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Gender Influences Bacterial Contamination of Reusable 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. Third year undergraduate Master of Pharmacy students were invited to voluntarily participate in the study. Prior to their sampling a consent form giving a written explanation of the procedure was presented to all volunteers. Each volunteer signed the consent form prior to taking part in the study. This form also allowed the individual to discreetly opt out of the study. Furthermore, beyond gender, no private, confidential or personal information was recorded.

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Abstract

Background

Operators are the primary source of cleanroom contamination, with the majority of their detritus identified as skin squames and their associated microorganisms. To reduce contamination, operatives are required to wear a specific arrangement of specialist garments. However, bacteria can evade this clothing and tarnish outer surfaces whilst operators work, with adverse implication for cleanroom environment and product sterility. Gender plays a significant role in bacterial dispersion, with male rates being in excess of female counterparts. Currently there is a lack of published literature evaluating the effect of gender on contamination of cleanroom garments. Such information would assist cleanroom facilities to more robustly assess and mitigate operator-associated contamination risks.

Aim

To compare bacterial contamination on the surface of cleanroom operators' garments, specifically with respect to gender.

Method

Levels of bacteria on garments worn by male and female operators working under two conditions (30 minutes: Grade A/B cleanroom and 60 minutes: Grade C cleanroom) were compared. Immediately following the operators' exit from the cleanroom, a direct agar contact method was undertaken at several sites on the surface of their garments.

Findings

Bacteria were recovered from the surface of garments worn by both genders. Bacterial levels on garments worn by male operators were almost always in excess of those worn by females at all sites tested (Percentage of plates displaying growth: Grade A/B – 83.9%/63.3% and Grade C – 86.1%/70.1%, respectively) [* $p<0.05$]. Regardless of gender, bacterial levels at the chest and posterior cervicis region of suits were reduced with the donning of a hood, covering the head.

Conclusions

Gender plays a significant role in bacterial contamination of cleanroom garment surfaces, with bacteria on the surface of clothing worn by males being in excess of that on garments worn by female counterparts. In addition, the donning of a hood reduces bacterial numbers on suits. These findings add to the limited body of knowledge examining bacterial contamination of cleanroom garments and contribute towards understanding operator-associated contamination risks within cleanroom facilities.

Keywords: Gender, Cleanroom, Clothing, Operators, Bacteria, Contamination

1.0 Introduction

Operators are well established as the predominant source of cleanroom contamination [1-5]. This contamination is derived primarily from human skin squames, a result of continuous skin shedding [5, 6], a tenth of which typically host a small number of bacteria [7]. With cleanroom classification based entirely upon the cumulative concentration of airborne particles within the room [8], this is concerning.

1.1 Use of Cleanroom Garments

In seeking to maintain integrity of the cleanroom environment within defined limits, operators are required to wear a specific arrangement of specialist clothing [8, 9]. Although previous research has shown that these garments form an effective barrier between the operator and the environment, in one study reducing microbe carrying particle (MCP) dispersion rates by over 90% [1], the garments will not retain all sources of human detritus [1, 6, 10, 11]. In fact, gowned operators can shed up to 1.7×10^4 particles/min, elevating the airborne particulate concentration of the cleanroom environment by nearly 2×10^3 particles/m^3 [3].

1.2 Bacterial Contamination of Cleanroom Garments

The polyester fibres used to manufacture reusable cleanroom garments are vulnerable to bacterial adherence [12-14] and can become contaminated whilst operators work [2]. A previous study investigating bacterial numbers on the surface of surgical garments reports that these may be in excess of 1.28×10^4 cfu per surgical clothing system [15]. Of concern, cleanroom clothing may act as a mode for bacterial transmission, either directly contaminating a surface [16] or disseminating airborne organisms [17]. Furthermore, bacterial metabolism may cause textile degradation [12], potentially reducing barrier efficiency, an essential cleanroom garment property. Hence, bacterial contamination of cleanroom garments can have serious implications for environmental and product sterility.

1.3 Particulate and/or Microbial Dispersion Rates

Research studies quantifying particulate and/or microbial dispersion rates from people over a specified period, most commonly using a modified dispersal chamber [1, 6, 10, 11, 18-23], show these differ between individuals depending upon factors such as their clothing [6, 10, 11, 20, 21, 23], activity [6], skin pathology [18], care regimes [22], and gender [1, 18-23]. In fact, gender plays a significant role in particulate and microbial dispersion, with these past studies confirming male dispersion rates to be in excess of female counterparts [1, 18-23]. In 2007 Whyte and Hejab reported that whilst wearing cleanroom garments males disperse 1.5 times more MCPs than their female counterparts [1].

