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Identification of Bacterial Isolates Recovered from the Surface of Cleanroom Operators' Garments following Wear

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Conflict of Interest Declaration
The authors declare that they have no competing interests.

Ethical Approval

A research ethics self-assessment (RESA) form was submitted in respect to the Robert Gordon University's Research Ethics Policy. This application was reviewed and approved by the Ethical Review Panel of the School of Pharmacy and Life Sciences.

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Abstract

Background

Contamination of sterile pharmaceutical products can have serious consequences, in worst case scenario resulting in patient death. Cleanroom operators are the primary source of microbial contamination, where the surface of their specialist sterile clothing garments is subject to such contamination during wear. In turn these garments become a transmission vector for microorganisms within the cleanroom environment. Insight into identification of predominant bacterial isolates from garment surfaces would help to establish their original source and probable contamination route. This should assist possible intervention strategies to mitigate against this contamination.

Aim

The research aimed to determine identity of representative bacterial isolates recovered from the surface of cleanroom operators' garments following wear within a cleanroom.

Methods

Following isolation and purification of bacterial isolates, 16S rRNA gene sequencing was used to establish species identity for isolates recovered from the surface of male and female operators' garments following wear within the cleanroom environment.

Results

Of the 47 isolates recovered from the surface of garments, 16S rRNA gene sequencing successfully identified 94 % to genus level and 77 % to species level. Most were confirmed as Gram - positive bacteria; predominantly species of *Staphylococcus, Micrococcus* and *Bacillus*. The isolates recovered from the surface of female operatives' garments were more diverse than those retrieved from male counterparts.

Conclusion

Most isolates recovered from garments were found to be skin commensals, with nearly 70% attributed to the operators within the environment. The remainder were credited to contamination of garments with species of environmental origin. Whilst most bacteria identified present minimal threat to healthy individuals, certain of these are opportunistic pathogens, presenting a hazard for immunocompromised and/or those with underlying health conditions.

1. Introduction

Microbial contamination of pharmaceutical cleanrooms and subsequently products can have serious consequences including product recalls (1,2), shutdowns and increased expenditure (3), infection (4,5) and in worst case scenario death of product recipients (6–8). An effective environmental microbial monitoring programme is fundamental for ensuring contamination control (9). These programmes aid identification of habitual microflora, establishing predominant isolates and ascertaining probable source and routes of transfer into the cleanroom (10,11).

Findings from these monitoring programmes evidence a correlation between bacteria resident on human skin and those present within the cleanroom environment (12–17); attributing operators as the principal contamination source (18–23). Eighty percent of operator contamination is in the form of dead skin squames (24). A small percentage of these harbour skin associated microorganisms, so called microbe carrying particles (MCPs) (25). Failures of cleanroom garments to retain such squames and MCPs has been extensively reported (18–21,23,26,27). These studies confirmed properly gowned operatives disperse approximately 17,000 particles (21), including 180 MCPs per minute into the cleanroom environment (20). The polyester fibres of reusable garments were found to offer a substratum for microbial adherence, survival and growth (28–30) and that garment surfaces are subject to bacterial contamination following wear (31,32). In turn, garments become a vector for microbial dissemination within a cleanroom either via indirect airborne transmission (33) or direct surface transfer (34,35).

Several studies have investigated microbial populations within cleanroom environments (12–17,36–41), with over 70% of isolates originating from the human microbiome (17). Gram-positive bacteria are commonly observed in pharmaceutical cleanrooms, predominantly species of *Staphylococcus* and *Micrococcus* (12,15–17,39,40). Indeed, a 9-year review of cleanroom isolates investigating the types, trends, and patterns of cleanroom microflora, reports over 50% of isolates as such species (12). In addition, environmental *Bacillus* species account for 10-13% of isolates, whilst a much lower percentage of Gram-negative bacteria are present (12). However, few studies assess bacterial populations on operators and their garments (12,31,42), where just two have attempted to undertake identification to species level (31,42), one focused solely on *Staphylococcus* species using the API *Staph* identification system (42).
Microbial identification tests typically classify microorganisms to their genus or species level (43). Traditional phenotypic methods involve identification based on observable characteristics, derived from several biochemical tests. However, such techniques are labour intense and subject to limitations including reliance on expression of metabolic activities, interpretation of subjective results, reproducibility, and observation of similar phenotypic characteristics between isolates. Consequently, isolates can be misidentified (44) or remain unidentified (45). In contrast, genotypic sequence-based identification is considered more reliable with 16S rRNA gene sequencing reported to identify over 80 % of isolates to the species level (46). Despite the emergence of genotypic techniques, phenotypic methods are still commonly employed in cleanroom isolate identification (12).

