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ORIGINAL ARTICLE

Basic Study

In vitro study on the transmission of multidrug-resistant bacteria from textiles to pig skin

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Abstract

BACKGROUND

The survival of microorganisms on textiles and specifically on healthcare professionals' (HCP) attire has been demonstrated in several studies. The ability of microorganisms to adhere and remain on textiles for up to hours or days raises questions as to their possible role in transmission from textile to skin *via* HCP to patients.

AIM

To evaluate the presence, survival and transmission of different multidrug-resistant bacteria (MDRB) from HCP attire onto skin.

METHODS

Three MDRB [methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant *Enterococcus faecium* (VRE); carbapenem-resistant *Klebsiella pneumoniae*, CRKP)] were inoculated on textiles from scrubs (60% cotton-40% polyester) and

white coat (100% cotton) at concentrations of 10^8 colony-forming units (CFU), 10^5 CFU, and 10^3 CFU per mL. The inoculation of swatches was divided in time intervals of 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h. At the end of each period, textiles were imprinted onto pig skins and each skin square was inverted onto three different selective chromogenic media. Growth from the pig skin squares was recorded for the 3 MDRB at the three above concentrations, for the whole length of the 6-h experiment.

RESULTS

MRSA was recovered from pig skins at all concentrations for the whole duration of the 6-h study. VRE was recovered from the concentration of 10^8 CFU/mL for 6 h and from 10^5 CFU/mL for up to 3 h, while showing no growth at 10^3 CFU/mL. CRKP was recovered from 10^8 CFU/mL for 6 h, up to 30 min from 10^5 CFU/mL and for 1 min from the concentration of 10^3 CFU/mL.

CONCLUSION

Evidence from the current study shows that MRSA can persist on textiles and transmit to skin for 6 h even at low concentrations. The fact that all MDRB can be sustained and transferred to skin even at lower concentrations, supports that textiles are implicated as vectors of bacterial spread.

Key Words: Textiles; Attire; Multidrug-resistant bacteria; Methicillin-resistant *Staphylococcus aureus*; Vancomycin-resistant *Enterococcus faecium*; Extended-spectrum b-lactamase; Pig skin; Skin; Transmission

Core Tip: The current study aimed to evaluate the transmission of multidrug-resistant bacteria (MDRB) from attire (scrubs, white coats) onto skin. Three MDRB types [methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, carbapenem-resistant *Klebsiella pneumoniae*] were inoculated on textiles over various time intervals for up to 6 h and then imprinted onto pig skin. All MDRB were able to be sustained and transferred to skin, but at different concentrations and time. MRSA had the longest presence on textile and highest transmission potential even at lower concentrations. Evidence suggests that textiles can be implicated as vectors of MDRB spread in the healthcare setting.

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INTRODUCTION

It is well established that textiles can carry micro-organisms, a fact with raises concerns for their ability to transmit them either onto skin or to other textiles[1]. Previous studies and systematic reviews have demonstrated the ability of pathogens, including multidrug-resistant bacteria (MDRB), to survive on healthcare professionals' (HCP) attire, devices and surfaces in hospitals and long-term care facilities, thus raising the need to study their potential to contribute to transmission[1-4].

Cyprus is considered a high-prevalence country for MDRB. In fact, in healthcare-associated infections in Cyprus hospitals in 2020, methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) was detected in 49.1% of invasive infections by *S. aureus*, vancomycin-resistant *Enterococcus faecium* (*E. faecium*) (VRE) accounted for 44.1% of enterococci, and isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) exhibited multidrug resistance in 18.2% and carbapenem resistance in 19.8%[5]. Further to this, in a previous study, our group demonstrated the presence of several different MDRB on HCP uniforms in hospitals and long-term care facilities in Cyprus, including MRSA, VRE, extended-spectrum b-lactamase (ESBL)-producing bacteria and carbapenem-resistant bacteria[1]. These findings suggest that MDRB may be transmitted in different ways within healthcare settings, thus raising the need to identify additional areas for targeted interventions and improvement.

A demonstrable means to evaluate the role of attire and textiles in the transmission cycle, is by studying the degree of transmission of micro-organisms from fabrics to skin. To achieve this, it is necessary to use an alternative to human skin with similar properties, such as pig skin, in order to allow accurate *in-vitro* experimentation[6]. The similarities between human and pig skin lie in the structure of epidermis and thickness ratios between dermis and epidermis, as well as in hair follicle and blood vessel patterns. In contrast to the skin of other animals used in laboratory studies, the dermal collagen and elastic content of the pig skin are more similar to the skin of humans. Furthermore, Schmook *et al*[7] demonstrated in an *in-vitro* study of percutaneous absorption, that in the absence of human tissue, pig skin was the most suitable model.