2.0 Aim and Objectives

Examination of gender with respect to the bacterial contamination of cleanroom garments has not been investigated in any previous study. The sole study evaluating bacteria on cleanroom garments examines this with respect to time spent working in the cleanroom rather than gender as such [2]. Therefore, the purpose of this study was to compare levels of bacterial contamination on the surface of cleanroom operators' garments, specifically with respect to gender.

3.0 Method

A total of 497 contact plates were used to test the exterior surface of sterile reusable cleanroom garments worn by a subject group of 77 operators (26 male and 51 female) following a working period in a cleanroom environment. The periods were either 30 minutes working in a Grade A/B cleanroom (35 operators [8 male/27 female]) or 60 minutes working in a Grade C cleanroom (42 operators [18 male/24 female]).

3.1 Contact Plate Preparation

Contact plates were prepared in a laminar airflow cabinet by aseptically pipetting 13mL of molten sterile Nutrient Agar (Thermo Fisher Scientific Inc, Gloucester, UK) into the base of 55mm petri dishes (Fisher Scientific Ltd, Loughborough, UK) to form a convex surface. Once set these were stored at 4°C until two hours prior to their use, when they were permitted to acclimatise at room temperature.

3.2 Garment Donning

Operators entered the changing area of the cleanroom facility and donned over shoes, a facemask and a hair net (Critical Environment Solutions Ltd, Wiltshire, UK). Following a standardised hand washing protocol incorporating antibacterial soap (HiBiSCRUB® antimicrobial skin cleanser, Regent Medical Ltd, Lancashire, UK) each operator then donned non-sterile gloves (Fisher Scientific, Loughborough, UK) before systematically donning a sterile reusable antistatic polyester carbon filament suit. In addition, Grade A/B operators donned a sterile hood and boots of the same composition. All sterile garments (Chemsplash, Manchester, UK) were laundered to Class A-ASTM F51/00 specifications (Fishers Laundry Group, Aberfeldy, UK). Following garment donning, Grade C operators removed their non-sterile gloves and omitted to wear gloves, whilst Grade A/B operators replaced their non-sterile gloves with KIMTech G5 sterile latex sterile cleanroom gloves (Basan UK, Basingstoke, UK).

3.3 Garment Testing

After 30 minutes working in a Grade A/B cleanroom or 60 minutes working in a Grade C cleanroom, each operator exited the room and immediately had the surface of their garments tested at 6 sites (chest, umbilicus, posterior cervicis, lumbus, left carpus and right carpus [Figure 3] using contact plates. In addition, Grade A/B operators tested the oral cavity region of their hood. During testing the lid of the contact plate was removed and the surface of the agar was carefully applied to the test surface for five seconds using constant pressure. Contact plates were then incubated at 37°C for 48 hours.

3.4 Microbiological Analysis

Following incubation contact plates were examined and recorded as displaying either no growth (0 colony forming units (cfu)/plate), low (1-9 cfu/plate), moderate (10-20 cfu/plate) or high-level growth (>20 cfu/plate). All results were statistically analysed using Two-Way Analysis of Variance (ANOVA) at a 95% confidence level (GraphPad Prism 7.0 [GraphPad Software Inc., La Jolla, CA]).

4.0 Results

4.1 Comparison of Gender on the Level of Contamination on Cleanroom Operators' Garments

A significantly higher percentage of plates used to test cleanroom garments worn by male operators displayed growth, under both working conditions, compared to the percentage of plates displaying growth used to test garments worn by their female counterparts (Figure 1): Grade A/B – 83.9%/63.3% and Grade C – 86.1%/70.1%, respectively [* $p<0.05$].

