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Longitudinal Study on Antimicrobial Performance of a Polyhexamethylene Biguanide (PHMB) Treated Textile Fabric in Hospital Environment

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Abstract: Healthcare workers in the hospital environment can be exposed to the risk of infection and body fluids such as saliva, bacterial contamination, oral bacteria, etc. directly or indirectly. These bio-contaminants, when stick on the hospital linens and clothing, grow substantially as conventional textile products provide a favorable media for bacterial and viral growth which add risk of transmitting infectious disease in hospital environment. Textile with durable antimicrobial property prevents microbial colonization on its surface and helps containing the spread of pathogens. This longitudinal study aims to investigate the antimicrobial performance of PHMB-treated healthcare uniform upon prolonged usage and repetitive laundries in hospital. The PHMB-treated healthcare uniform displayed non-specific antimicrobial property and remains efficient (>99% against *S. aureus* and *K. pneumoniae*) after deploying to use for 5 months. With the fact that no antimicrobial resistance was reported towards PHMB, the presented PHMB-treated uniform may reduce infection in hospital settings by minimizing the acquisition, retention, and transmission of infectious diseases on textile products.

Keywords: PHMB; Antimicrobial clothing; Antimicrobial medical textile; Infection control; Contaminants; Infection transmission vehicles; Disinfection; Coronavirus

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1. Introduction

Infectious diseases pose a significant threat in hospital environments. Pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), *Clostridium difficile*, norovirus, coronavirus, and multidrug-resistant gram-negative rods, are known to survive on a dry surface for extended periods and are difficult to eliminate by simple cleaning and disinfection procedures [1]. Contaminated surfaces contributed a significant role among hospital environments in transmitting healthcare-associated infections (HCAI) and were evaluated through modelling simulation [2], microbiologic studies [3,4], observational studies [5–8], and intervention studies [9–13]. In particular, literature reported the plausible mechanisms of transferring HCAI-causing microorganisms from hospital textiles onto skin and other surfaces [14]. Durable hospital-grade antimicrobial textiles could play a crucial part in infection control. It provides an environment that discourage the growth of microorganisms and suppresses microbial colonization. As a result, applying antimicrobial textiles can potentially remove one of the

environmental sources of microorganisms. There are numerous antimicrobial treatment methods for textile reported [15], however most of them show limited biocidal efficacy. To make the matter worst, their antimicrobial properties deteriorate upon abrasions from daily use and repetitive hospital-grade laundries [16]. As a result, they are not ideal for use in the hospital environment.

Polyhexamethylene biguanide, also known as PHMB, is a polymer containing multiple cationic biguanide repeating units separated by hydrophobic hexamethylene hydrocarbon chains (Figure 1). When present at a low concentration (<0.3% wt/wt) [17], PHMB is biocidal and widely applied in various products, such as wet wipes [18], cleansing solutions of contact lenses [19], pharmaceutical hygiene products for wound and burns treatment [20,21], and water treatment [22]. It is generally accepted that the positively charged biguanides bind to the negatively charged phosphate group of the bacterial cell wall or virus envelope. This interaction breaks the membrane integrity, leading to cell lysis and subsequent cell death [23]. A recent molecular dynamic study on PHMB revealed another antimicrobial mechanism where the PHMB polymer translocates across the membrane bilayer, binds to the DNA, and potentially blocks the DNA replication and repair pathway [24]. Regardless of the antimicrobial mechanisms, PHMB demonstrated non-specific biocidal property on microorganisms, and no bacterial resistance against PHMB has ever been reported [25]. Attributed to the electrostatic interaction and hydrogen bonding, PHMB shows a strong binding affinity toward cotton fiber [26]. Utilizing the fact that PHMB is a broad-spectrum biocide and the binding mechanism with cotton, Kan et al. and Avalon SteriTech have demonstrated a practical pad-dry-cure treatment method to adhere PHMB on cotton-based fabrics [27]. To our knowledge, the application of cotton-based antimicrobial uniforms in hospital environment for infection control is rarely investigated. In this context, a longitudinal study on the antimicrobial performance of PHMB-treated cotton-based healthcare uniform over a 5-month period was presented.

