIC.

Fractional inhibitory concentrations (FICs) of silver additive with biological detergent and bleaching agent

Antimicrobial interactions between combinations of silver additive, biological detergent and industrial laundering bleaching agent were investigated as a potential strategy to reduce the dose of each agent within the wash while retaining the antimicrobial activity. Antimicrobial interactions were determined using a checkerboard method as described previously (Owen and Laird 2019). A series of dilutions of silver additive (1–0.01% w/v for \textit{S. aureus}, 4–0.06% w/v for \textit{E. coli}), biological detergent (1–0.01 g l\(^{-1}\) for \textit{S. aureus}, 4–0.06 g l\(^{-1}\) for \textit{E. coli}) and industrial bleaching agent (0.03–0.0003% v/v for \textit{S. aureus}, 0.06–0.0006% v/v for \textit{E. coli}) were prepared in nutrient broth and inoculated to 10^6 CFU per ml \textit{S. aureus} or \textit{E. coli} type or clinical isolate. Aliquots of silver additive (100 \(\mu\)l) were added to a 96-well plate in order that the concentration varied along the columns of the plate, before solutions of either biological detergent or the bleaching agent (100 \(\mu\)l) were added to the plate so their concentration varied along the rows. Controls of broth with micro-organism were included.

The optical density (595 nm) of the plates were measured before and after 24 h incubation at 37°C using a Spectramax Plus 384 microplate reader (Molecular Devices) to determine bacterial growth. The fractional inhibitory concentration indexes (FICIs) of the combinations were determined using Eqn (1):

\[
\text{FICI} = \frac{\text{MIC of Component 1 in Combination}}{\text{MIC of Component 1 Alone}} + \frac{\text{MIC of Component 2 in Combination}}{\text{MIC of Component 2 Alone}}
\]

where FICI \(\leq 0.5\) is synergistic, 0.5–4 is indifferent, and >4 is antagonism.

Antimicrobial activity of silver additive-coated textile

Antimicrobial textile treatment

Polycotton samples were treated with the silver additive (Micro-Fresh 1911) in an Indesit IWSD61251 Eco machine using a 40°C standard domestic 2 kg wash cycle. The cycle temperature was measured using an iButton Thermochron data logger (Measurement Systems Ltd, Newbury, UK). The mean peak temperature was 40.12 ± 0.31°C, time to peak temperature was 25.78 ± 1.11 min, and holding time at peak temperature ±0.5°C was 16.06 ± 1.15 min
(n = 19 ± standard error (SE)). For testing of silver additive in combination with biological detergent or industrial bleaching agent, the silver additive was added to the detergent compartment (wash phase) to achieve a 0-06–0-5% (w/v) solution in the wash water (4-4 l ± 0-08; n = 3 ± SE) and bath ratio of 2-20. The volume of wash water for the 40°C standard cycle was determined by stopping the cycle after filling for the wash phase was completed and measuring the volume of drained water.

For investigation of silver additive alone, textile was treated with a 2% (w/v) solution (4-39% w/w silver additive per dry weight of the textile) in the wash water as described above. Due to a lack of activity under dirty conditions, the silver additive was also investigated in the rinse phase of the wash; the presence of soiling or dirt may affect the antimicrobial coating, yet this would be minimised during the rinse phase due to prior washing with detergent. A concentration of 4-39% w/w per dry weight of the textile was tested as per the wash phase (2% w/v solution in wash water), which was equivalent to a 0-5% w/v solution of silver additive in the rinse water (16-9 l). The silver additive was added to the detergent compartment (wash phase) to produce a 2% w/v solution or added to the fabric conditioner compartment (rinse phase) of the washing machine to achieve a 0-5% w/v solution for tests against E. coli and S. aureus. Tests were also conducted on 2% w/v silver additive-treated polycotton that was subject to a further wash cycle with water alone at 40°C or 73°C. All textiles were air dried within an autoclave bag in a room at 23°C and 47% relative humidity for 18–24 h prior to testing.