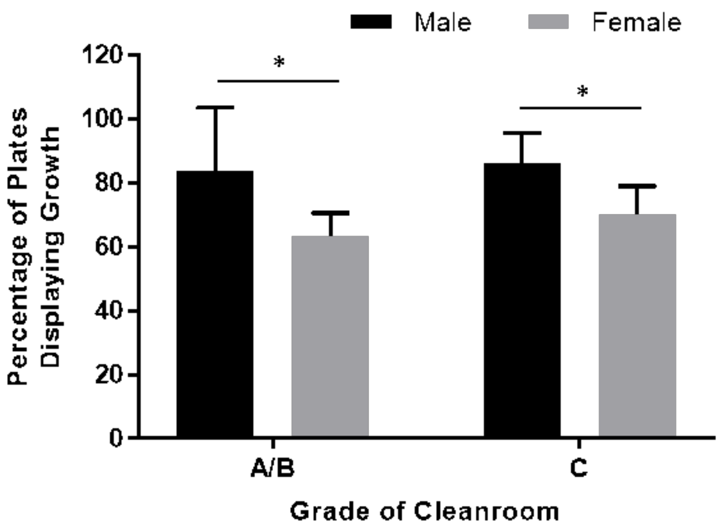


Figure 1: Comparison of gender against the percentage of contact plates displaying growth, used to test the surface of cleanroom garments worn by operators working in either a Grade A/B or Grade C cleanroom [* $p<0.05$].

A comparison of gender against the levels of growth displayed on plates was also investigated. The percentage of plates displaying low (1-9 cfu/plate), moderate (10-20 cfu/plate) or high (>20 cfu/plate) levels of growth used to test males garments were in excess of those used to test garments worn by female operators working under the same condition (Figure 2): Grade A/B – no growth - 16.1%/36.7% [** $p<0.01$], low - 69.6%/54.5% [* $p<0.05$], moderate - 3.6%/3.2% and high-level growth - 10.7%/5.8%, respectively; Grade C – no growth - 13.9%/29.9% [* $p<0.05$], low - 61.6%/54.9%, moderate - 7.4%/1.4% and high-level growth - 17.6%/13.8%, respectively.

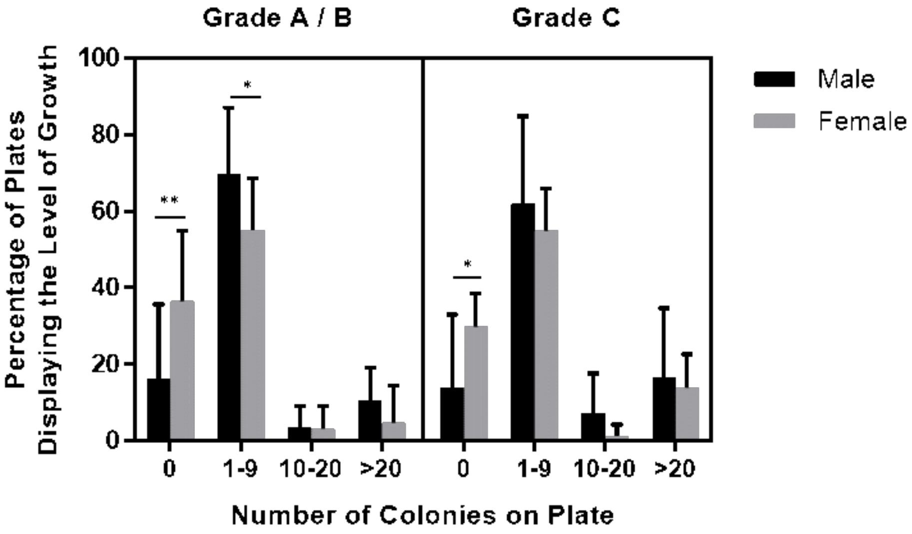


Figure 2: Comparison of gender against the total percentage of contact plates displaying either 0 (no growth), 1-9 (low), 10-20 (moderate) or >20 (high-level growth) cfu/plate used to test the surface of cleanroom garments worn by operators working in a Grade A/B or Grade C Cleanroom [**p< 0.01; *p< 0.05].

4.2 Comparison of Gender against the Contamination of Specific Garment Sites

Bacterial contamination of specific garment sites, with respect to gender, was also investigated (Figure 3).