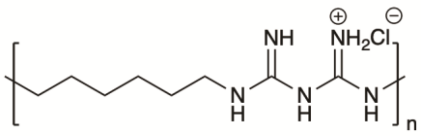

Figure 1. Structure of PHMB.

2. Materials and Methods

2.1. Antimicrobial Textile Fabric

Cotton/polyester blended fabric was used for making the textile fabric used in hospital environment. The fabric consisted of 35% cotton and 65% polyester with weight of 175 g/m^2. The antimicrobial recipe consists of different ingredients: (i) polyhexamethylene biguanide (PHMB) (15% (weight/volume (*w*/*v*); original purity at least 20%), (ii) poly(ethylene glycol) (PEG) (5% (*w*/*v*); molecular weight: 400, purity > 99%) and (iii) binder (8% (*w*/*v*); (polyurethane based, polyurethane content at least 20%). The antimicrobial formulation is applied to the fabric via pad-dry-cure process in which padding was wet pick-up of 80%; drying was 90 °C for 5 min and curing was 130 °C for 3 min. This process was applied to the fabric form.

2.2. Antibacterial Property

The antibacterial property of the PHMB-treated textile fabric was tested according to the standard method AATCC TM100-2019 using *S. aureus* (ATCC 6538) and *K. pneumoniae* (ATCC 4352). The antibacterial rate of the PHMB-treated textile fabric was calculated using the formula:

$$\textit{Antibacterial Rate}\ (\%) = [(A - B) / A] \times 100\% \quad (1)$$

where A is the CFU/mL for sample at 0 h and B is the CFU/mL for sample after a contact time for 24 h.

2.3. Antiviral Property

The antiviral property of the treated textile was evaluated according to the standard ISO 18184:2019 on H1N1 and Human coronavirus 229E.

The antiviral activity rate was determined by

$$\textit{Antiviral Activity Rate}\ (\%) = [(V_a - V_c) / V_a] \times 100\% \quad (2)$$

where V_a is the common average of infectivity titer value immediate after inoculation of the control specimen, and V_c is the common average of infectivity titer value after 2 h contacting with the treated textile fabric.

2.4. Longitudinal Study Design

The study was conducted in an Accident & Emergency Department of a hospital in Hong Kong from December 2021 to May 2022 (with research ethical approval from: (i) The Hong Kong Polytechnic University (ref: HSEARS20200629003-02) and (ii) Hospital Authority, Hong Kong (ref: KC/KE-21-0154/ER-3)). Before the start of the longitudinal study, the antibacterial property of the unused PHMB-treated textile fabric was verified, as baseline, by testing against a Gram-positive bacteria, *S. aureus* (ATCC 6538), and a Gram-negative bacteria, *K. pneumoniae* (ATCC 4352). The antiviral property was verified, as baseline, by testing against an envelope virus H1N1, and human coronavirus 229E. It was found that the unused PHMB-treated textile fabric inactivates >99.99% of both *S. aureus* and *K. pneumoniae* (Table 1). In terms of the antiviral property, it inactivates >99% of Human coronavirus 229E and >99.99% of H1N1 (Table 2). The PHMB-treated textile fabric was then manufactured into antimicrobial healthcare uniform set as shown in Figure 2.

Figure 2. PHMB-treated antimicrobial uniform set for hospital.

Table 1. Antibacterial performance (AATCC TM100-2019) of unused PHMB-treated cotton/polyester (35/65) textile fabric for healthcare uniform manufacturing.

Sample	Test Organisms	% Reduction
Control textile fabric	*S. aureus*	N.A.
PHMB-treated textile fabric		>99.99%
Control textile fabric	*K. pneumoniae*	N.A.
PHMB-treated textile fabric		>99.99%

Table 2. Antiviral performance (ISO 18184:2019) of unused PHMB-treated cotton/polyester (35/65) textile fabric for healthcare uniform manufacturing.

