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ORIGINAL ARTICLE

Antimicrobial Efficacy of Trushield™ Gloves: an In vitro Experimentation-Based Review

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Abstract

Wearing gloves in hospital setting protect the public and healthcare workers from pathogen transmission and associated diseases. Recently, the concept of antimicrobial gloves has been emerged. In the present study, we evaluated and compared the antibacterial efficacy of Trushield™ gloves to that of sterile surgical latex gloves. Inoculums of differential microbes (gram-positive bacteria, gram-negative bacteria, yeast, and fungus) with the final concentration of 1×10^8 colony forming units (CFU)/mL were obtained. The tested glove swatches were inoculated with a microbial sample in a Petri plate, followed by incubation for different time periods (0, 5, 10, 30, 60, 240, and 480 minutes (min)). The sample was processed in duplicate. The microbial colonies were counted and log reduction was calculated. A p value of < 0.05 was set for statistical significance. At 0 min, both Trushield™ and latex gloves demonstrated the growth of differential microbes in the range of 5.6×10^5 to 7.8×10^5 CFU/mL. The growth of all test microorganisms was significantly impeded with the Trushield™ glove sample in a time-dependent manner ($p < 0.05$). Moreover, Trushield™ antimicrobial gloves achieved the value of zero CFU/mL in 1 hour (h), as compared to sterile latex surgical gloves ($p < 0.05$). Furthermore, Trushield™ antimicrobial gloves demonstrated antimicrobial efficacy to the extent of 2 log reduction at 5 min, 3 log reduction at 10 min, 4 log reduction at 30 min, and > 4 log reduction at 1 h and beyond. Trushield™ gloves could be a promising and user-friendly advancement in the healthcare sector by providing additional protection against pathogens.

Keywords Trushield™ gloves · Latex gloves · Antimicrobial efficacy · Microbe reduction

Introduction

Healthcare workers are at a high risk of pathogen-associated infections due to their daily and direct contact with the blood and other body fluids of the patients [1]. Thus, the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) have recommended alternate ways of gloving and hand hygiene to prevent such infections, especially for healthcare workers [2, 3]. In India, the National Health Mission implemented "Kayakalp" initiative, launched by the Ministry of Health and Family Welfare in 2015 to promote hygiene and infection control practices in public healthcare [4]. Wearing gloves is considered as a prima facie for the protection of patients as well as healthcare workers from potential microbial exposure and associated infections [1].

Gloves in hospital settings are widely used during medical examinations and surgical procedures to prevent cross-contamination. In an observational study, Pittet et al. (1999) found that healthcare workers with gloves (3 colony forming units per minute [CFU/min]) were less prone to bacterial contamination than those without gloves (16 CFU/min) [5]. Several other clinical studies in the literature confirmed the efficacy of gloves in protecting and reducing microbial transmission in healthcare; thus, the hospital sector should have an over-reliance on gloves [3, 6]. With increasing demand, an ample number of medical gloves synthesized from different polymers such as latex, polyvinyl chloride, nitrile rubber, and neoprene have been projected in the market. They may

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be available as either powdered or non-powdered and sterile or non-sterile. The gloves are powdered with talcum powder or corn starch for lubrication purposes [7]. In addition, medical gloves are also used in chemical and biochemical laboratories for protection against corrosives and surface contamination. The features of different types of medical gloves are listed in Table 1.

Medical or surgical gloves act as protective barriers to reduce the risk of microbial contamination from surface to humans and transmission of diseases viz. human-to-human infection. However, prolonged and inappropriate use of gloves may increase the possibility of microbial transmission, and cause adverse reactions, skin sensitivity, and dermatitis. Loveday et al. (2014) reported that healthcare workers touch surfaces or clinical equipment, patient notes, or objects with their gloved hands before performing patient care activities [8]. Another study by Wilson et al. (2017) revealed that cross-contamination occurred in 50% of care episodes in clinical routine due to inappropriate use of gloves [9]. In view of the different practices at healthcare facilities and patient workloads, it is difficult to comply with the WHO recommendations, i.e., avoid the extended use of gloves. Based on this scenario, a new concept of antibacterial gloves has emerged as the need of the hour. Proper hand hygiene has been shown to be the most effective procedure for prevention of cross infection, however, even in the best centers, the compliance rates are low [3].