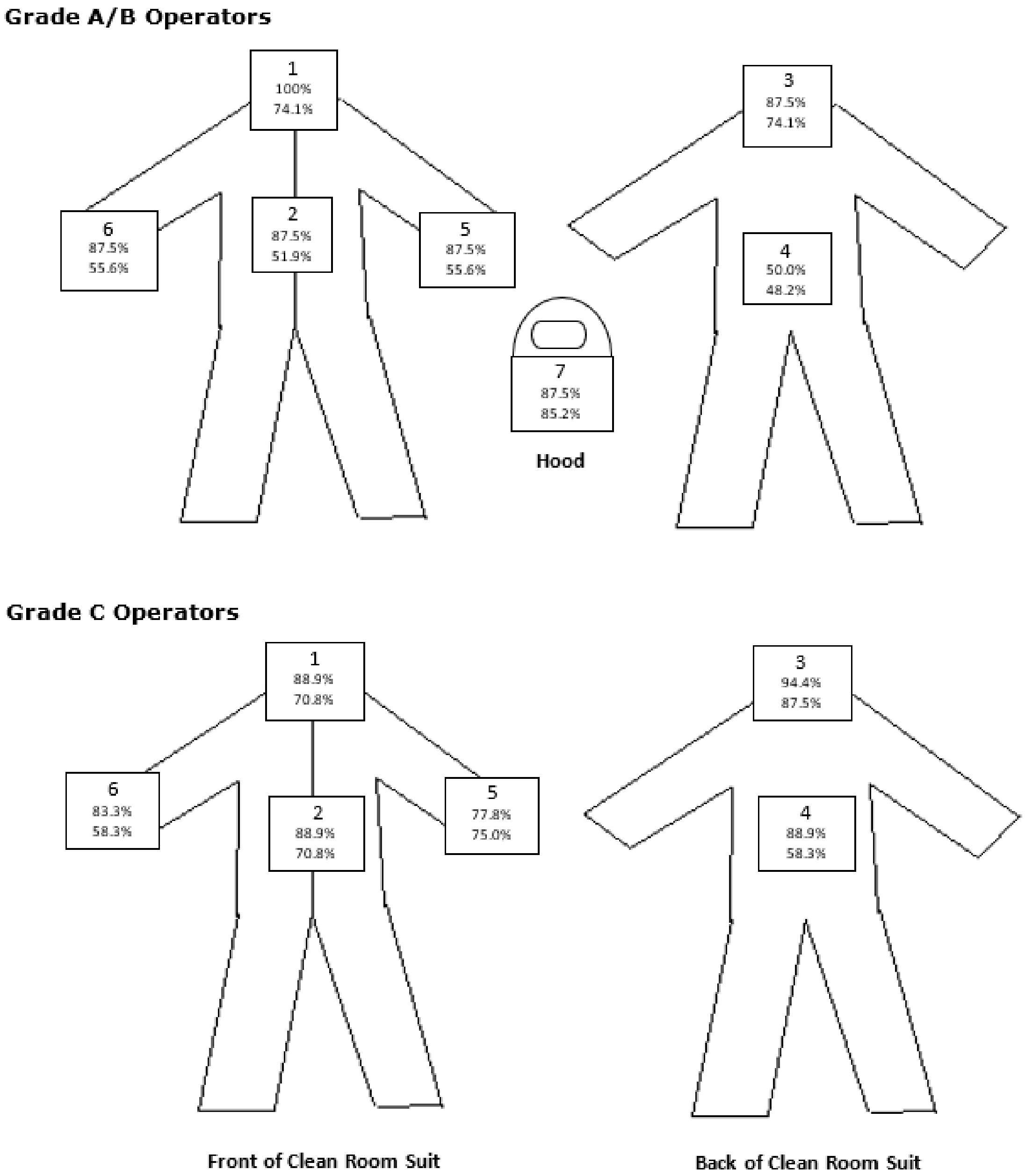


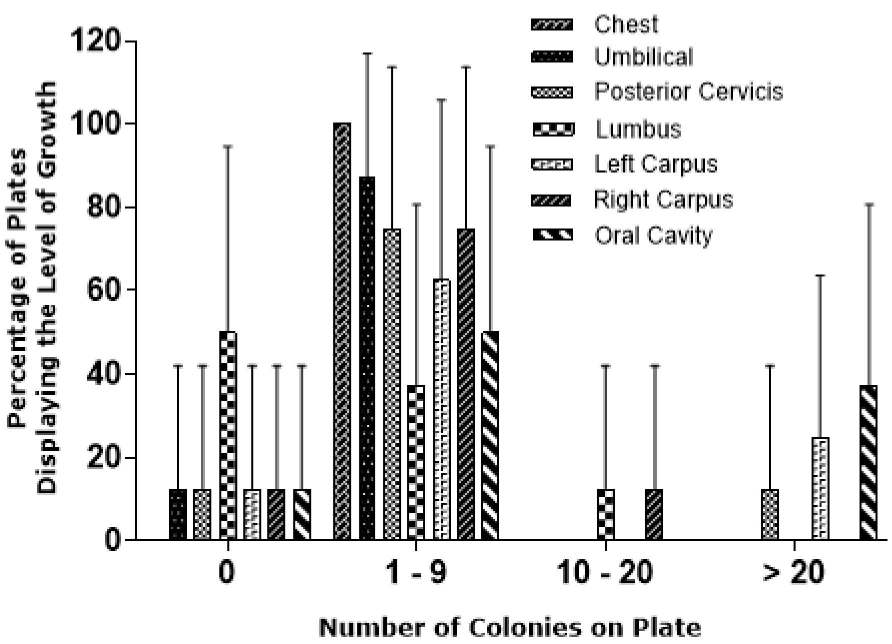
Figure 3: Comparison of gender against the total percentage of contact plates displaying growth at each site tested: 1 - chest, 2 - umbilicus, 3 - posterior cervicis, 4 - lumbus, 5 - left carpus, 6 - right carpus and 7 - oral cavity, of garments worn by male (upper value) and female (lower value) operators working in either a Grade A/B or Grade C cleanroom.

A higher percentage of plates used to test the surface of male operators' garments displayed growth at every single one of the 6 or 7 sites tested compared to the percentage displaying growth used to test the same sites of garments worn by female counterparts, under the same conditions: Grade A/B – chest - 100%/74.1%, umbilicus - 87.5%/51.9%, posterior cervicis - 87.5%/74.1%, lumbus - 50.0% / 48.2%, left carpus - 87.5%/55.6%, right carpus - 87.5%/55.6%, hood - 87.5%/85.2%, respectively; Grade C – chest - 