Identification of isolates on cleanroom garments and determination of their origin is vital in assessing operator associated environmental and product risks and foreseeing incidences of contamination. Therefore, the aim of this study was to use 16S rRNA gene sequencing to seek identification and origin of bacterial isolates recovered from the surface of cleanroom operators' garments following wear.

2. Methods

2.1 Bacterial Recovery

Bacterial isolates were recovered during our study examining influence of gender on the surface contamination of clean room operators' garments following wear (32). During this investigation the surface of clean room operators' garments were tested within the exit area of the changing area, prior to garment removal and immediately following their period of working in the cleanroom environment. The method used to recover bacteria from garments involved a direct agar contact method, where the surface of a nutrient agar contact plate was depressed against the surface of the garments at specific sites for a period of 5 seconds at constant pressure (Figure 1). Following colony enumeration from the incubated contacts plates for the study reported earlier (32), a total of 47 representative colonies were selected for this study based upon morphological characteristics. Of the total, 36 isolates were obtained from contact plates examining female operatives' garments with 11 isolates taken from garments worn by their male counterparts.

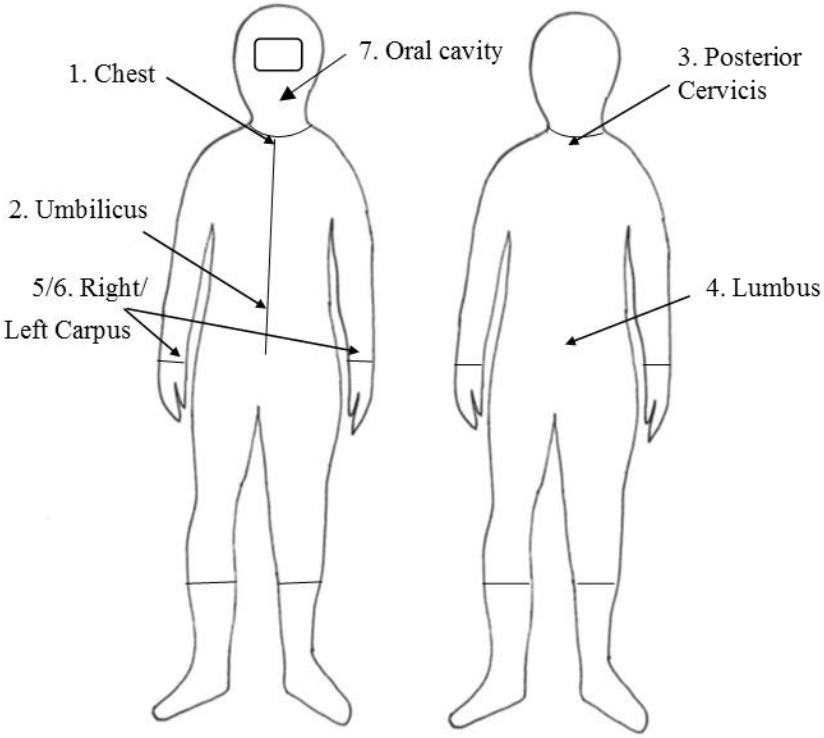


Figure 1: Garment sites tested: 1- chest, 2 - umbilicus, 3 - posterior cervicis, 4 - lumbus, 5/6 - left and right carpus and 7 - oral cavity.

2.2 Bacterial Purification and DNA Extraction

Colonies of isolates were picked, streaked and then re-streaked to purity on nutrient agar plates incubated at 37 ^{o}C, then stored at 4 ^{o}C prior to DNA extraction. To permit DNA extraction 1 mL of overnight culture in nutrient broth (prepared from a well isolated colony of the isolate) was centrifuged in a sterile 1.5 mL micro centrifuge tube for 5 minutes at 13,500 rpm. After supernatant was removed the pellet was re - suspended in 100 µL of 10 mg/mL lysozyme solution in 0.1 M TE Buffer for 45 minutes at 37 ^{o}C. Following this, 1 µL of 20 mg/mL Proteinase K (Sigma Aldrich, Dorset, UK) and 1 µL of 10 % SDS solution were added and incubated for a further 30 minutes, before 100 µL