The introduction of antimicrobial gloves in the healthcare sector is an approach to protect the public and healthcare workers from pathogen transmission and associated diseases [10]. The antimicrobial glove is designed with an advanced feature that inhibits the growth of different microbial species including bacteria, viruses, and fungi, using a suitable antimicrobial agent. In a study, Tyagi et al. (2000) used 40% iodination to produce antimicrobial natural rubber films [11]. Iodine is widely used as a disinfectant; it reacts with the functional groups and double bonds of biomolecules present in a microbial cell [11]. Babadi et al. (2016) described the use of metal ions and antiseptic dyes as antimicrobial agents in natural rubber blend films [12]. Another study by Lee et al. (2017) found that 92.5% of gram-positive bacteria, such as *Staphylococcus aureus* were killed on blending palm kernel oil (70%) and soybean oil (30%) in natural rubber [13]. Other cationic antibacterial agents include chlorhexidine gluconate (CHG) and polyhexamethylene biguanide (PHMB). The antimicrobial agent is incorporated into natural or synthetic rubber gloves by dispersion or coating techniques [14]. In the dispersion technique, the antimicrobial agent is prepared as a dispersion, followed by incorporating it into the latex compound. In contrast, in the coating technique, as the name suggests, the surface of the glove is coated with a thin layer of an antimicrobial agent by dipping. Another aspect is the protection of healthcare workers against contaminated fluids that may penetrate through gloves due to manufacturing defects or needlesticks. For instance, Modak et al. (1992) developed CHG gloves, where CHG acts as an instant release matrix on the inner surface and provides additional protection to healthcare workers against contaminated fluids [15]. In such conditions, antimicrobial gloves are highly recommended due to their protective efficacy against contaminated fluids entering through pinholes.

Based on this background, antimicrobial gloves namely Trushield™ gloves were developed by Healthium Medtech Ltd., India, to overcome the challenges of pathogen transmission. The development of these gloves is based on a unique antimicrobial technology that is highly effective and everlasting. The antimicrobial shield is formed by a long molecular chain of carbon atoms with positively charged nitrogen attached to a silica atom. This agent is covalently bonded to the material of the gloves. When a negatively charged pathogen comes in contact with the positively charged molecular chain bonded to the glove, the pathogen undergoes lysis. This mechanism provides continuous and everlasting defense against pathogens. This property does not deplete on washing the gloves and neither leach into the tissues. This glove is lightly powdered with dusting powder-corn starch for easy donning. Moreover, these

Table 1 Different types of medical gloves

Glove type	Natural/synthetic	Material	Features/strengths	Limitations
Latex	Natural	Rubber tree	-Excellent sensitivity -Good elasticity -Highly comfortable -Durable -Biodegradable	-Highly allergic -Little chemical protection
Nitrile	Synthetic	Acrylonitrile butadiene	-Latex-free -High strength -Puncture resistance	-Low elasticity -Less comfortable -Non-biodegradable
Vinyl	Synthetic	Polyvinyl chloride	-Latex-free -Highly durable	-Non-biodegradable
Neoprene	Synthetic	Chloroprene	-Latex-free -High strength	-Low elasticity -Less comfortable

antimicrobial gloves are cost-effective. In the present study, we evaluated and compared the antibacterial efficacy of Trushield™ gloves to that of sterile surgical gloves against gram-positive bacteria, gram-negative bacteria, yeast, and fungi. This study not only provides in vitro evidence of antimicrobial properties of Trushield™ gloves but also gives an overview of antimicrobial gloves, their underlying mechanism of action, advantages, and associated challenges in the healthcare system.

Materials and Methods

Microbial Samples and Reagents Used

A broad spectrum of test microorganisms including *Bacillus subtilis*, *Clostridium sporogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Aspergillus brasiliensis*, and *Candida albicans* were purchased from the American Type Culture Collection (ATCC), Virginia, USA. *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* (MRSA) were purchased from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Trushield™ antimicrobial gloves evaluated in this study was obtained from Healthium Medtech Ltd., Bengaluru, India. Soybean casein digest agar, Sabouraud dextrose agar, Letheen broth, and 0.9% saline were procured from Himedia Pvt. Ltd., India.