88.9%/70.8%, umbilicus - 88.9%/70.8%, posterior cervicis - 94.4%/87.5%, lumbus - 88.9%/58.3%, left carpus - 77.8%/75.0%, right carpus - 83.3%/58.3%, respectively.

4.3 Comparison of Gender on the Level of Contamination of Specific Garment Sites

A comparison of suit site against the levels of growth displayed on plates used to test garments worn by each gender, under each working condition, was also investigated (Grade A/B cleanroom, Figure 4; Grade C cleanroom, Figure 5). Generally, levels on plates used to test male operators' garments were in excess of those detected on plates use to test female operators' garments.

Male Grade A/B Operators



Female Grade A/B Operators

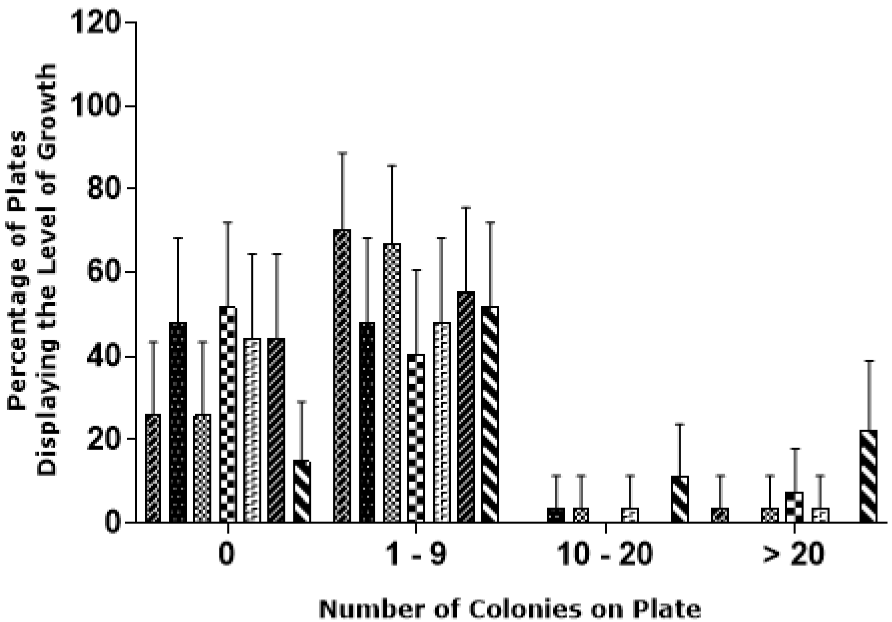


Figure 4: Comparison of garment site against the percentage of contact plates displaying either 0 (no growth), 1-9 (low), 10-20 (moderate) or >20 (high-level growth) cfu/plate, used to test the surface of garments worn by Grade A/B operators.