Qualitative antimicrobial textile efficacy screening method
A method adapted from BS EN ISO 20645:2004 (British Standards Institute 2004) was used to screen polycotton washed with silver additive alone or in combination with biological detergent and industrial bleaching agent for antimicrobial activity.

Circular samples (25 mm) of treated textile were placed on agar plates previously spread with 100 µl E. coli or S. aureus (approximately 10⁶ CFU per ml). After incubation at 37°C for 24 h, agar plates were inspected for zones of inhibition (ZoI) of bacterial growth. Controls were untreated polycotton and textile pre-treated with 0-3% w/v Micro-Fresh 2611.

Quantitative antimicrobial textile efficacy method
A method adapted from BS EN ISO 20743:2013 (British Standards Institute 2013) was used to quantify the antimicrobial activity of polycotton washed with 2% w/v silver additive.

Treated textile samples weighing 0-4 g were placed in vials and inoculated with 200 µl 10⁵ CFU per ml E. coli or S. aureus (type and clinical isolates). Immediately after inoculation, 20 ml shake-out saline (8-5 g l⁻¹ sodium chloride and 2 g l⁻¹ Tween 80) was added, and the samples were vortexed for 5 × 5 s intervals. The number of surviving microorganisms were determined by spread plating or spiral plating (Interscience, Saint Nom la Bretêche, France) the supernatant on to nutrient agar. Identical samples were incubated for 18–24 h, vortexed in 20 ml shake-out saline and the surviving micro-organisms enumerated as described above. Controls of untreated polycotton were included.

The antimicrobial activity of treated textiles was determined by calculating the antibacterial activity value (A), which is a measure of log₁₀ reduction compared to the negative control, as per the BS EN ISO 20743:2013 method (British Standards Institute 2013):

\[ A = F - (\text{Test Log}_{10} \text{CFU at 24h}) - (\text{Negative control Log}_{10} \text{CFU at 0h}) \]  

(2)

where

\[ F = (\text{Negative control Log}_{10} \text{CFU at 24h}) - (\text{Negative control Log}_{10} \text{CFU at 0h}) \]  

(3)

An A value ≥2 is considered to indicate significant antimicrobial activity according to BS EN ISO 20743:2013 (British Standards Institute 2013).

Survival of micro-organisms during a domestic wash cycle

Overnight cultures of S. aureus or E. coli (type or clinical isolates) in nutrient broth were washed thrice in phosphate buffered saline (PBS; Oxoid) and resuspended to 10⁶ CFU per ml in PBS for clean conditions or defibrinated sheep blood (TCS Biosciences, Botoloph Claydon, UK) for dirty conditions. For each wash, two sterile swatches (25 cm²) of polycotton (65% polyester/35% cotton; Carrington Textiles Ltd, Adlington, UK) were contaminated with 500 µl of either S. aureus or E. coli washed cell suspension and incubated for 24 h at room temperature. Inoculated polycotton swatches and two kg sterile polycotton makeweights (AATCC Ballast Type Three, James Heal, Halifax, UK) were placed into the washing machine along with two sterile polycotton swatches to measure cross contamination. Under dirty conditions, 25 ml sterile defibrinated sheep blood was also added to the washing drum before laundering. Silver additive was added to either the detergent compartment (wash phase) to produce a 2% w/v solution in the wash water or fabric conditioner compartment (rinse phase) to produce a 0-5% w/v solution in the rinse water (equivalent to 4-39% w/w dry weight of textile in both the wash
and rinse phases) with or without biological detergent powder (35 g). Washes were conducted during the rinse phase as a strategy to allow gross soiling to be removed in the wash phase prior to the addition of the antimicrobial supplement to allow disinfection/sanitisation to occur. All washes were conducted using a standard 40°C cycle. Control washes using water or biological detergent alone were also conducted. Upon completion of the wash cycle, fabric swatches were vortexed in 30 ml PBS for one min and the supernatant was either spread plated or membrane filtered onto nutrient agar to enumerate surviving micro-organisms.

Statistical analysis
Wash tests were conducted on two separate occasions using two biological samples per wash (n = 4). All other tests were repeated twice on two separate occasions (n = 4).