Based on current literature, including experimental and observational studies, we hypothesize that MDRB can carry the ability to survive on textiles to a considerable extent to be isolated and to transmit onto skin. Our aim was to evaluate the survival potential of different MDRB on different types of textiles and to evaluate their potential for transmission onto skin, using representative strains such as MRSA, VRE and carbapenem-resistant *K. pneumoniae* (CRKP).

MATERIALS AND METHODS

Aims

The aim of the current study was to determine the viability of MDRB over a 6-h period, which mimics the usual duration of a hospital shift, on two types of textiles and to evaluate whether these inoculated textiles can colonize pig skin using three MDRB (MRSA, VRE and CRKP) at different concentrations. Study approval was not required as it did not involve human subjects or animals. The strains were retrieved from a previous study that was approved by the Cyprus National Bioethics Committee (decision number "ΕΕΒΚ ΕΠ 2018.01.155").

Materials

Fresh MRSA, VRE and CRKP cultures were grown from previously isolated organisms from HCP uniforms from a previous study, that were stored in -20 °C. After 24 h of incubation, the organisms were prepared at 0.5 MacFarland standard each. Their precise concentrations were calculated: 1.18×10^8 CFU/mL MRSA, 1.6×10^8 CFU/mL for *E. faecium*, and 1.14×10^8 CFU/mL for *K. pneumoniae*. All 3 organisms were serially diluted from 10^8 CFU down to 10^2 CFU. The concentrations of 10^8, 10^5, and 10^3 CFU per mL were used during the process.

Textile swatches were cut in squares of 1.5 cm × 1.5 cm and sterilised in 100 °C in dry oven for 2 h. The swatches were taken from uniforms and scrubs used in healthcare settings in order to mimic txextiles used in real-life conditions, with a composition of 60% cotton and 40% polyester (T1, taken from a used scrub uniform of a nurse, who had worn it for 6 mo), and 100% cotton (T2, taken from a white coat used by a physician for 6 mo). Aluminium foil squares of 1.5 cm × 1.5 cm were cut and heat sterilised in the same manner as the cloth swathes. These would be used as a barrier when pressure would be applied on cloth or skin.

Fresh pig skin (commercially retrieved 24 h after slaughtering), cleared from most of the fat under the dermis, was scrubbed and washed with chlorhexidine soap and then with alcohol. The cleaned skin was then dried in examination paper and kept covered to avoid drying. When the experiment was ready to begin, square pieces of 1.5 cm × 1.5 cm of skin were cut using sterilized blades. Negative control skin samples were incubated on the 3 chromogenic media to ensure the absence of the 3 microorganisms used. The specialized chromogenic media used were CHROMO agar, MRSA medium, chromo VRE, and chromo ESBL.

Experimental design

The current experimental study was designed in duration and environmental conditions that mimic the working conditions within a 6-h HCP shift. Temperature of the laboratory room was kept constant at 22.9-23.5 °C and humidity of 49%-53% and the experiment was conducted on the bench.

Each microbial concentration had 10 stations allocated at time intervals of 1 min, 5 min, 15 min, 30 min, 1 h, and then every hour for a total of 6 h and at three different concentrations. Each station had in place: 2 cloth swatches (T1 & T2), 2 respective skin squares (S1 & S2) and chromogenic plates, for each of the 3 dilutions of the microorganisms (Figure 1).

Firstly, 2 cloth swatches in each station were inoculated with 50 μL of each dilution in each column. The inoculation started from 6 h up to 1 min, guided by stopwatches. At the end of each time slot, each cloth swatch was placed on top of the skin square. The cloth was applied onto skin with a pressure of 100 Gr (friction asset) for 5 s and foil was placed over it for protection. Afterwards and following the same technique, each skin square was pressed onto the chromogenic agar. The plates were then incubated at 37 °C. After 24 h, the incubated plates were checked and incubated for an additional 24 h. When the 48-h incubation period ended, the chromogenic plates were inspected, and the presence of growth was recorded. We did not perform a concentration count at each station; the results indicated presence or not. Thus, the experiment was performed based on the assumption that even a small amount of bacteria could be transmitted and possibly cause infection.