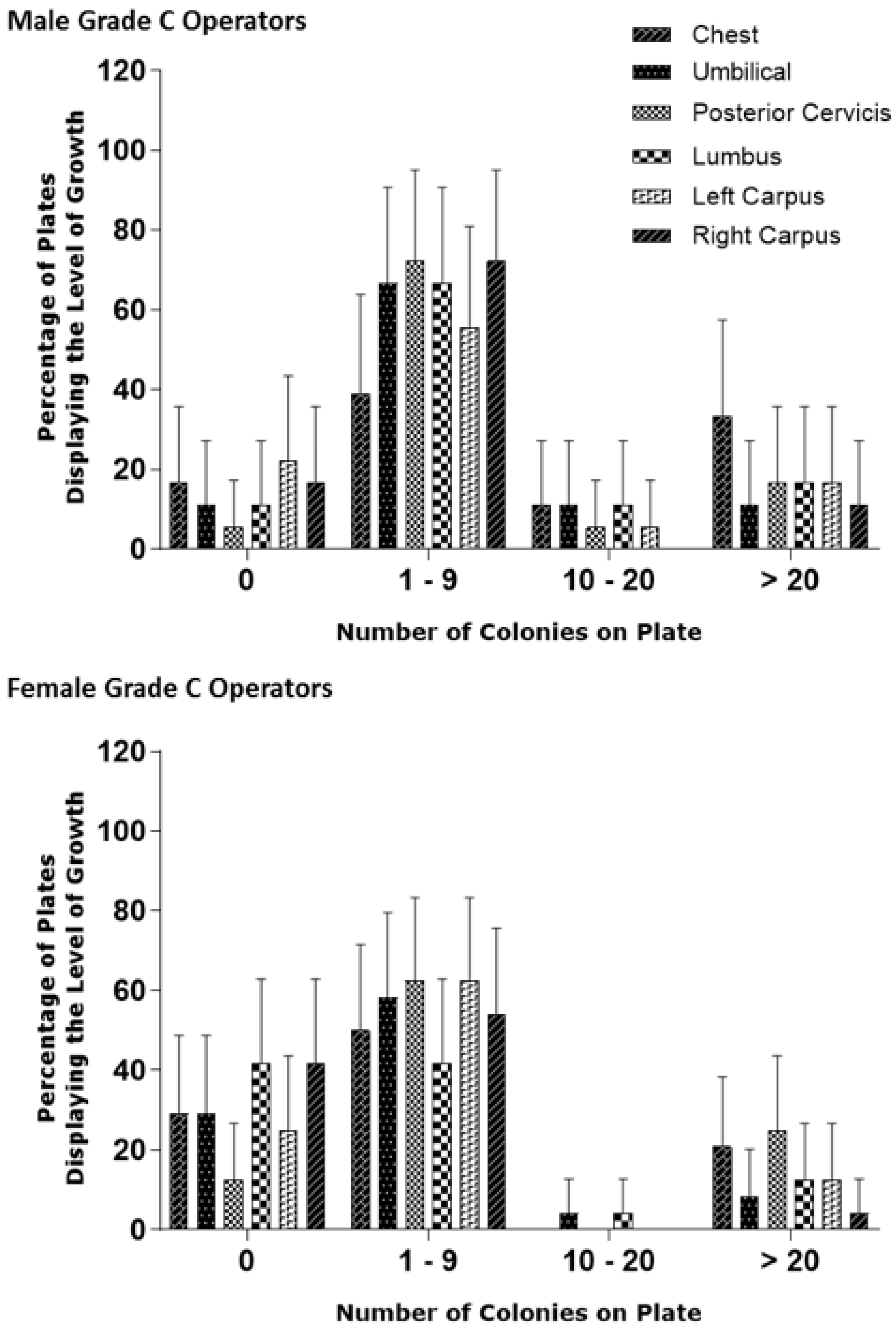


Figure 5: Comparison of garment site against the percentage of contact plates displaying either 0 (no growth), 1-9 (low), 10-20 (moderate) or >20 (high-level growth) cfu/plate, used to test the surface of garments worn by Grade C operators.