Regulatory Compliance of Microbiological Testing

The microbiological testing protocols were performed by Trustin Analytical Solutions Pvt. Ltd., Tamil Nadu, India. The study was conducted in accordance with the International Organization for Standardization (ISO) 22,196:2011.

Microbial Sample Preparation

Inoculums of a wide spectrum of drug-resistant bacteria (gram-positive and gram-negative bacteria) and yeast (*Candida albicans*) were prepared by harvesting their respective cultures using sterile 0.9% saline (pH 7.2). The fungal (*Aspergillus brasiliensis*) culture was harvested from slants using 0.9% saline (pH 7.2) with 0.5% polysorbate 80. The turbidity units were measured for the suspension/dilution of each culture, except for the fungus. The cultures were serially diluted to obtain a final inoculum concentration of 1×10^8 CFUs/mL in each suspension. The pour plate method was used to enumerate the microbial suspensions [16]. The list of tested microorganisms along with their media and laboratory culture conditions are given in Table 2.

Sample Processing

The tested material was cut from Trushield™ antimicrobial gloves and sterile latex surgical gloves, and the test swatches were placed in 90 mm sterile Petri plates. The test swatches were inoculated with 400 µL of prepared and diluted microbial sample. The inoculums were immediately covered with a 4×4 cm sterile thin film to prevent evaporation of the suspension. The samples were incubated for control (0 minutes (min)) and required experimental periods (5 min, 10 min, 30 min, 1 hour (h), 4 h, and 8 h), followed by adding 10 mL of neutralizer (Letheen broth). Further, tenfold serial dilutions were prepared in serial diluents (up to 10^{-6}), and samples were incubated at specified temperatures and periods, as given in Table 2. The samples were processed in duplicate. At the end of the incubation period, the microbial colonies were counted, and the arithmetic mean of the duplicate plates was calculated.

Table 2 List of tested microorganisms, their classification, and laboratory culture conditions

Microbial species	Classification	ATCC no	Culturing media	Incubation temperature	Incubation time
Staphylococcus aureus	Gram-positive bacteria	6538	SCDA	30–35 °C	24–48 h
Methicillin-resistant Staphylococcus aureus	Gram-positive bacteria	BAA-44	SCDA	32.5 °C	24–48 h
Clostridium sporogenes	Gram-positive bacteria	19,404	SCDA	30–35 °C	24–48 h
Bacillus subtilis	Gram-positive bacteria	6633	SCDA	30–35 °C	24–48 h
Escherichia coli	Gram-negative bacteria	8739	SCDA	30–35 °C	24–48 h
Pseudomonas aeruginosa	Gram-negative bacteria	9027	SCDA	30–35 °C	24–48 h
Salmonella typhimurium	Gram-negative bacteria	14,028	SCDA	30–35 °C	24–48 h
Candida albicans	Yeast	10,231	SDA	20–25 °C	48–72 h
Aspergillus brasiliensis	Fungus	16,404	SDA	20–25 °C	48–72 h

SCDA Soybean casein digest agar, *SDA* Sabouraud dextrose agar, *h* hours

Evaluation of Different Microbial Colonies at Different Time Points

The average of the common logarithm of the number of viable colonies recovered from the contact time of Trushield™ antimicrobial gloves (A) and sterile latex surgical gloves (B) was calculated. Further, microbial $\log_{10}$ reduction values were calculated by subtracting B from A at different time periods. In addition, the percentage reduction was calculated using the following formula:

$$\text{Percentage reduction } (\%) = ([B - A]/B) \times 100$$

A colonies obtained by Trushield™ antimicrobial gloves in CFU/mL
B colonies obtained by sterile latex surgical gloves in CFU/mL

Statistical Analysis

Data were analyzed to determine the statistical differences between the colonial growths from both types of gloves, i.e., Trushield™ antimicrobial gloves and sterile latex surgical gloves using GraphPad Prism (v.8). The data between the test samples were compared using *t*-test. Repeated measures analysis of variance (ANOVA) was used for comparing the antimicrobial efficacy at different time periods. A p value of <0.05 was considered as statistically significant.