of 30 % (w/v) BT Chelex® 100 resin (BioRad Laboratories, CA, USA) was added. After addition of resin the tube was vortex mixed. To optimise DNA yield the tube was heated to 56 ^{o}C for 30 minutes in a heating block then boiled for 10 minutes in a water bath to further promote bacterial cell lysis. The tube was centrifuged for 5 minutes at 13,500 rpm and ~ 200 µL of supernatant transferred into a fresh sterile 1.5 mL micro centrifuge tube. Gel electrophoresis using 10 µL of supernatant on a 1.5 % (w/v) agarose gel was performed to establish successful DNA extraction. The remainder was frozen at -20 ^{o}C for future analysis.

2.3 Polymerase Chain Reaction (PCR) Amplification of Extracted Bacterial DNA

16S rRNA forward and reverse primers (synthesised by Sigma Aldrich, Dorset, UK) were used to amplify the 320 bp hypervariable region at the 5'- end of the 16S rRNA gene in each of the isolates selected for study. The sequence and melting temperatures of the primers to obtain the anticipated PCR amplicon size of 320 bp are shown in Table 1.

Table 1: 16S rRNA forward and reverse primer sequence and melting temperatures.

Primer	Sequence	Melting Temperature Tm (°C)
Forward 16S F	5' – GCTCAGATTGAACGCTGG – 3'	62.3
Reverse 16S R	5' – TACTGCTGCCTCCCGTA – 3'	60.6

Primers were stored at -20 °C and thawed at room temperature prior to dilution with sterile Milli–Q water down to a working concentration of 10 mM. For each extracted DNA sample 50 µL of PCR master mix was prepared by addition of 10 µL of 5 x PCR buffer, 3 µL of 25 mM $MgCl_2$, 1 µL of 10mM dNTPs (all Bioline Ltd, London, UK), 1 µL of each forward and reverse primer and 35 µL of Milli–Q water in a sterile 1.5 mL micro centrifuge tube. Thereafter, the PCR reaction contained 50 µL of PCR master mix, 5 µL of extracted bacterial DNA and 1 µL Taq polymerase (5 units / µL) (Bioline Ltd, London, UK).

PCR was undertaken in a Jencons – PLS Techne TC – 312 thermo cycler (Jencons Scientific Ltd, Bedfordshire, UK). Initially, samples were denatured at 94 °C for five minutes before undergoing 30 thermal cycles of denaturing at 94 °C for 1 minute, annealing at 63 °C for 1 minute and elongation at 72 °C for 2 minutes. A final elongation stage of 10 minutes at 72 °C was undertaken. To determine successful amplification and DNA concentration within each sample 10 µL from the reaction was subjected to gel electrophoresis on a 1.5 % (w/v) agarose gel.

2.4 Purification of Amplified Bacterial DNA Samples

Amplified DNA samples obtained by PCR were purified using the Wizard® SV Gel and PCR Clean Up System (Promega, Southampton, UK). In a sterile 1.5 mL micro centrifuge tube 40 µL of PCR product was added to 40 µL of membrane binding solution. Following mixing, an SV mini column was inserted into a collection tube and the 80 µL of PCR product/membrane binding solution transferred onto the column. This was incubated for 1 minute at room temperature, before being centrifuged at 16,000 *x g* for a further 1 minute. The column was removed from the collection tube and the flow-through discarded. The column was re-inserted into the collection tube and washed using 700 µL of membrane wash solution and centrifuged at 16,000 *x g* for 1 minute. The flow through was once again discarded and the column re-inserted into the collection tube. This washing process was repeated with 500 µL of membrane binding solution and centrifuged for 5 minutes at 16,000 *x g*. The collection tube was again emptied and the column re-centrifuged at 16,000 *x g* to allow evaporation of any residual ethanol. To elute the PCR product from the mini column this was placed into a sterile 1.5 mL micro centrifuge tube and 50 µL of nuclease free water added. This was incubated at room temperature for 1 minute and centrifuged at 16,000 *x g* for a further 1 minute. Purified DNA products were all stored at -20 °C for future analysis.