Significant (P ≤ 0.05) differences in log10 CFU reduction of bacteria and log10 CFU count on previously sterile textiles between wash treatments was determined using an independent sample t-test assuming unequal variances, one-way ANOVA with Tukey’s post-hoc test or univariate general linear model with Tukey’s post-hoc test on SPSS ver. 25 (IBM). Where assumptions of normality were not met according to the Shapiro–Wilks test, the Mann–Whitney U test or Kruskal–Wallis tests with pairwise comparisons were conducted.

Results

MICs and FICIs of silver additive, biological detergent and bleaching agent alone

The MIC of silver additive ranged from 0.125–0.25% against S. aureus type and clinical isolates to 1% against E. coli type and clinical isolates. MICs of biological detergent ranged from 0.25–1 g l⁻¹ and industrial bleaching agent from 0.005 to 0.01% against all test species (Table 1). The combinations of silver additive with biological detergent or bleaching agent were indifferent, except silver additive with bleaching agent against S. aureus type strain, which was synergistic (FICI = 0.5; Table 1). Despite a lack of synergism, the MIC of silver additive was reduced in combination, indicating that the required dose in the wash could be decreased. The antimicrobial efficacy of polycotton washed with the lowest doses in combination to inhibit all test species (0.5% silver additive with 1 g l⁻¹ biological detergents; 0.25% silver additive with 0.005% bleaching agent), E. coli only (0.5% silver additive with 1 g l⁻¹ biological detergent; 0.25% silver additive with 0.005% bleaching agent) or S. aureus only (0.06% silver additive with 0.125 g l⁻¹ biological detergent; 0.06% silver additive with 0.0025% bleaching agent).

Antimicrobial activity of silver additive, biological detergent and industrial bleaching agent-coated textile

Polycotton treated with 0.25% silver additive inhibited S. aureus and E. coli type strains and 0.5% silver additive-treated polycotton inhibited E. coli type strain and S. aureus type and clinical isolates. Washing with biological detergent or bleaching agent did not impart antimicrobial activity onto polycotton. Of the combined treatments, only 0.5% silver additive and 1 g l⁻¹ biological detergent treated polycotton possessed some antimicrobial activity, inhibiting E. coli type strain. The combinations were therefore not taken forward for further investigation.

Polycotton washed with water alone was not antimicrobial (Table 2).

Survival of micro-organisms during a domestic wash cycle with silver additive

The survival of E. coli and S. aureus type and clinical isolates during laundering was investigated at the concentration of silver additive required to inhibit both E. coli and S. aureus (1%; Table 1). Silver additive significantly (P ≤ 0.05) reduced S. aureus and E. coli compared to water alone (6.17–8.09 vs 2.21–4.25 log10 CFU reductions) and ≤0.27 log10 CFU cross contamination to previously sterile textile was observed (Table 3).

Soiling significantly (P ≤ 0.05) reduced the antimicrobial activity of 1% silver additive in the wash against all test species. Under dirty conditions, S. aureus type and clinical isolates were reduced by 2.14–3.59 log10 CFU in a wash with 1% silver additive (Table 3). The cross contamination of sterile textiles with S. aureus was significantly (P ≤ 0.05) increased under dirty conditions to 2.97–6.66 log10 CFU (Table 3). Similarly, 2.70–3.49 log10 E. coli type and clinical isolates was removed under dirty conditions and 2.26–4.22 log10 cross contamination of sterile textiles was observed (Table 3).

The dose of silver additive was increased to 2% to potentially improve the antimicrobial activity under dirty conditions (Table 4). The microbial reduction and cross contamination on to sterile textiles under clean conditions were not significantly different (P > 0.05) between 1 and 2% silver additive for both S. aureus and E. coli type and clinical isolates (Table 4). The antimicrobial activity of 2% silver additive was significantly (P ≤ 0.05) reduced against E. coli type and clinical isolates under dirty conditions, with 2.76–3.09 log10 CFU reductions and 3.26–4.24 log10 cross contamination (Table 4), yet
activity was maintained against S. aureus (7.99–8.08 log_{10} reduction, P < 0.05; Table 4).