Results

Different Microbial Growth at Different Time Periods

At 0 min, both the test samples, Trushield™ antimicrobial gloves and sterile latex surgical gloves, demonstrated growth of different microbes (gram-positive bacteria, gram-negative bacteria, yeast, and fungus) in the range of 5.6×10^5 to 7.8×10^5 CFU/mL. The growth of all test microorganisms was found to be significantly impeded with the Trushield™ antimicrobial glove sample in a time-dependent manner ($p < 0.05$). Moreover, Trushield™ antimicrobial gloves achieved the value of zero CFU/mL in 1 h, as compared to sterile latex surgical gloves ($p < 0.05$) (Fig. 1). However, the microbial growth increased in the case of sterile latex surgical gloves, which was statistically insignificant ($p > 0.05$).

Further, the average logarithm of viable microbes recovered from both test samples was calculated, which was in the range of 5.44 to 5.84 at 0 min. The Trushield™ antimicrobial glove sample exhibited log reductions in a wide range of microbes including gram-positive bacteria, gram-negative bacteria, yeast, and fungus in a time-dependent manner. However, insignificant changes in log values were observed with sterile latex surgical glove samples (Fig. 2).

In vitro Log Reduction and Percentage Reduction

Log reduction was calculated at different time periods of incubation, i.e., 0 min, 5 min, 10 min, 30 min, 1 h, 4 h, and 8 h. The log reduction value of <1 was found at zero min for all microbial samples. Trushield™ antimicrobial gloves demonstrated antimicrobial efficacy to the extent of 2 log reduction at 5 min, 3 log reduction at 10 min, 4 log reduction at 30 min, and >4 log reductions at 1 h and beyond (Table 3).

Lastly, the percentage reduction data showed $<90\%$ of antimicrobial efficiency of Trushield™ antimicrobial gloves at 0 min, which increased to 99.8% at 5 min, 99.97% at 10 min, 99.99% at 30 min, and $>99.99\%$ at 1 h, 4 h, and 8 h (Table 4), thus indicating the antimicrobial efficacy of Trushield™ antimicrobial gloves.

Discussion

Prolonged use of contaminated gloves and inadequate hand hygiene is considered the leading cause of healthcare-related infections and diseases [3, 17]. In addition, the recommended guidelines that mandate the use of gloves while handling the blood and body fluids of the patients are largely ignored [3, 17]. In a study, Thompson et al. (1997) stated that 75% of potential microbial transmissions occur due to failure to change or remove the gloves after interacting with patients [18]. The traditional approach of educating the public and healthcare workers is a futile process, and other approaches need to be considered. Thus, the emergence of antimicrobial technology in medical/surgical gloves appears as a prudent solution to this problem [11]. In the present study, we evaluated the antimicrobial efficacy of Trushield™ gloves compared to that of sterile latex gloves against gram-positive bacteria, gram-negative bacteria, fungi, and yeast.

Traditionally, antimicrobial gloves were needed to be activated by light or moisture for creating disinfecting micro atmospheres of chlorine dioxide (ClO_2). Such gloves can reduce *S. aureus* to 2.4-log after 45 min, which is clinically insufficient [19]. Therefore, new antimicrobial technology of clinical relevance needs to be created that also avoided the additional step of activation. The present study revealed that Trushield™ gloves provide a simple and significant method of preventing pathogen transmission. These gloves achieve $>99.9\%$ of antiseptic efficacy against *S. aureus* in 1 h and do not require any activation by light or moisture.