RESULTS

Over a period of 6 h and at different time points, a total of 3 MDRB (MRSA, VRE, CRKP) were inoculated in 3 different concentrations (10^8 CFU/mL, 10^5 CFU/mL, 10^3 CFU/mL) on 2 different cloth textiles (T1, T2) and then each cloth was applied on a pig skin square (S1, S2 respectively). The skin inoculates were then cultured on special medium for 48 h and MDRB growth was recorded.

All findings demonstrating the recovery of the 3 MDRB from skin and textiles are presented in Table 1. Recovery times for all 3 MDRB were the same in both skin swatches S1 and S2 that were in contact with T1 (60% cotton and 40% polyester) and T2 (100% cotton), respectively. Overall, no differences were observed in terms of growth for each MDRB between the two types of textiles. MRSA exhibited the highest recovery. In specific, recovery of MRSA was successful at all time intervals and for all 3 concentrations (10^8 CFU/mL, 10^5 CFU/mL and 10^3 CFU/mL). MRSA remained detectable and could be transmitted throughout the 6-h experiment duration. VRE was recovered from the highest concentration of

Table 1 Growth indicated in skin swatches S1 from textile T1 (cotton-polyester cloth) and S2 from T2 (100% cotton cloth), when imprinted on chromogenic agars at three concentrations

Time	MRSA (CFU/mL)						VRE (CFU/mL)						CRKP (CFU/mL)					
	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)	S1 (10^8)	S2 (10^8)	S1 (10^5)	S2 (10^5)	S1 (10^3)	S2 (10^3)
1 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	G	G
5 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
15 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
30 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	G	G	/	/
60 min	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
2 h	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
3 h	G	G	G	G	G	G	G	G	G	G	/	/	G	G	/	/	/	/
4 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/
5 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/
6 h	G	G	G	G	G	G	G	G	/	/	/	/	G	G	/	/	/	/

CFU: Colony-forming units; CRKP: Carbapenem-resistant *Klebsiella pneumoniae*; G: Growth; MRSA: Methicillin-resistant *Staphylococcus aureus;* VRE: Vancomycin-resistant *Enterococcus faecium*.

10^8 CFU/mL for the whole duration of the 6-h period and for up to 3 h from the 10^5 CFU/mL concentration. No recovery of VRE was recorded from the lowest concentration of 10^3 CFU/mL. CRKP was also recovered from the highest concentration of 10^8 CFU/mL for the total duration of 6 h (duration of the experiment) and for 30 min from the second highest concentration (10^5 CFU/mL), whereas it was recovered for only up to 1 min from the lowest concentration of 10^3 CFU/mL.

Conclusively, our results support the sustainability of MRSA for the maximum of the duration of the study in all concentrations. The other gram-positive coccus (VRE) also remained for the whole duration of the study but only in the highest concentration, whereas VRE was not isolated for the lowest concentration. In contrast, the gram-negative bacterium (CRKP) remained for less time in the concentration of 10^5 CFU/mL and for only 1 min at the lowest concentration.

DISCUSSION

In our study, involving the transfer and presence of MDRB as a result of contact between textiles and pig skin, MRSA exhibited the longest persistence out of the 3 studied MDRB and over the duration of 6 h of the experiment. VRE and CRKP were both detected at the highest concentration of 10^5 CFU/mL, for up to 3 h and 30 min, respectively. The presence of MDRB, as recorded on both textile types that were used in the study, was also confirmed on pig skin, which provides evidence of potential for transfer of bacteria and MDRB onto skin from contaminated textiles and furthermore, suggesting this as a transmission mode to patients. The study results show that gram-positive cocci, such as staphylococci and enterococci, are more resilient on textiles and in the environment over time, whereas CRKP also showed prolonged presence at higher concentrations.

