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# Microbiome of Clothing Items Worn for a Single Day in a Non-Healthcare Setting

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**Abstract:** When worn, clothing acquires a microbiome of bacteria and fungi derived from the wearer's skin and from the environment. The types of bacteria and fungi that may be recovered from clothing in healthcare settings have been well characterized, but less is known regarding the microbiome of clothing worn in non-healthcare settings and the possible roles that such clothing may play in microbial exchange. Culture-based methods and culture-independent genomic sequencing were used to enumerate and identify bacteria and fungi recovered from T-shirts, baby onesies, socks, and underwear worn for a single day after having been purchased new, washed, and dried. The highest bacterial loads were recovered from socks, underwear, and onesies (>$10^6$ colony-forming units [cfu]/sample) and the highest fungal loads were obtained from socks and underwear (>$5 \times 10^2$ cfu/sample). The sequencing method identified opportunistic pathogens present in the samples, including members of genus *Staphylococcus* and *Corynebacterium*, as well as anaerobic members of the family *Clostridiales*. The opportunistic fungal pathogen *Candida parapsilosis* was identified in a high proportion of worn clothing samples. These results suggest that clothing may represent a pathogen reservoir and a vector for microbial exchange between household occupants or the community outside of the home.

**Keywords:** clothing microbiome; microbial cross-contamination; opportunistic pathogens; infectious agents



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## 1. Introduction

The term fomite (or fomes) was coined in the 1500s. At the time, the term referred to "seeds of disease" that were thought to be found in the clothing of infected individuals that could spread illness through indirect human contact [1]. Fomites as disseminators of infectious agents in the healthcare setting remain relevant in the 21st century, as clothing and linens have been shown to harbor pathogens, even after laundering [2–4]. Importantly, contaminated textiles have been shown to play a role in the spread of antibiotic-resistant bacteria [5,6]. Less is known about the roles contaminated clothing may play in microbial exchange and, therefore, infection dissemination, outside of healthcare settings. The recent characterizations of the microbiome of worn T-shirts [7] and the microbiomes of domestic [8–10] and public [11] washing machines have provided results suggesting that contaminated clothing may, indeed, harbor the proverbial "seeds of disease". In the contexts of the ongoing COVID-19 pandemic and continuing concerns regarding the generation of antibiotic resistance in microorganisms, understanding the degrees to which fomites, such as clothing items, may harbor microorganisms, and devising strategies to mitigate such risks, is of increasing importance.

The types of microbes recoverable from clothing items is strongly linked to the microbiome of the adjacent skin [10], which hosts an enormous diversity of bacteria, fungi, and viruses. For instance, depending on the skin properties (i.e., oily, moist, dry), and

anatomical location (e.g., foot), between $10^2$ to $10^7$ bacterial cells per $cm^2$ skin surface may be present, representing up to 1000 different species from 19 different bacterial phyla [12,13]. A garment's microbiome is heavily influenced by the microbiome of its wearer [7] but is also influenced by the fabric composition and various environmental factors. For example, polyester garments are prone to the accumulation of malodor (and odor-causing bacteria) more so than garments composed of cotton or wool, which has been attributed to the hydrophobicity of polyester [7]. Environmental factors include contributions from potentially contaminated indoor surfaces (common spaces within the home, in healthcare settings, and in food service/hospitality settings), indoor or outdoor contact with other people and with animals, and outdoor exposure to soil, water, etc. Finally, there is a possible contribution from the machine laundering process itself, as in the absence of appropriate sanitizing conditions, a microbiome becomes established within certain parts of the washing machine. Evidence is accumulating [8–11] that the washing machine microbiome may transfer (exchange) to the clothing being washed.

Studies of domestic laundering processes have demonstrated that the microbial communities of clothing are not disturbed (either with regard to composition or biomass) by washing in mild detergent in the absence of enzymes [7]. For instance, a study of the survival and transfer of *Staphylococcus epidermidis*, *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* on cotton textiles found that washing in standard detergent under cold-water conditions (30 °C, 12-min wash cycle) resulted in substantial microbial survival [14]. This suggests a need for more research on strategies for achieving sanitization of clothing during household- and public-laundering processes, rather than simply removing stains.