Further, inappropriate use of medical gloves not only risks the lives of patients but also of healthcare workers and

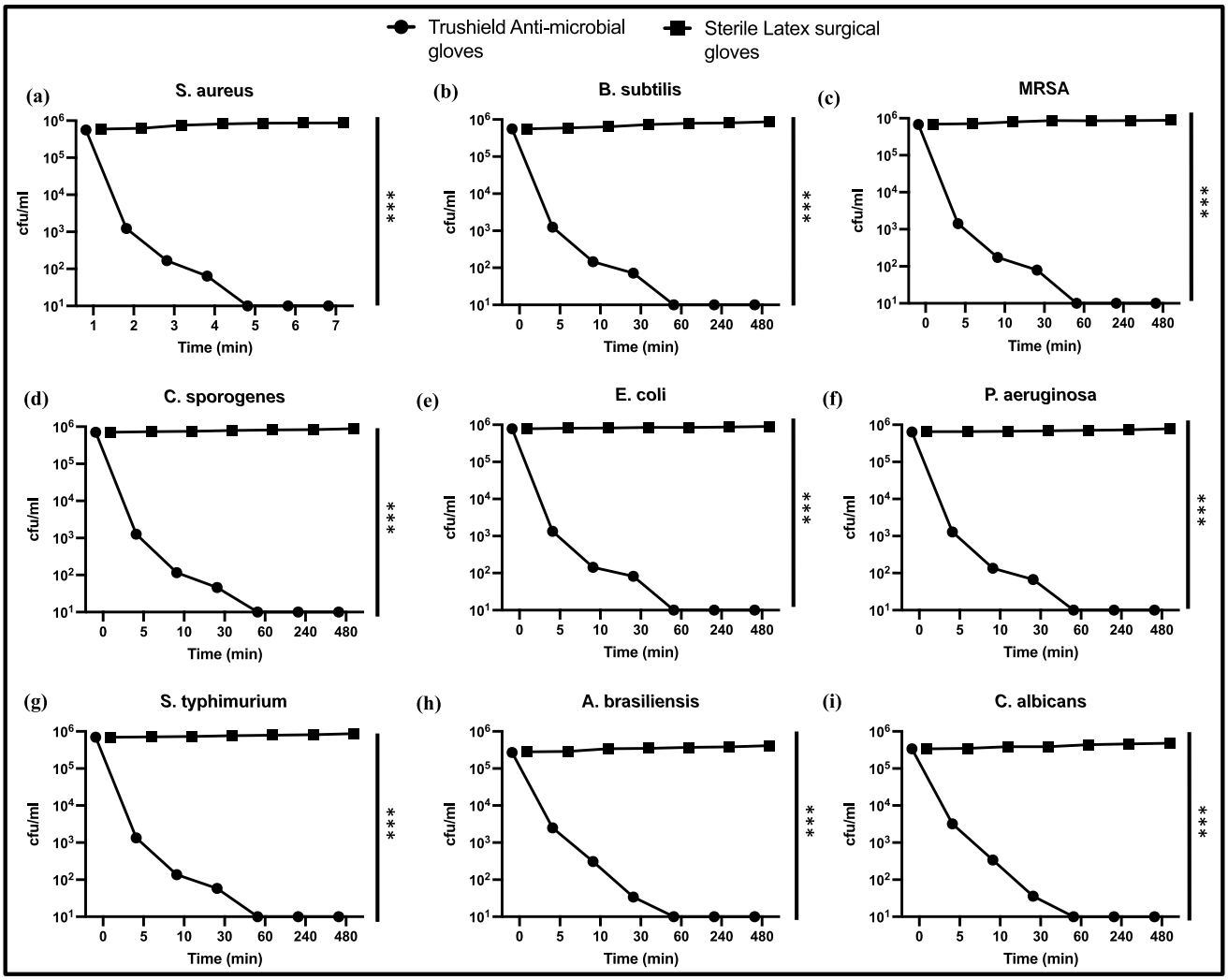

Fig. 1 Antimicrobial efficacy of Trushield™ antimicrobial gloves vs. sterile latex surgical gloves at different time periods. Line plots representing and comparing the antimicrobial efficacy of Trushield™ antimicrobial gloves vs. sterile latex surgical glove for colony formation by differential microbes (a) *Staphylococcus aureus*, (b) *Bacillus subtilis*, (c) *Methicillin-resistant Staphylococcus aureus* (MRSA), (d) *Clostridium sporogenes*, (e) *Escherichia coli*, (f) *Pseudomonas aeruginosa*, (g) *Salmonella typhimurium*, (h) *Aspergillus brasiliensis*, and (i) *Candida albicans,* expressed in CFU/mL at different time periods, 0 min, 5 min, 10 min, 30 min, 60 min (1 h), 240 min (4 h), and 480 min (8 h). ***$p < 0.001$