To our knowledge, only few previous studies have evaluated the transferability of bacteria onto skin. Butler *et al*[8] conducted a study on the transfer of bacteria onto pigskin by use of white coats. Specifically, MRSA, VRE and *Acinetobacter baumannii* (*A. baumannii*) (reported as pan-resistant) were inoculated on cloth swatches and rubbed on sanitized pigskin. All 3 MDRB exhibited ability to transfer from cloth to pig skin at time intervals of 1, 5 and 30 min, whereas *A. baumannii* was transferred up to a dilution of 1:1000. Desai *et al*[9] also confirmed sustainability and transmission of MRSA from cotton towels and bedsheets to pig skin for long periods reaching up to 14 d, whereas Sattar *et al*[10] reported transfer of MRSA from textiles (cotton and polycotton) to other textiles and to finger skin. The transfer was fivefold higher when the textile was moist and when there was friction. Varshney *et al*[11] in a study transfer between textiles of *Acinetobacter calcoaceticus, Escherichia coli* (*E. coli*) and *S. aureus,* also reported that cell transfer varied between 5%-61% when friction was applied compared to non-friction, which suggested the importance of the role of rubbing between same textiles. Malnick *et al*[12] examined the transfer of bacteria between fabric and surrogate skin taking into consideration the effect of surface energy and surface roughness of fabrics, while using *E. coli* and *S. aureus* against 100% cotton, 100% polyester and a 50-50 blend of both. Their conclusion was that the 100% polyester attracted the highest number of bacteria.