Sample	Test Virus	Antiviral Activity Rate (%)
Control textile fabric	H1N1	N.A.
PHMB-treated textile fabric		>99.99%
Control textile fabric	Human coronavirus 229E	N.A.
PHMB-treated textile fabric		>99%

On the first day of the field study, healthcare worker in the Accident & Emergency Department who enrolled in the study were provided with a set of unused PHMB-treated antimicrobial uniform. After the shift work, the uniforms were washed according to the existing washing practice in the hospital laundry, i.e., at least 75°C for 5 min with the presence of hydrogen peroxide and detergent. Subsequently, the uniform sets were recycled for use. Three set of clean uniforms were randomly collected from the hospital laundry on day 30, day 60, day 90, day 120 and day 150 which represent continuous usage for 1 to 5 months, respectively. Each set of collected uniform (including both jacket and trousers) was tested against bacteria, *S. aureus* and *K. pneumoniae*, and against an indicative virus, Human coronavirus 229E, to evaluate if the antimicrobial property of the PHMB-treated uniform show any changes upon continuous usage.

3. Results and Discussion

Overall, 74 healthcare workers from the Accident & Emergency department of the hospital participated in the longitudinal study. The antibacterial and antiviral properties of the used PHMB-treated healthcare uniform was evaluated (**Table 3**).

Table 3. Summary of antimicrobial performance on used PHMB-treated healthcare uniforms upon different usage periods in a hospital.

Conditions	Antibacterial performance		Antiviral performance against Human coronavirus 229E
	S. aureus	*K. pneumoniae*	
Untreated uniform	N.A.	N.A.	N.A.
Unused uniform	>99%	>99%	>99%
30-days usage	>99%	>99%	>95%
60-days usage	>99%	>99%	>95%
90-days usage	>99%	>99%	>95%
120-days usage	>99%	>99%	>95%
150-days usage	>99%	>99%	>95%

The reduction value of the unused uniform were >99% on all test microorganisms, which demonstrated the superior antimicrobial performance of the PHMB-treated textile fabric which ensure the validity of the testing. It was found that the antibacterial and antiviral efficacy of the jacket did not show any deterioration after over 30 days and remained >99%. Furthermore, the antiviral property of the uniform fabric did not show any significant deterioration after 150 days, with up to 95% inactivation rate on coronavirus. Excellent antibacterial and antiviral properties were retained. They were likely attributed

to the broad biocidal spectrum of PHMB and the strong affinity of PHMB on cotton fabrics using the specific antimicrobial treatment method and formulations. It is well established that infectious pathogens remains active on textile for a pro-longed period, for example SARS-CoV-2 can survive on textile for 48 h [28]. Based on the experimental results in this study of uniform fabric treated with PHMB after 150 days of being deployed for usage, it is believed that application of such antimicrobial textile fabric effectively removes biocontamination sources in high-risk area such as hospital environment. The wide adapability of the presented antimicrobial technology on textile fabric was also tested on multiple cotton-based materials [27,29]. Therefore it is expected that the technology and treatment method is transferrable to other hospital linens, including bedsheets, pillowcases, and curtains for infection control in hospital environment.

With the potential widespread use of PHMB-treated antimicrobial textile fabrics in the hospital environment for a prolonged period, the risk of microbial resistance development to PHMB should be considered. In terms of mechanistic consideration, the non-specific inhibition of cell metabolism by PHMB makes resistance development highly unlikely. Indeed, no bacterial resistance to PHMB has ever been reported from clinical samples [20,25]. Nevertheless, it is recommended to develop a continuous monitoring program on the use of PHMB-treated antimicrobial textiles in hospitals.

4. Conclusions

Hospital linens are frequently contaminated with pathogenic microorganisms. The present study demonstrated an antimicrobial TC textile (cotton 35% / polyester 65%) developed by pad-dry-cure treatment with PHMB is a practical means to develop durable medical-grade antimicrobial uniform for hospital usage to reduce a source of microbial colonization and hence contain the transmission of pathogens, including SARS-CoV-2, in high-risk places.

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