medical practitioners. A study was conducted in a French University hospital by Girou et al. (2004) who reported that microbial transmission occurred in 18% of all patient contacts due to prolonged use of gloves [20]. Removal or change of contaminated gloves after performing patient care activities is considered a good clinical practice that reduces the risk of contamination [3]. However, such a strategy is difficult to maintain in clinical settings; thus, the role of antimicrobial gloves came into the picture. Leitgeb et al. (2013) evaluated in vitro skin-to-surface recovery of four bacterial species (*S. aureus*, *Klebsiella oxytoca*, *E. coli*, *Enterococcus faecium*, and *Staphylococcus epidermidis*) in PHMB-coated nitrile gloves and nitrile gloves [21]. Another study by Bador et al. (2015) is an extension of the in vitro study in which they compared the efficacy of antibacterial nitrile gloves coated with PHMB and control nitrile gloves in an ICU setting [22]. Both the studies revealed that the use of antibacterial nitrile gloves was associated with significantly less bacterial contamination in 57% of clinical activities as compared with the use of control gloves. Our data are in accordance with these studies. We observed a significant decline in the growth of microbes with the use of Trushield™ antimicrobial gloves in a time-dependent manner as compared to the use of latex surgical gloves ($p < 0.05$). PHMB covers a broad spectrum of microbes and acts against gram-positive bacteria, gram-negative bacteria, and fungi; however, it is inefficient against *Pseudomonas* spp. [21, 22]. In contrast, Trushield™ antimicrobial gloves were found