The addition of biological detergent was investigated as a potential means to improve the activity of 2% silver additive in the wash by lifting gross soiling from the fabric. Washing with biological detergent and 2% silver additive significantly (P < 0.05) increased the removal of E. coli (Table 5) compared to 2% silver additive alone under dirty conditions (Table 4), however a similar number of E. coli cells (P > 0.05) were deposited onto sterile textile in the wash (Tables 4 and 5). The addition of biological detergent to the wash with 2% silver additive did not significantly (P ≤ 0.05) increase the reduction of S. aureus (Tables 4 and 5). Biological detergent alone removed 5.75–6.53 log_{10} CFU E. coli and S. aureus type and clinical isolates (Table 5) and limited cross-contamination to 0.00–1.36 log_{10} CFU (Table 5), thus 2% silver additive with biological detergent was not significantly (P ≤ 0.05) more antimicrobial than biological detergent alone under dirty conditions.

The addition of silver additive to the rinse phase of the wash following washing with biological detergent was investigated as a strategy to remove gross soiling prior to the addition of the antimicrobial supplement to allow disinfection/sanitisation to occur. The dose used was equivalent to that used in the wash phase per dry weight of the textile (4.39%) which produced a 0.5% solution in the rinse water; due to the removal of soiling during the wash phase by detergent and the large volume of water used in the rinse phase (16.9 l), the excessive amounts of silver additive required to achieve the same solution in water as the wash phase (2%; 4.1 l) were expected to be unnecessary to inhibit E. coli and S. aureus. Indeed, silver additive significantly (P ≤ 0.05) increased the removal of E. coli and S. aureus in the rinse phase compared to the wash phase, (7.98–8.40 log_{10} CFU reductions; Table 5). Silver additive in the rinse phase was also significantly (P ≤ 0.05) more antimicrobial than biological detergent alone against all species except for S. aureus type strain (Table 5). Cross contamination was not significantly (P > 0.05) different between biological detergent and silver additive in the wash or rinse phases against all test species, and between silver additive in the wash and rinse phases across S. aureus type and clinical isolates and E. coli clinical isolate, however there was a significant (P ≤ 0.05) reduction in cross contamination of E. coli type strain from 2.28 log_{10} CFU with silver additive in the wash phase to 0.00 log_{10} CFU in the rinse phase (Table 5).

**Antimicrobial activity of silver additive-coated textile**

Polycotton washed with 2% silver additive inhibited E. coli and S. aureus type and clinical isolates according to the qualitative antimicrobial textile screening method (Table 6). The positive control of textile padded with 0.3% Micro-Fresh 2611 was also antimicrobial whereas the untreated polycotton control did not produce ZoIs.

The qualitative antimicrobial textile assay indicated that 2% silver additive treated polycotton was significantly antimicrobial against E. coli and S. aureus (Table 7). The antimicrobial activity of 2% silver additive-treated...
Table 2. antimicrobial activity of polycotton washed with silver additive, biological detergent, blanching agent or combination (vol % of wash water) according to the qualitative antimicrobial textile efficacy screening method (µ = 4; mean ± SE).

| Micro-organism | Water alone | 0.05% Silver | 0.05% Silver additive | 0.2% Silver | 0.2% Silver additive | 0.2% Silver + 0.005% bleaching agent | 0.2% Silver + 0.005% bleaching agent | 0.0025% Silver | 0.0025% Silver additive | 0.0025% Silver + 0.005% bleaching agent | 0.0025% Silver + 0.005% bleaching agent |
|---------------|------------|---------------|-----------------------|-------------|----------------------|---------------------------------|---------------------------------|----------------|-----------------------|---------------------------------|---------------------------------|
| Enterococcus faecalis | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 26 ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm |
| E. coli ATCC 11775 | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm |
| S. aureus | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm | 0 mm ± 0 mm |

Discussion

A silver-based antimicrobial textile coating product inhibited E. coli and S. aureus, including clinical isolates, at a dose of 1–2% in a 40°C domestic wash under clean conditions. A dose of 2% silver additive reduced microbial contamination of the inoculated textile (E. coli and S. aureus type and clinical isolates) by 7.14–8.08 of an eight log_{10} CFU inoculum and cross contamination to other fabric in the wash was prevented (Table 4).