2.5 Preparation of PCR Samples for Sequencing

After determining the concentration of DNA within each sample from the agarose gel electrophoresis detailed in section 2.3 the concentration of each sample was adjusted to give between 10 - 20 ng in 30 µL of Milli-Q water (0.33 - 0.67 ng / µl). DNA sequencing was performed by the MRC Protein Phosphorylation and Ubiquitylation Unit at the University of Dundee (MRC PPU

Unit, School of Life Sciences, University of Dundee, Scotland - https://www.dundee.ac.uk/facilities/mrc-ppu-dna-sequencing-and-services).

2.6 Basic Local Alignment Selection Tool (BLAST) Analysis of Bacterial DNA Sequences

DNA sequences were converted to the FASTA (text – based) format and entered into BLAST (http://blast.ncbi.nlm.nih.gov/) for comparison to sequences stored in the 16S rRNA sequence database. Identification to species level was considered successful when a similarity of > 97 % was achieved to a sequence in the database. For those sequences which did not meet these criteria they were only identified to the genus level where a > 95 % similarity was observed (47).

3. Results

To permit genotypic identification of the 47 bacterial isolates recovered from the surface of cleanroom operators' garments following working in the cleanroom environment, 16S rRNA gene sequencing was undertaken. Of these, 44 isolates were identified to at least the genus level (> 95% similarity to a sequence in the BLAST database). Two further isolates could not be identified to the genus level as both achieved only 85% similarity within the BLAST database. Furthermore, somewhat surprisingly, one of the isolates had a sequence for which no significant similarities were found within the BLAST database.

3.1 Characteristics of Bacterial Isolates Identified to Genus Level

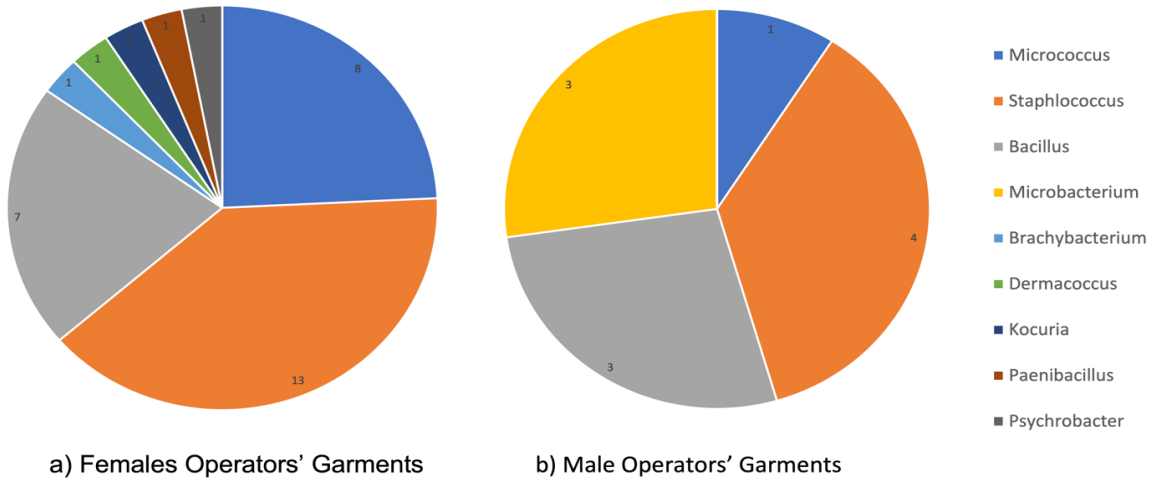
Of the 44 isolates identified via BLAST, 28 were confirmed as Gram - positive cocci, 14 as Gram - positive rods, and 1 being a Gram - negative coccus. The last of this batch of isolates was identified as a species of *Brachybacterium*, where cell shape varies and exhibits an unusual rod-coccus cycle of growth (48). The common habitat of these bacteria, as well as their predicted source for contamination of the cleanroom garments, are reported in Table 2. As shown, most bacterial isolates were identified as species of *Staphylococcus*, *Micrococcus* and *Bacillus*. Smaller numbers of *Microbacterium* were found along with individual isolates of *Brachybacterium, Dermacoccus*, *Kocuria*, *Paenibacillus* or *Psychrobacter* species. Based on genera, isolates were identified as either common members of human skin microbiome or the outdoor environment. Indeed, 28 isolates (64 %) were therefore determined to have originated from the wearer of the garments, whilst 16 isolates (36 %) could be attributed to environmental contamination.