Regardless of gender, the material covering the oral cavity region of hoods was the most contaminated region of garments worn by Grade A/B operators, with the greatest percentage of plates displaying high-level growth (>20 cfu/plate): male – 37.5%; female – 22.5% (Figure 4). In comparison, the chest region of suits worn by male and female Grade C operators and the posterior cervicis region of suits worn by female operators working under the same condition were the most highly contaminated areas, with the greatest percentage of plates displaying high-level growth; chest: male – 33%; female – 20.8% and posterior cervicis: female – 25% (Figure 5).

5.0 Discussion

The results of the study confirm that bacteria, probably attached to skin particles, permeate reusable antistatic carbon filament cleanroom garments, contaminating their outer surface whilst operators work. The migration of bacterial cells and/or MCPs through cleanroom garments is supported by findings from earlier studies which identify isolates recovered from the surface of cleanroom garments as human in origin [2] and predominant cleanroom isolates as skin commensal species of *Staphylococcus* and *Micrococcus* [24-27]. Results from the current study further validate the finding that although cleanroom garments will reduce operator associated particulate and microbial environmental contamination [1, 6, 10, 11], such clothing will not retain all contaminants.

5.1 Gender Influences Contamination of Operators' Garments

The main finding from the study confirms that gender plays a significant role in bacterial and associated MCP dispersion rates of cleanroom operators and subsequent contamination of cleanroom garments. In virtually every instance, bacteria on the surface of male operators' garments were in excess of values obtained for females' garments. Increased contamination levels may be due to males shedding more organisms [18, 19, 23] and particulates [1, 19] than females. Indeed, previous research established that males shed up to 5 times more MCPs [19] and increased levels of skin commensal organisms, including twice as much *S. aureus* [23], than do females.

This may be a direct consequence of skin squame size, with previous research establishing the mean diameter of male skin squames to be 35.3µm, significantly smaller than for females at 40.0µm [28]. Although garment fabric pore size was not investigated during this study, this would none the less suggest greater potential for permeation. However, further clarification is required, as an unpublished study reported in Whyte and Hejab failed to find a relationship between squame size and gender [1]. In addition, increased dispersion may be a direct consequence of overall skin surface area, with males in general proportionally larger than females. Therefore, in this respect, dispersion may be related to larger skin surface area rather than gender as such. However, the same unpublished study failed to find a correlation between surface area and particulate dispersion rates [1], suggesting this is not the case.

5.2 Influence of Gender on Human Skin Microbiome

Gender directly influences the diversity of the human skin microbiome [29, 30] with males harbouring a greater array of microorganisms and these predominantly being bacterial in origin [29]. Therefore, the increased bacterial levels observed on male operators' garments may be in part as a consequence of their more varied skin microbiota.

Skin is a complex organ, host to a number of gender specific physical and chemical features within its structure and influenced by varying types and concentrations of sex hormones [31]. Varying physiological skin factors include sebum, sweat and collagen levels, as well as differences in pH [31, 32], thickness and tone [31], some or all of which may affect the skin's microbiome.

The exact mechanism between gender diversity and dispersion differences remain unclear. Increased dispersion maybe be linked to gender specific physiological skin differences, although studies which assess these factors have been contradictory. For example, whilst Wilhem and colleagues found that gender does not significantly affect skin pH [33], conversely Ehlers *et al.* latterly reported the pH of male skin to higher than that of their female counterparts [34].

5.3 Reducing Operator Associated Cleanroom Contamination

Several approaches which may reduce operator associated contamination levels within the cleanroom environment are reported in the literature [10, 20, 21, 35-37]. The workforce could wear garments designed to reduce the flow of air between their skin and the garments' fabric. Such as those with secure close-fitting plackets [20, 21] or those constructed of tightly woven, occlusive fabric [20, 21, 35-37], previously shown to reduce male bacterial dispersion rates by over 70% [21]. In addition to over-garments, operators could wear specialist underwear or non-linting scrubs, reported to reduce bacterial dispersion by up to 75% [10]. However, a previous study found that an additional textile layer can reduce air permeability by approximately 50%, with additional layers decreasing this further [37]. Of concern, poorly ventilated garments can reduce operator thermal comfort [4,38] and negatively impact on wellbeing and associated productivity and accuracy of output. Another option would be for operators to wear cleanroom garments constructed of fabric treated with an antimicrobial finish. Such treatment has previously been shown to reduce microbial survival on polyester fibres [39-41].