Washing with 2% silver additive also deposits an antimicrobial coating on to the textile which achieved the British Standards Institute (2013) criteria for antimicrobial activity of treated textiles and is stable for at least one further wash at 40 or 73°C (Tables 6 and 7). The silver additive may therefore be useful for the prevention or reduction of healthcare worker uniform contamination during use and cross contamination of other textiles during laundering. In accordance, laundering with the silver-based product SilvaClean (1.3–1.4 mg kg^{-1} silver) significantly reduced the total aerobic, S. aureus and MRSA counts of hospital sheets and gowns before and after patient use, suggesting an antimicrobial coating was deposited on the textile (Openshaw et al. 2016). The antimicrobial activity of silver is well known and has been attributed to the perturbation of the bacterial cell membrane, protein denaturation and generation of reactive oxygen species (Kedziora et al. 2018).

In the clinical environment, microbial contamination of textiles is expected to mainly occur within organic material (Loveday et al. 2007) which can support the growth of micro-organisms on textiles (Mitchell et al. 2015) and interferes with their removal and from textiles during laundering (Bockmühl 2017; Nandy et al. 2019). Enterococcus sp. were significantly (P < 0.05) more resistant to thermal disinfection on cotton in the presence of artificial soil than without soil, with a more pronounced resistance occurring after drying in soiling for 24 h (Diab-Elschahawi et al. 2010). In the presence of interfering substances, the antimicrobial activity of 1% silver additive alone was significantly (P < 0.05) reduced against all test species (Table 3) and at a dose of 2% the antimicrobial activity was significantly (P < 0.05) reduced against E. coli type and clinical isolates (Table 4).
Table 3  $\text{Log}_{10}$ CFU reduction of *Escherichia coli* and *Staphylococcus aureus* type and clinical isolates* and cross contamination onto previously sterile textile during laundering with 1% w/v silver additive under clean or dirty conditions ($n = 4$; mean ± SE)

| Micro-organism                  | Water alone   | 1% Silver additive (clean conditions) | 1% Silver additive (dirty conditions) |
|---------------------------------|--------------|---------------------------------------|---------------------------------------|
|                                 | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination |
| *E. coli* type strain           | 2.21 ± 0.18  | 3.18 ± 0.18                           | 6.17 ± 0.25                          | 0.15 ± 0.15                           | 3.49 ± 0.72                          | 2.26 ± 0.45                           |
| *E. coli* clinical isolate      | 3.79 ± 0.20  | 3.43 ± 0.47                           | 6.93 ± 0.11                          | 0.27 ± 0.10                           | 2.70 ± 0.13                          | 4.22 ± 0.23                           |
| *S. aureus* type strain         | 4.25 ± 0.33  | 3.13 ± 0.40                           | 8.09 ± 0.00                          | 0.25 ± 0.17                           | 3.59 ± 0.46                          | 3.66 ± 0.23                           |
| *S. aureus* clinical isolate    | 3.81 ± 0.27  | 4.01 ± 0.11                           | 7.57 ± 0.24                          | 0.00 ± 0.00                           | 2.14 ± 0.36                          | 2.97 ± 0.22                           |

*An initial inoculum of 8 $\text{Log}_{10}$ CFU was used.