Our goal in this work was to characterize the abundance and diversity of bacteria and fungi recoverable from worn clothing exposed to different skin locations and soiling challenges (T-shirts, socks, underwear, and baby onesies). This information should inform as to the possible role of clothing in the transfer of opportunistic bacterial and fungal pathogens, and to identify the need for risk mitigation strategies to prevent potential infection dissemination through contaminated clothing.

## 2. Materials and Methods

### *2.1. Experimental Design*

New articles of cotton clothing were purchased and washed in a cold-water cycle with Tide detergent and dried in a domestic clothes dryer. The clothing items, including Hanes brand cotton T-shirts, underwear, and socks, and Gerber brand organic cotton baby onesies, were provided to volunteers in individual, previously unused, Ziplock brand bags. Internal company volunteers ($n$ = 10) were recruited via site-wide email. Volunteers external to Reckitt ($n$ = 10) were recruited according to standard company protocols, using a consumer database (automated recruitment communication system, ARCS). It was determined that, due to the nature of the study and the plan to present only anonymized data, an internal review board was not necessary. Instead, a general release of liability form was executed by each study volunteer and retained by Reckitt. The internal and external volunteers were asked to wear the supplied clothing items for $\geq$12 h and then to return them to the lab for processing the following day. In the case of socks, both worn socks of the pair were returned for analysis. Worn clothing analysis included quantification of microbial and fungal colonies on appropriate media, as well as sample preparation for molecular identification, as described below. Molecular analyses (bacterial 16S RNA sequencing and fungal internal transcribed spacer [ITS] sequencing) of samples were performed by Zymo Research (Irvine, CA, USA). Sample analyses were blinded prior to analysis.

### *2.2. Recovery of Microbial Load and Total Viable Counting Procedures*

Analysts wearing sterile gloves and using sterile scissors treated with Decon ELIMINase™ (Thermo Fisher Scientific, Waltham, WA, USA) processed the various clothing items as follows. For worn socks, 2″ × 2″ squares were cut from three separate areas of each sock: (1) the ball of the foot; (2) the heel; and (3) the ankle. The resulting fabric

pieces (six per volunteer) were pooled in a sterile Stomacher bag with 30 mL of sterile 1× phosphate-buffered saline (PBS) (Becton Dickinson GmbH, Heidelberg, Germany). For worn T-shirts, 3″ × 3″ squares were cut from five areas: (1) the right armpit; (2) middle of back; (3) top of back; (4) chest; and (5) center of stomach. The various pieces (five per volunteer) were pooled in a sterile Stomacher bag with 30 mL of sterile PBS. For worn underwear, the entire article was placed in a sterile Stomacher bag with 150 mL of sterile PBS. For worn baby onesies, 2″ × 2″ squares were cut from four areas: (1) the buttocks area; (2) stomach; (3) upper chest; and (4) center of back. The various pieces (four per volunteer) were pooled in a sterile Stomacher bag with 30 mL of sterile PBS.

The various clothing samples were processed in a Seward Stomacher 3500 at 180–260 rpm for 10 min to adequately mix the samples and the PBS. Following this, the clothing samples were manually squeezed of remaining buffer using sterile gloved hands. The clothing items were then discarded. The resulting extracts were serially diluted and plated (in duplicate) on trypticase soy agar plates (TSA, BD Difco, Sparks, MD, USA) for total bacterial enumeration, and malt extract agar plates (with chloramphenicol) (Hardy Diagnostics, Santa Maria, CA, USA) for fungal enumeration. TSA plates were incubated for at least 48 h at 35–37 °C, and malt extract agar plates were incubated for 7–18 days at 29–31 °C before colonies were counted. The use of the different incubation temperatures for bacteria vs. fungi was predicated on the preferences of the two microorganism types for the higher or lower temperature ranges, respectively.

### *2.3. Sample Processing for DNA/RNA Extraction*

After plating for microbial enumeration, aliquots of remaining liquid PBS sample from Stomacher processing were centrifuged at 10,000× *g* for 15 min in a Labnet Prism microcentrifuge. The resulting pellets were pooled and resuspended in additional PBS sample aliquots, divided into smaller aliquots, and pelleted again under the same conditions. The remaining sample pellets were resuspended in approximately 1 mL of DNA/RNA Shield solution (Zymo Research) and sent to Zymo for sequence-based identification.