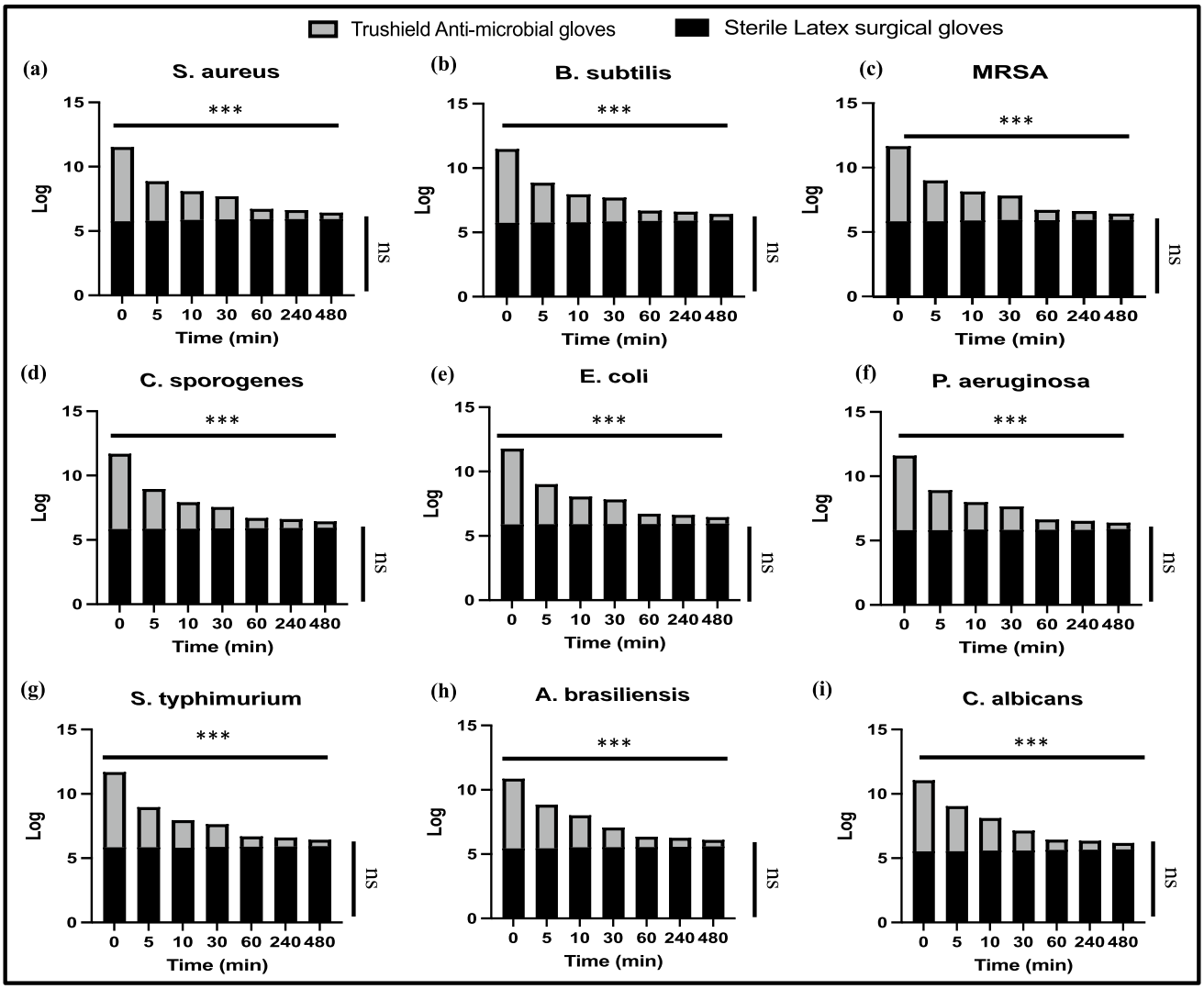

Fig. 2 Log representation of differential microbes with Trushield™ antimicrobial gloves vs. sterile latex surgical gloves in a time-dependent manner. Bar-grouped plots representing colony formation by different microbes in log values. (a) *Staphylococcus aureus*, (b) *Bacillus subtilis*, (c) *Methicillin-resistant Staphylococcus aureus* (MRSA), (d) *Clostridium sporogenes*, (e) *Escherichia coli*, (f) *Pseudomonas aeruginosa*, (g) *Salmonella typhimurium*, (h) *Aspergillus brasiliensis*, and (i) *Candida albicans*. Non-significant changes were observed in microbial colony formation with latex surgical gloves, represented with a vertical line. ns: non-significant; ***$p < 0.001$

Table 3 Log reduction at different time periods (heat map)

Microbial species	0 min	5 min	10 min	30 min	1 h	4 h	8 h
Staphylococcus aureus	<1	2.71	3.65	4.10	>4	>4	>4
Methicillin-resistant Staphylococcus aureus	<1	2.70	3.67	4.03	>4	>4	>4
Bacillus subtilis	<1	2.68	3.64	4.01	>4	>4	>4
Clostridium sporogenes	<1	2.76	3.81	4.23	>4	>4	>4
Escherichia coli	<1	2.78	3.76	4.01	>4	>4	>4
Pseudomonas aeruginosa	<1	2.70	3.69	4.01	>4	>4	>4
Salmonella typhimurium	<1	2.73	3.73	4.12	>4	>4	>4
Aspergillus brasiliensis	<1	2.07	3.04	4.01	>4	>4	>4
Candida albicans	<1	2.04	3.06	4.04	>4	>4	>4

min minutes, *h* hours

Table 4 Percentage reduction at different time periods (heat map)

Microbial species	0 min (%)	5 min (%)	10 min (%)	30 min (%)	1 h (%)	4 h (%)	8 h (%)
Staphylococcus aureus	<90	99.80	99.97	99.99	>99.99	>99.99	>99.99
Methicillin-resistant Staphylococcus aureus	<90	99.80	99.97	99.99	>99.99	>99.99	>99.99
Bacillus subtilis	<90	99.70	99.97	99.99	>99.99	>99.99	>99.99
Clostridium sporogenes	<90	99.80	99.98	99.99	>99.99	>99.99	>99.99
Escherichia coli	<90	99.83	99.98	99.99	>99.99	>99.99	>99.99
Pseudomonas aeruginosa	<90	99.80	99.97	99.99	>99.99	>99.99	>99.99
Salmonella typhimurium	<90	99.81	99.98	99.99	>99.99	>99.99	>99.99
Aspergillus brasiliensis	<90	99.10	99.90	99.99	>99.99	>99.99	>99.99
Candida albicans	<90	99.00	99.91	99.99	>99.99	>99.99	>99.99