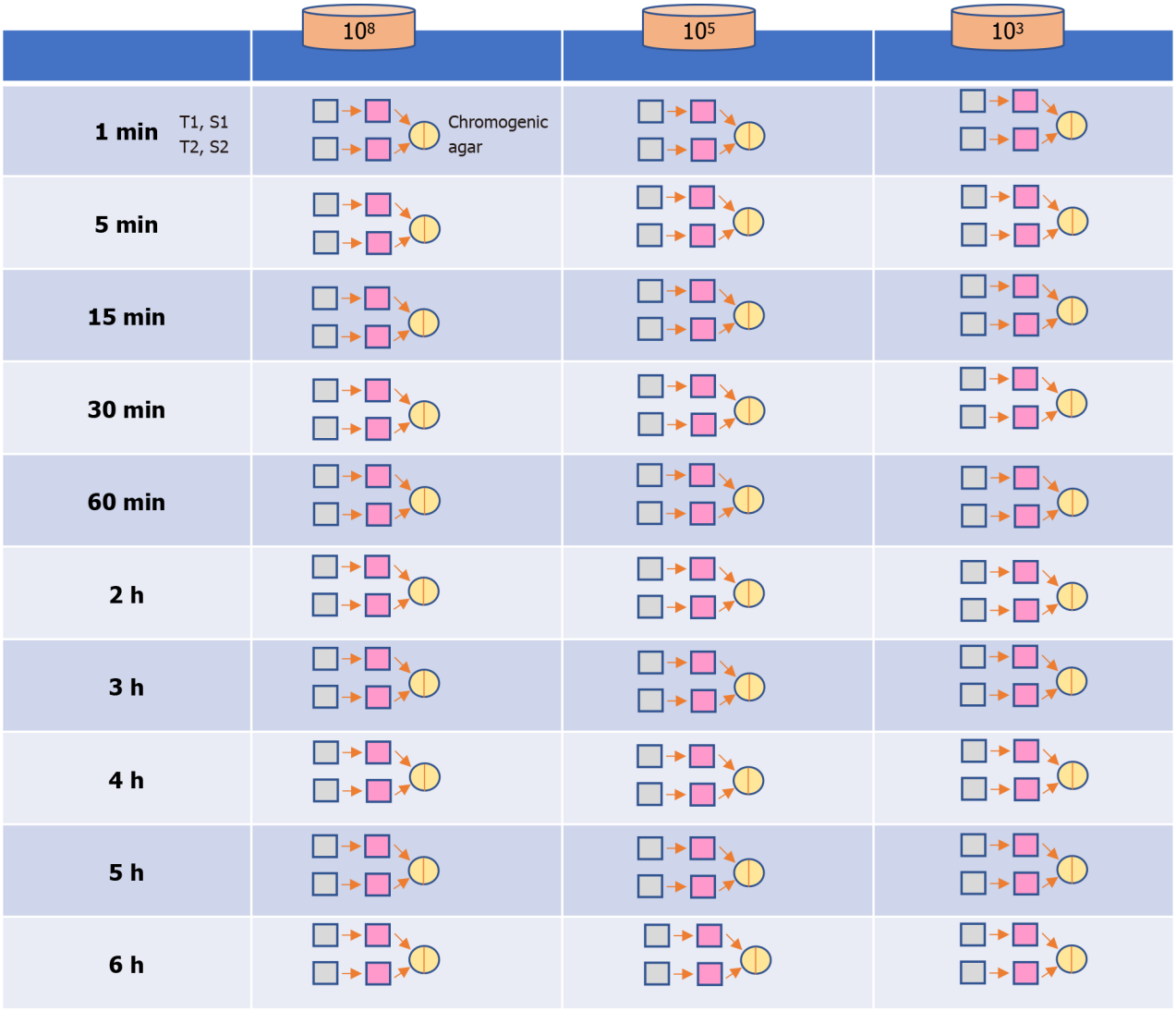

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Figure 1 Experimental set up: Each station contains 2 cloth swatches (T1 & T2), 2 pig skins (S1 & S2), and one chromogenic agar.

The survival ability of microorganisms on textiles has been previously addressed by several studies. Koca *et al*[13] used different fabrics, such as cotton, cotton-polyester, silk, and wool. To assess persistence of a variety micro-organisms, including yeasts, fungi, MRSA, VRE and ESBL-producing bacteria. Results showed that VRE had its longest survival on cotton-polyester fabrics (51 d, and 49 d on 100% cotton, silk and wool). MRSA had the longest survival period of 41 d on wool and 37 d on cotton silk and cotton-polyester. Respectively *E. coli* showed its longest survival for 45 d on cotton, silk and wool, and on cotton-polyester 37 d. These findings were confirmed by Malnick *et al*[12] who showed that 50% of VRE-swabbed pyjamas and bed sheets yielded micro-organisms overnight. Similarly, Neely and Maley[3] studied the survival of strains of MRSA and VRE on five different types of materials used commonly in hospitals: 100% cotton (which represented cloths and towels), cotton-polyester, 100% polyester, and 100% polypropylene plastic (represented which splash aprons). MRSA had the longest survival on polypropylene plastic (> 51 d), followed by 40 d (polyester), and 21 d (cotton). VRE exhibited long survival periods of > 80 d on polypropylene plastic and polyester, and 22 d on the other materials.

In agreement with the above findings, a literature review supported that contaminated textiles can harbour bacteria and consequently transmit infection for weeks[14]. Current evidence supports survival and persistence on textiles not only of bacteria, but also mycobacteria, fungi and viruses. Specifically, VRE can survive for more than 90 d, MRSA for up to 56 d and *K. pneumoniae* for 11 d. Survival at room temperature favoured polyester against cotton. Even though there is only a limited number of reported healthcare-associated infection outbreaks associated with contaminated textiles[15-19], their involvement may be undermined, and not sought out during incidences or as part of the general infection control process.

Taken cumulatively, the findings of our study add insight to the literature and clearly suggest that staff attire, including uniforms and scrubs, when contaminated, can transmit pathogenic micro-organisms onto patients' skin, thus acting as bacterial vectors. It can also be assumed that contaminated hands can in turn contaminate uniforms[10]. Hence, strict and targeted preventive measures targeting textile and attire are needed to break the chain of transmission. Current evidence from various studies suggests against the use of white coats and ties in healthcare settings, as they are more

frequently contaminated compared to other HCP attire[4]. On the other hand, short-sleeved uniforms can be more beneficial as regards to lower transmission of pathogenic micro-organisms[20]. However, these measures alone seem insufficient to control MDRB spread and support the need for additional control measures, such as ensuring application of appropriate home laundering practices, use of a hospital laundry service, wearing single-use protective aprons or gowns wherever applicable, promoting hand hygiene before and after patient interaction, daily uniform change, and application of contact precautions particularly in high-prevalence settings[21,22]. Our experimental study was performed under the same environmental conditions for all 3 studied MDRB. Therefore, the different observations in regard to time intervals and concentrations among the 3 studied MDRB in our study, probably reflect the properties of these bacterial strains.

The strength of the current study lies in its design, which aimed to resemble as much as possible a real-life situation. Specifically, in addition to evaluating the possibility of textile inoculation and transfer onto pig skin, we confirmed the viability of clinically important MDRB over time, in an attempt to mimic the environmental conditions in healthcare settings during HCP shifts. Our findings demonstrate that 6 h after MDRB inoculation of textiles, transfer from a dry textile surface on pig skin was possible. Simulation of real-life conditions showed that MDRB can survive, grow and transmit from textiles of two different compositions onto pig skin, which is the closest parallel to human skin.