Table 4  $\text{Log}_{10}$ CFU reduction of *Escherichia coli* and *Staphylococcus aureus* type and clinical isolates* and cross contamination of *E. coli* and *S. aureus* type and clinical isolates onto previously sterile textile during laundering with 2% w/v silver additive under clean or dirty conditions ($n = 4$; mean ± SE)

| Micro-organism                  | Water alone   | 2% Silver additive (clean conditions) | 2% Silver additive (dirty conditions) |
|---------------------------------|--------------|---------------------------------------|---------------------------------------|
|                                 | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination |
| *E. coli* type strain           | 2.21 ± 0.18  | 3.18 ± 0.18                           | 7.98 ± 0.15                          | 0.00 ± 0.00                           | 3.09 ± 0.20                          | 3.26 ± 0.13                           |
| *E. coli* clinical isolate      | 3.79 ± 0.20  | 3.43 ± 0.47                           | 7.14 ± 0.44                          | 0.00 ± 0.00                           | 2.76 ± 0.22                          | 4.24 ± 0.42                           |
| *S. aureus* type strain         | 4.25 ± 0.33  | 3.13 ± 0.40                           | 8.08 ± 0.00                          | 0.00 ± 0.00                           | 7.57 ± 0.44                          | 0.45 ± 0.45                           |
| *S. aureus* clinical isolate    | 3.81 ± 0.27  | 4.01 ± 0.11                           | 7.99 ± 0.39                          | 0.00 ± 0.00                           | 6.39 ± 0.10                          | 1.37 ± 0.10                           |

*An initial inoculum of 8 $\text{Log}_{10}$ CFU was used.

Table 5  $\text{Log}_{10}$ CFU reduction of *Escherichia coli* and *Staphylococcus aureus* type and clinical isolates* and cross contamination of *E. coli* and *S. aureus* type and clinical isolates onto previously sterile textile during laundering with 2% w/v silver additive in the wash phase or 0.5% w/v silver additive in the rinse phase with biological detergent under dirty conditions* ($n = 4$; mean ± SE)

| Micro-organism                  | Biological detergent alone | 2% Silver additive (wash phase) + biological detergent | 0.5% Silver additive (rinse phase) + biological detergent |
|---------------------------------|----------------------------|-------------------------------------------------------|--------------------------------------------------------|
|                                 | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination | $\text{Log}_{10}$ reduction | $\text{Log}_{10}$ cross contamination |
| *E. coli* type strain           | 6.53 ± 0.48                | 0.92 ± 0.55                                           | 5.56 ± 0.20                          | 2.28 ± 0.38                           | 8.31 ± 0.43                          | 0.00 ± 0.00                           |
| *E. coli* clinical isolate      | 5.91 ± 0.54                | 1.36 ± 0.17                                           | 6.65 ± 0.54                          | 1.11 ± 0.54                           | 8.40 ± 0.00                          | 0.48 ± 0.48                           |
| *S. aureus* type strain         | 7.56 ± 0.00                | 0.00 ± 0.00                                           | 6.30 ± 0.83                          | 0.49 ± 0.28                           | 8.24 ± 0.43                          | 0.74 ± 0.47                           |
| *S. aureus* clinical isolate    | 5.75 ± 0.45                | 1.18 ± 0.61                                           | 6.32 ± 0.46                          | 0.82 ± 0.52                           | 7.98 ± 0.27                          | 1.42 ± 0.17                           |

*An initial inoculum of 8 $\text{Log}_{10}$ CFU was used.

*Doses of silver additive used in wash and rinse phases were both equivalent to 4.39% w/v dry weight of textile.

Biological laundry detergent contain enzymes to degrade proteins and surfactants to lift soiling from textiles (Bajpai and Tyagi 2007) and was thus investigated as a means to improve the antimicrobial activity of 2% silver additive in the wash phase under dirty conditions. The removal of *E. coli* type and clinical isolates was significantly ($P \leq 0.05$) improved from 2.76–3.09 $\text{Log}_{10}$ CFU by 2% Micro-Fresh 1911 alone (Table 4) to 5.56–6.65 $\text{Log}_{10}$ CFU in combination with biological detergent under dirty conditions (Table 5); this was not significantly different ($P \geq 0.05$) to washing with biological detergent alone, and the level of cross-contamination remained similar ($P \geq 0.05$) to washing without biological detergent (1.11–2.28 $\text{Log}_{10}$ CFU vs 3.26–4.24 $\text{Log}_{10}$ CFU). This suggests that biological detergent enhances the removal of organic soiling and bacteria from the...
textile, while the antimicrobial activity of silver additive remains limited. The antimicrobial interactions between silver additive and biological detergent in solution were indifferent (Table 1) suggesting that the biological detergent is unlikely to be reducing the antimicrobial activity of silver additive in the wash. Organic matter (6 g l⁻¹ bovine serum albumin) reduced the antimicrobial activity of 12.5 mg l⁻¹ ionic and biogenic silver, in support of the current findings; *E. coli* was reduced by approximately 7.5 log_{10} in 2 h by ionic or biogenic silver in water, compared to respective reductions of approximately two and four log_{10} under dirty conditions (Sintubin et al. 2011).