min minutes, *h* hours

competent against all microbial species covering *S. aureus*, *MRSA*, *C. sporogenes*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, *C. albicans*, and *A. brasiliensis.* Our data suggest that Trushield™ gloves provide additional protection in healthcare settings as compared to other commercially available gloves.

Surgical latex gloves can be a source of microbial contamination and infection [23]. Ferreira et al. (2011) quantified microbial load on latex gloves in the beginning, middle, and end of the container opening procedures and observed the mean colony density ranging from 4.7 to 6.2. The predominant microorganisms were *Staphylococcus* spp. Further, the authors found no significant difference in the microbial load during the beginning or end of procedures [24]. In another study, Moore et al. (2013) evaluated MRSA transmission from a contaminated glove to a clean surface and found that latex gloves were associated with 0.01 to 19.5% of MRSA transmissions in controlled conditions. The authors further observed that the MRSA transmission was significantly more in contaminated conditions when bacteria were suspended in blood, ranging from 8 to 50.5% [25]. Both the studies are in favor of our data. In our study, microbial colonies were found to be increasingly growing with sterile latex gloves in the range of 5.6×10^5 to 7.8×10^5 CFU/mL, which might remain a major source of cross-contamination.

Furthermore, Trushield™ antimicrobial gloves significantly impeded the bacterial growth in a time-dependent manner ($p<0.05$) and achieved the value of zero CFU/mL from 5.6×10^5 to 7.8×10^5 CFU/mL in 1 h. The efficacy of these gloves was checked until 8 h and we observed zero CFU/mL of microbial growth during this period. Thus, the results obtained from this study indicate that Trushield™ gloves are highly efficient in significantly reducing microbes in a short period of time, i.e., 5 min and erode > 99.99% of microbes in 1 h.

Lastly, log reduction was calculated at different time periods of incubation, i.e., 0 min, 5 min, 10 min, 30 min, 1 h, 4 h, and 8 h. Trushield™ antimicrobial gloves demonstrated antimicrobial efficacy to the extent of 2 log reduction with 99.8% percentage reduction at 5 min, 3 log reduction with 99.97% percentage reduction at 10 min, 4 log reduction with 99.99% percentage reduction at 30 min, and > 4 log reduction with > 99.99% percentage reduction at 1 h and beyond until 8 h. Suchomel et al. (2018) measured the magnitude of antibacterial suppression with chlorhexidine-coated antimicrobial surgical gloves over a period of 3 h and observed a log reduction of 2.67, which is considerably less than the value obtained with Trushield™ antibacterial gloves [11]. Hence, Trushield™ gloves can be considered valuable in the healthcare setting as an alternate for the prevention of pathogen transmission.

This study has few limitations. The study provides only in vitro evidence of the effective reduction of microbes by inoculating microbial suspension in a Petri plate containing a patch of the glove. The gloves have not been tested in surgical procedures, which is another major limitation of our study. Further in vivo or clinical studies are required to determine the antimicrobial efficacy of Trushield™ gloves in reducing microbial contamination or transmission.

Conclusion

In conclusion, the replacement of commercially available sterile surgical gloves with antimicrobial medical gloves can be an efficient strategy to prevent or reduce cross-contamination and transmission of micro-organisms in hospital settings [26]. Based on our results, Trushield™ gloves may be a promising and user-friendly advancement in the healthcare sector globally by providing additional protection against pathogens.

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Declarations

Conflict of Interest Manu Shankar, Shahzad Marghoob Ali, and Mahesh Gupta have not received any financial aid from Healthium Medtech Limited. Ashok Kumar Moharana and Deepak TS are full time employees of Healthium Medtech Limited, who are the manufacturers of Trushield™ Antimicrobial Gloves.

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