Table 2: Characterisation of Bacterial Isolates Recovered from Worn Cleanroom Garments.

Genus	No. of Isolates	Common Habitat	Contamination Source	Ref
Staphylococcus	17	Microbiome of human skin	Operator	(49)
Bacillus	10	Inhabitant of the natural environment – primarily soil	Outdoor Environment	(50)
Micrococcus	9	Microbiome of human skin	Operator	(51)
Microbacterium	3	Inhabitant of the natural environment	Outdoor Environment	(52)
Brachybacterium	1	Inhabitant of a variety of environments	Outdoor Environment	(53)
Dermacoccus	1	Microbiome of human skin and mucus membranes	Operator	(54)
Kocuria	1	Microbiome of human skin, mucosa and oropharynx	Operator	(55)
Paenibacillus	1	Inhabitant of the natural environment – primarily soil	Outdoor Environment	(56)
Psychrobacter	1	Inhabitant of cold environments	Outdoor environment	(57)

3.2 Effect of Gender on Microbial Diversity of Cleanroom Garments

The effect of gender on the diversity of isolates on the surface of cleanroom operators' garments following wear was reviewed. The relative proportions of isolates by genera and with respect to gender is illustrated in Figure 2. This represents 33 isolates recovered from the surface of female operatives' garments and 11 isolates recovered from the surface of garments worn by their male counterparts.



a) Females Operators' Garments b) Male Operators' Garments

Figure 2: Proportions of bacterial isolates recovered from the surface of male and female cleanroom operators' garments.

As shown in Figure 2, staphylococci were most commonly recovered from the surface of garments worn by both male and female operators, whilst similar proportions of *Bacillus* spp. were noted in each case. A greater proportion of *Micrococcus* spp. were identified from garments worn by female operators compared to those worn by their male counterparts. *Microbacterium* spp. were recovered from male operatives' garments whereas the same genus was not retrieved from garments worn by their female counterparts. A greater diversity of bacteria was identified from the surface of garments worn by female operators including species of *Brachybacterium, Dermacoccus, Kocuria, Paenibacillus* and *Psychrobacter*. Of course, it must be acknowledged that the smaller original number of isolates examined from male operators' garments make this comparison less reliable.

3.3 Identification of Bacterial Isolates to Species Levels.

Of the 44 isolates identified to the genus level, 36 of these proved possible to be identified to species level (> 97% similarity to a sequence in the BLAST database). Their site of garment recovery with respect to gender are reported in Table 3.

Table 3: Species level identification of bacterial isolates recovered from the surface of female or male cleanroom operators' garments.

Genus	Species	No. of Isolates	Garment Site
Female Operators' Garments			
Micrococcus	*luteus*	7	Oral cavity, lumbus, umbilicus, left & right carpus, chest.
Staphylococcus	*succinus*	3	Oral Cavity, lumbus, umbilicus
	cohnii subsp. urealyticus	3	Oral Cavity, lumbus, umbilicus
	capitis	2	Posterior cervicis, umbilicus
	equorum	2	Posterior cervicis, chest
	hominis	1	Umbilicus
	saprophyticus	1	Umbilicus
Bacillus	*anthracis*	2	Oral cavity
	safensis	1	Left carpus
	pumilus	1	Umbilicus
Brachybacterium	*conglomeratum*	1	Oral cavity
Kocuria	*gwangalliensis*	1	Umbilicus
Psychrobacter	*pulmonis*	1	Lumbus
Male Operators' Garments			
Micrococcus	*luteus*	1	Right Carpus
Staphylococcus	*succinus*	2	Umbilicus
	cohnii	1	Umbilicus
	saprophyticus	1	Chest
Bacillus	*anthracis*	2	Chest, umbilicus
	safensis	1	Posterior Cervicis
Microbacterium	*martipicum*	2	Right carpus, Chest.

The most abundant species recovered from the surface of garments worn by female operatives was *Micrococcus luteus*. Several species of *Staphylococcus* were also recovered from garments worn by both genders with *S. succinus* and *S. cohnii* most commonly found. Environmental *Bacillus* species, including *B. anthracis,* were retrieved from garments worn by both genders.