*Staphylococcus aureus* was more susceptible to silver additive alone and in combination with biological detergent than *E. coli* (Tables 4 and 5) in accordance with their MICs in solution (0.125–0.25% vs 0.5–1% respectively; Table 1). There are conflicting reports on the differences in susceptibility of *S. aureus* and *E. coli* to silver. In a similar pattern to the current study, silver nanoparticles extracted from *Aloe vera* and *Portulaca oleracea* were more antimicrobial against *S. aureus* (MICs 1.5–2.8 mg l⁻¹) than *E. coli* (MICs 3.4–4.35 mg l⁻¹) while those extracted from *Cynodon dactylon* were equally active against *S. aureus* and *E. coli* with MICs of 5–5.5 mg l⁻¹ (Abalkhil *et al.* 2017), suggesting that the antimicrobial activity of silver is dependent on its source and formulation.

Biological detergent alone was antimicrobial under dirty conditions, reducing *E. coli* and *S. aureus* by 5.75–7.56 log_{10} CFU and limiting cross contamination to 0.00–1.36 log_{10} CFU, with greatest activity against *S. aureus* type strain (Table 5). However, the addition of silver additive to the rinse phase added value over that of biological detergent by significantly (P ≤ 0.05) increasing the removal of *S. aureus* clinical isolate and *E. coli* type and clinical isolates (Table 5). Riley *et al.* (2017) also reported that *S. aureus* was more susceptible to biological detergent than *E. coli* in solution at 40°C for 15 min; *S. aureus* was reduced by 3.75 log_{10} CFU while *E. coli* was not significantly reduced (7.68 log_{10} CFU survival). In this study, both *S. aureus* and *E. coli* were reduced to a greater extent in a 40°C wash with biological detergent than reported by Riley *et al.* (2017), where approximately three log_{10} CFU *E. coli* and *S. aureus* were removed and deposited onto sterile polycotton under clean conditions. The current study highlights the importance of cleaning in the removal of micro-organisms from textiles and indicates that biological detergent is useful for the decontamination of laundry.

The removal of *S. aureus* and *E. coli* from polycotton by silver additive under dirty conditions was significantly (P ≤ 0.05) improved by employing it in the rinse phase of the laundering cycle following a wash phase with biological detergent (7.98–8.40 log_{10} CFU reduction; Table 5). Due to the high volume of water used in the rinse phase (16 l), excessive amounts of the silver additive would be required to achieve a 2% solution in the rinse water; this

### Table 6

Antimicrobial activity of polycotton laundered with silver additive (2% v/w), untreated polycotton or textile padded with 0.3% v/w Micro-Fresh 2611 according to the qualitative antimicrobial textile efficacy screening method (n = 4; mean ± SE)

| Micro-organism                  | 2% Silver additive | 0.3% Micro-Fresh 2611 | Untreated polycotton |
|--------------------------------|---------------------|------------------------|----------------------|
|                                | ZoI (mm)            |                        |                      |
| *Escherichia coli* type strain | 33.25 ± 0.38        | 31.63 ± 0.52           | 0.00 ± 0.00          |
| *E. coli* clinical isolate     | 33.77 ± 0.66        | 30.54 ± 0.24           | 0.00 ± 0.00          |
| *Staphylococcus aureus* type strain | 35.59 ± 0.46        | 31.71 ± 0.26           | 0.00 ± 0.00          |
| *S. aureus* clinical isolate  | 33.30 ± 0.55        | 31.68 ± 0.51           | 0.00 ± 0.00          |