Limitations of our study include the small spectrum of micro-organisms used and the fact that we used only two types of fabrics. We also acknowledge the fact that during our experiment, we didn't quantify the bacterial colony concentrations after inoculation. However, we performed the experiment under the hypothesis that bacterial persistence through time and transmission is evidence that textiles can be infectious, regardless of the growth amount. In addressing the current research gaps in the literature and the limitations of our study, future studies can include inoculation of textiles not solely with pure bacterial cultures, but also mixed with organic matter such as bodily fluids, to better emulate real-life conditions. Furthermore, the role of skin flora could be investigated in the survival of microorganisms on textiles and to evaluate their effect on MDRB binding capacity and replication.

Of note, only few similar studies have attempted to associate textiles with the transmission and survival of microorganisms. In line with the general perception that the risk of transmission from textiles is low, there is not enough emphasis given to the importance of cleaning and decontamination of textiles, compared to other inanimate surfaces[22,23]. However, our findings are supportive that textiles can be responsible for the transmission of pathogens in healthcare settings and thus, they should be managed accordingly. Moreover, our study strongly suggests that textiles should be included in the transmission triad "patient - healthcare professional - environment". To this end, healthcare settings should opt to analyse all possible steps in the chain of transmission and introduce appropriate action plans that include reduction of transmission risk through textiles and HCP attire. In the absence of protocols for testing uniform cleanliness and compliance, our findings provide evidence towards enforcing appropriate measures to reduce bacterial reservoirs in healthcare settings.

CONCLUSION

In conclusion, the current experimental study using 3 types of MDRB, provides evidence of their sustainability and transmission from textiles to skin. MRSA exhibited maximum sustainability for the whole duration of the 6-h study in all concentrations, VRE also remained for the whole duration of the study but only in the highest concentration, whereas CRKP remained for less time overall. Cumulatively, our data adds support to increasing evidence that textiles should be considered as vehicles of transmission in the healthcare setting.

ARTICLE HIGHLIGHTS

Research background

The isolation of microorganisms from textiles, including healthcare professionals' (HCP) attire, has been previously demonstrated in several studies.

Research motivation

The ability of microorganisms to adhere and survive on textiles, raises questions as to their possible role in transmission from textile to skin in healthcare environments.

Research objectives

The present experimental study aimed to evaluate the presence, survival and transmission of different multidrug-resistant bacteria (MDRB) from HCP attire onto skin.

Research methods

Inoculation of 3 MDRB [methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant *Enterococcus faecium* (VRE); carbapenem-resistant *Klebsiella pneumoniae*, CRKP)] on textiles from two types of textiles (60% cotton-40% polyester and 100% cotton) at 3 different concentrations (10^8 CFU, 10^5 CFU and 10^3 CFU per mL) and at different time intervals ranging from 1 min to 6 h. At the end of each time period, textiles were imprinted onto pig skins and each skin

square was inverted onto selective chromogenic media. Growth from the pig skins was recorded for the 3 MDRB at the three above concentrations, for the whole length of the 6-h experiment.

Research results

Recovery of MDRB from pig skins differed for each strain, with MRSA recording the longest and most sustained recovery at all concentrations and for up to 6 h. VRE showed no growth from 10^3 CFU/mL and was recovered from 10^8 CFU/mL for 6 h and from at 10^5 CFU/mL for up to 3 h. CRKP was recovered from 10^8 CFU/mL for 6 h, up to 30 min from 10^5 CFU/mL and for only 1 min from 10^3 CFU/mL.

Research conclusions

Evidence from the current study shows that all 3 studied MDRB can be sustained and transferred onto skin, with MRSA showing the highest level of persistence on textiles and transmission to skin even at low concentrations.

Research perspectives

Our findings support that textiles can be implicated as vectors of bacterial spread.

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FOOTNOTES

Author contributions: Lena P contributed to the conceptualization of this study; Lena P, Liatsou M, Lamnisos D, Papageorgis P, and Tsioutis C involved in the methodology of the manuscript; Lena P, Karageorgos S, Agouridis AP, Spernovasilis N, Lamnisos D, Papageorgis P, and Tsioutis C participated in the formal analysis and investigation of this manuscript; Lena P, Karageorgos S, and Tsioutis C drafted the manuscript; Lena P, Karageorgos S, Agouridis AP, Spernovasilis N, and Tsioutis C contributed to the review and editing of this manuscript; Lena P, Papageorgis P, and Tsioutis C involved in the supervision of this study; and all authors approved final article version published.

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