Bacteria recovered from the surface of female operators' garments were slightly more diverse than those from males, with single isolates of Brachybacterium *conglomeratum, Kocuria gwangalliensis* and *Psychrobacter pulmonis* all identified. However, two isolates recovered from garments worn by male operatives were identified as *Microbacterium martipicum*, a species not recovered from females. Overall, there did not appear to be a correlation between garment site and the bacterial species recovered, with a diverse population of bacteria across garment sites observed.

4. Discussion

The results confirm that sterile barrier clothing worn by operators within a cleanroom facility to guard against product contamination, becomes rapidly contaminated with bacteria and thereby presents a potential contamination risk. Whilst most bacterial organisms identified from the garments present minimal threat to healthy individuals, others identified are known opportunist pathogens. The latter harbour ability to cause infection and disease in immunocompromised persons or those with underlying health conditions.

Of those bacterial isolates identified in this study, other than one, all were found to be Gram - positive organisms; primarily cocci. This might have been anticipated due to the abundance of Gram – positive bacteria on human skin (58), their previously reported prevalence within the cleanroom environment (12,15–17,39), and their incidence on the surface of cleanroom operators' garments (12,31). Of note, species of *Bacillus* identified in this study produce spores which survive extreme habitat fluctuations for extended periods of time, also withstanding desiccation and disinfection (59). These present increased risk to the cleanroom, due to potential to remain inactive for a prolonged period before sporulating back to vegetative growth under appropriate conditions.

Most isolates were identified as species of Gram - positive cocci, either *Staphylococcus* or *Micrococcus.* The presence of these particular bacteria confirms contamination of sterile barrier clothing by the operators during gowning or working within the cleanroom. Findings from this study indicate approximately two thirds of isolates recovered from the garments can be attributed to this source. As noted by Grangé *et al.* (2010) (31) and an earlier study reported from our laboratory (32) bacteria will contaminate the outer surface of operators' garments whilst they work. The remaining one third of isolates identified are non - human in origin where contamination probably arose from an environmental source. Several isolates which derive from soil were identified, their presence attributed to operator transfer and / or materials being brought into the cleanroom and subsequently transferred onto the garment via surface contact. In their study of cleanroom contamination routes Moissi - Enichinger *et al.* (2015) (60) found the operator changing area to be the primary location for bacterial transmission into the cleanroom environment, with 68 % of cleanroom isolates originating from this area (60). This relates to movement of personnel and transfer of materials from contaminated unclassified areas, coupled to elevated level of activity within this area (including garment donning process). Increased movement promotes particles including MCPs to be disseminated from operators. Complete mitigation of such activities is both impractical and unavoidable, where we previously reported contamination of cleanroom garments at the point of their donning, critically being prior to actual cleanroom entry (61).

The single most abundant isolate observed was the skin commensal *Micrococcus luteus*, an organism responsible for 25 % of all cleanroom contamination events (62). Although an opportunist pathogen causing nosocomial infections such as meningitis (63), septic shock (64), endocarditis (65) and brain abscess (66), diseases are rare and limited to those with lowered immunity. A range of *Staphylococcus* species isolates including *S. succinus, S. cohnii, S. equorum, S. capitis, S. hominis* and *S. saprophyticus* were also identified. Whilst the majority of these are skin commensal and attributed to the wearer / operators within the environment, *S. equorum and S. succinus* are not reported to originate from humans (67,68) and not in keeping with expected operator and / or

environmental transmission sources. Were the bar for species identification increased from 97% sequence identity of 16S rRNA sequence, it might be the case these could have been found to be different species that are human in origin.

Environmental *Bacillus* were also recovered, with species of ***B.*** *anthracis, B. safensis* and *B. pumilus* identified, the former the cause of anthrax (69). These bacteria are common soil inhabitants (70) and as such the route onto the clothing garment is thought to be through operator transfer from the external environment, probably via footwear. These species are commonly found in pharmaceutical cleanrooms and are regarded as problematic (71). Whilst the pathogenicity of *B. safensis* is not clearly evidenced (72), although rare, *B. pumilus* has been reported to cause infection (73–77). However, it is worth noting that using 16S rRNA gene sequencing some *Bacillus* species exhibit more than 99 % sequence similarity (78) and in this study *Bacillus* isolates may not be identified accurately beyond genus level.