### Table 7

Antibacterial activity of polycotton laundered with silver additive with or without biological detergent according to the qualitative antimicrobial textile efficacy test (n = 4)

| Micro-organism                  | 2% Silver additive (wash phase) | 0.3% Micro-Fresh 2611 | 2% Silver additive, washed again at 40°C | 2% Silver additive, washed again at 73°C | 0.5% Silver additive (rinse phase) with biological detergent |
|--------------------------------|---------------------------------|------------------------|------------------------------------------|------------------------------------------|----------------------------------------------------------|
|                                | F value                         | A value                | F value                                  | A value                                  | F value                                                  |
| *Escherichia coli* type strain | 1.5                             | 5.5                    | 1.5                                      | 5.7                                      | 4.3                                                      | 7.3                                                      | 4.3                                                      | 6.8                                                      | 5.4                                                      | 2.8                                                      |
| *E. coli* clinical isolate     | 4.3                             | 8.9                    | 4.3                                      | 8.9                                      | 4.5                                                      | 9.2                                                      | 4.5                                                      | 9.2                                                      | 5.4                                                      | 1.8                                                      |
| *Staphylococcus aureus* type strain | 0.4                             | 4.0                    | 0.4                                      | 4.5                                      | 0.3                                                      | 4.1                                                      | 0.3                                                      | 3.8                                                      | 5.5                                                      | 8.9                                                      |
| *S. aureus* clinical isolate  | 0.0                             | 4.1                    | 0.0                                      | 4.0                                      | 0.5                                                      | 4.1                                                      | 0.5                                                      | 4.6                                                      | 5.0                                                      | 8.9                                                      |

F value = log_{10} increase in positive control over 24 h incubation; A value = antibacterial activity value (log_{10} reduction over 24 h compared to the control). A ≥2 = significant antimicrobial activity; A ≥ 3 = strong antimicrobial activity (British Standards Institute 2013).
would not be commercially viable and was not necessary to achieve a reduction in *S. aureus* and *E. coli*, due to the removal of soiling prior to the rinse phase by the biological detergent. In order to draw comparisons between the data from the wash and rinse phases, the same concentration of silver additive to dry weight of textile (4.39%) was applied. Cross contamination was not significantly (\(P > 0.05\)) reduced when employed in the rinse phase compared to the wash phase with the exception of cross contamination of *E. coli* type strain which was reduced (\(P \leq 0.05\)) from 2.28 log_{10} CFU by silver additive in the wash phase to 0.00 log_{10} CFU in the rinse phase (Table 5). Polycotton treated with biological detergent and 0.5% silver additive in the rinse phase was significantly antimicrobial (A values 1.8–8.9; Table 7), suggesting that cross contamination remaining after the wash could be inhibited by the antimicrobial textile finish.

This study provides an insight into the most common low temperature washing parameters used by nurses (Riley et al. 2015). There are a number of factors that may affect laundering performance, including the type of detergent, temperature and washing machine programme used; the parameters employed within this study may not represent those used by all healthcare workers. Further research on a wide range of washing parameters would provide greater insight into the antimicrobial activity of the silver additive within domestic environments.

A limitation of this study is the effect of dilution and agitation alone on the removal of *S. aureus* and *E. coli* from contaminated textile swatches during the wash, which prevents the determination of the microbial reduction by the silver additive alone within the wash. This could be accounted for in future studies by enclosing contaminated textiles in materials that prevent bacterial cells from flowing away from the swatch while allowing the silver additive to come in to contact with the swatches.

This study demonstrates that the silver additive exhibits antimicrobial activity against *E. coli* and *S. aureus*, including clinical isolates, during low temperature domestic laundering in the presence and absence of soiling and as an antimicrobial textile finish. Silver-based formulations could be developed as dual-function laundry additives to sanitize healthcare laundry and reduce contamination during wear, in the wash phase against lightly soiled textiles or rinse phase against heavily soiled textiles. In this manner, silver additive may reduce microbial cross contamination of textiles in domestic and clinical settings.

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**Conflict of Interest**

No conflict of interest declared.

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