5.4 Contamination of Specific Garment Sites

The current study found that bacterial levels on the surface of male operators' garments were in excess of those on garments worn by females at all the 6 or 7 sites tested. Regardless of gender, the highest bacterial numbers were detected at the chest region of suits worn by Grade C operators, as well as the oral cavity region of hoods worn by Grade A/B operators. There are limited studies which have assessed microbial contamination or dispersion rates with respect to specific body sites [15, 19], with only one of these considering gender [19]. Whilst Ullman and colleagues could not assess gender with respect to the surface contamination of surgical clothing, due to biased subject numbers [15], the group of Noble *et al.* found that males dispersed greater bacterial numbers from the thighs and abdomen, with females dispersing higher levels from the shin area [19]. However, this previous research is historic and considered bacterial release with respect to variability of normal clothing type between genders such as wearing of tights, a factor less apparent nowadays.

5.4.1 Chest

High-levels of bacterial contamination at the chest region of suits detected in the current study may be due to a combination of factors including hand-borne bacterial transfer during the donning process [16], direct skin contact [42] and contamination arising from the oral cavity [43]. These may also result from operator movement, whereby billowing of the suit can result in particles travelling upward in the direction of the neck placket [44].

5.4.2 Posterior Cervicis

High levels at the posterior cervicis region of suits worn by female operators are thought to arise from bacteria residing in the scalp and hair. In general females possess more abundant quantity and longer lengths of hair in comparison to males. The increased bacterial levels detected in females are most probably connected to this gender difference, where shedding from hair would be logically detected in this specific location.

5.4.3 Oral Cavity

The high bacterial numbers recovered from the oral cavity region of hoods worn by Grade A/B operators are thought to be due to the sheer volume of bacteria residing in the oral cavity [43]. Although many species of the oral microbiome are anaerobic, there are also abundant facultative and aerobic species present [43] that were recovered from the fabric on the contact plates incubated aerobically

5.4.4 Hood Use in the Cleanroom Environment

Although facemasks have previously been shown to reduce the dispersion of airborne bacteria [23], the effectiveness of cleanroom head coverings such as hoods is an area that lacks investigation, with this study appearing to be the first. The reduction in bacterial numbers at the chest and posterior cervicis region of suits worn by Grade A/B operators, in addition to the high bacterial levels detected on their hoods, suggests the donning of the hood reduces bacterial contamination on cleanroom suits. The pattern of data in figures 4 and 5 would support such a conclusion, although there was time difference of 30 minutes between grade A/B and grade C activities that likely also contributed. A more rigorous testing of this specific phenomenon is needed. Overall, our study concurs with an earlier report that highlights the importance of using cleanroom garments to cover as much of the body as possible, including the head [45].

6.0 Study Limitations

Unfortunately, this study was limited by a number of factors primarily out with the research group's control. With respect to subject population, the lower number of male operators participating in the study is attributed to the voluntary recruitment strategy. A larger number of female volunteers led to a disproportionate ratio of male to female operators' garments being tested. Importantly, the overall numbers of both genders within the study nonetheless permit statistically valid data interpretation and conclusions to be drawn. Another limitation related to working duration where time spent working in the cleanroom was also a variable in this research study. Previous research found an increased working duration led to higher bacterial numbers on garments [2]. Unfortunately, the necessity to employ two time points was a restraint due to the working schedule of the facility, therefore further investigation using other time points is warranted. Additionally, the results of the study may be constrained by the choice of sampling sites, with no lower extremities examined. With previous research reporting the dissemination of large numbers of bacteria from the lower extremities [15, 20], a future investigation of the bacterial contamination of garment sites below the waist would allow for a comparison of garment contamination between the upper and lower extremities.

7.0 Conclusions

It was previously known that bacteria from the human skin microbiome permeate reusable antistatic carbon filament cleanroom garments, contaminating the outer surface of the clothing whilst operators work. Our study provides the first definitive evidence that gender plays a significant role in the bacterial contamination of the surface of cleanroom garments, with bacterial levels on the surface of clothing worn by males being in excess of those worn by females. In addition, a hood worn over the head reduces the bacterial contamination of the chest and posterior cervicis regions of cleanroom suits. These findings warrant further investigation, but importantly they contribute greater understanding of bacterial contamination sources within cleanroom facilities.

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