In this study isolates exhibiting a > 97% similarity to a sequence within the BLAST database were considered positively identified to species level, as previously recommended by Schloss and Handelsman (47). However, it is also reported that over 97 % the differentiation between species is not entirely clear, as noted previously with some *Bacillus* species sharing > 99.5% sequence similarity (71). As such, Stackebrandt and Ebers (79) recommend a 98.7 – 99 % gene sequence similarity threshold to successfully identify isolates to the species level (79). Critically, although this increased threshold would reduce species identification efficiency in this study to just below 50 %, the 97% sequence similarity requirement selected permits bacterial genus identification with complete certainty. Indeed, if the primary purpose of identification is determination of isolate source, speciation of *Bacillus* spp. would not add any more value. Furthermore, misidentification of potentially pathogenic species such as *B. anthracis* may lead to unnecessary concern. In such cases phenotypic identification of isolates to the genus level may prove just as helpful while being less onerous and less costly. However, at the onset of this investigation the genus of the recovered isolates were unknown and 16S rRNA gene sequencing gave insight into the genus, as well as provisional speciation. Undeniably, the authors recognise that despite the advantages of 16S rRNA gene sequencing over phenotypic approaches, no bacterial identification method other than complete genome sequencing can be 100% accurate (80)**.**

Bacteria recovered from the surface of female operators' garments were more diverse than those retrieved from their male co-workers'. Gender has been previously shown to affect diversity of the skin microbiome, with greater variety observed on female skin (81,82) in keeping with the findings from the present study. The reason behind gender-linked skin microbiome differences is not well documented but thought to be attributed in part to physiological skin differences (82). The skin microbiome has also been shown to vary with host and environmental factors (58) and whilst wearing cleanroom garments body temperature and microclimate relative humidity have been shown to vary between genders (83,84). As well as an increased variety of bacterial genera identified, some of those recovered were unique to female operatives' garments, including environmental *Brachybacterium* and *Psychrobacter*, and skin commensal *Kocuria*. Whilst first reported in 1995 (85) *Brachybacterium conglomeratum* is an understudied organism with the limited literature restricting explanation for its presence on the garment. In contrast, *Microbacterium martipicum* was uniquely recovered from male workers clothing. Aside from the identification of this species in soil (86), there is little further information about this organism within the literature. However, it should be noted that as with *Bacillus*, species of *Microbacterium* have been shown to exhibit very similar 16S rRNA gene sequencing profiles and may be difficult to identify accurately at species level (87). In respect to the sample site from which the bacterial isolates were recovered there was no relationship identified between organism identity and the location where the sample

was taken from the garment. However, due to the large number of sites relative to the number of isolates, it would be unwise to suggest that no such correlation could exist.

In addition to the matter of similarity threshold applied for determination of bacterial species identity, other limitations for this study existed, primarily the inability to undertake a completely quantitative study for identification of all bacterial isolates. To achieve a semi-quantitative outcome, isolates selected were based upon colony morphology which sought to achieve proportionate representation. The study did not consider the exact grade of cleanroom in which the garments were worn beyond being isolates from operatives working within the facility, or the environmental parameters therein. Furthermore, although the operator numbers were always within standard operating procedure for the facility, the exact numbers of people within the cleanroom were not recorded. Previously Sandle (2011) reported that such factors will influence microbial diversity within the cleanroom environment (12). It is acknowledged this would have impacted upon the range and types of bacteria recovered from the surface of cleanroom operators' garments. Another was the variable length of time for which the garments were worn, being within periods of no less than 30 minutes to no more than 90 minutes, where bacterial number on cleanroom garments is known to increase with time (31,32). Collectively these all place limitations on the study findings and each would be worthy of separate further investigation with respect to the identity of bacterial contaminants recovered from cleanroom operators' garments.

5. Conclusion

Using 16S rRNA gene sequence comparisons this successfully identified 44 of 47 bacterial isolates recovered from the surface of cleanroom operators' garments to genus level and 36 down to species level. Virtually all isolates were identified as Gram - positive species, most being *Staphylococcus, Micrococcus* and *Bacillus*. Nearly 70 % of identified isolates were attributed to the operator contamination within the cleanroom facility and the remainder resulting from environmental source of contamination. The specialist garments worn by cleanroom operators eventually become contaminated with bacteria and thereafter represent a risk during sterile product manufacture. Whilst most bacteria identified would present little threat to healthy individuals, others are opportunist pathogens that are hazardous for at risk patient groups who typically receive sterile pharmaceutical products.

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