### *2.4. Sequence-Based Analysis of Microbial Flora in Worn Clothing Extracts*

DNA Extraction: One of three different DNA extraction kits was used, depending on the sample type and sample volume. In most cases, the ZymoBIOMICS® DNA Miniprep Kit (Zymo) was used. For low biomass samples, the ZymoBIOMICS® DNA Microprep Kit (Zymo) was used, as it allowed for a lower elution volume, resulting in more concentrated DNA samples. For larger sample volumes, the ZymoBIOMICS®-96 MagBead DNA Kit (Zymo) was used to extract DNA using an automated platform.

Targeted Library Preparation: Bacterial 16S ribosomal RNA (rRNA) gene sequencing was performed using the *Quick*-16S™ NGS Library Prep Kit (Zymo). The bacterial 16S primers amplify the V1-V2 and V3-V4 regions of the 16S rRNA gene. Fungal ITS gene-targeted sequencing was performed using the *Quick*-16S™ NGS Library Prep Kit (Zymo), with custom ITS2 primers substituted for 16S primers. The sequencing libraries were prepared in real-time polymerase chain reaction (PCR) machines to control cycles and limit PCR chimera formation. The final PCR products were quantified by means of quantitative PCR (qPCR) fluorescence readings and were pooled together based on equal molarity. The final pooled libraries were cleaned up with the Select-a-Size DNA Clean & Concentrator™ (Zymo), then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA, USA) and Qubit® (Thermo Fisher Scientific, Waltham, MA, USA).

Sequencing: Libraries were sequenced using an Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with >10% PhiX bacteriophage spike-in.

Bioinformatics Analysis: Unique amplicon sequences were inferred from raw reads using the DADA2 pipeline [15]. Chimeric sequences were also removed with DADA2. Taxonomy assignments were performed using Uclust from Qiime v.1.9.1 [16] with the Zymo Research Database, a 16S database that is internally designed and curated, as reference. Composition visualization, alpha-diversity, and beta-diversity analyses were performed

with Qiime v.1.9.1 [16]. If applicable, taxonomic assignments that had substantial abundance among different groups were identified by LefSe [17], using default settings. Other analyses, such as heatmaps, Taxa2SV Decomposer, and PCoA plots, were performed with internal scripts.

### *2.5. Statistics (Viable Counts)*

Certain values (colony-forming units [cfu]) obtained for viable bacterial or fungal counts were reported as "<x cfu/ sample" or ">x cfu/ sample". To enable statistical analysis, we truncated these values to "x cfu/sample". Summary statistics were generated using the statistical programming language, R [18]. The mean cfu/sample (bacterial) and standard deviation (SD) of the mean in logarithmic form have been reported. For fungal counts, the mean cfu/sample and SD of the mean have been reported.

## 3. Results

Newly purchased, washed, and dried clothing items (T-shirts, underwear, socks, and baby onesies) were worn for a single day for ≥12 h by volunteers and then analyzed for viable bacterial and fungal burden using culture-based enumeration. Since the clothing items provided to the volunteers were not sterilized by (for instance) autoclaving, an initial baseline survey of the microbial burden of the clothing items was conducted for items purchased, washed, and dried as described above, but not worn by volunteers, using the methods described above. This included both enumeration of microbial counts and molecular analyses for identification of the baseline microbial burden of the clothing items.

### *3.1. Results of Baseline Microbial Survey of Unworn (Control) Clothing Items*

Table 1 displays the results of the microbial enumeration (bacteria, fungal) for control (unworn) clothing items.

**Table 1.** Bacterial and fungal counts from control clothing items (washed and dried but not worn).

| Control Clothing Item | Underwear | | T-Shirt | Socks | | Baby Onsie |
|---|---|---|---|---|---|---|
| | Mens | Womens | Unigender | Mens | Womens | Unigender |
| Total Bacterial cfu ($log_{10}$) [1] | 150 (2.18) | 750 (2.88) | 90 (1.95) | 930 (2.97) | 120 (2.08) | 240 (2.38) |
| Total Fungal cfu ($log_{10}$) | No data | No data | <30 (<1.48) | 30 (1.48) | <30 (<1.48) | <30 (<1.48) |

[1] cfu, colony-forming units isolated using media and incubation conditions appropriate for bacteria or fungi.

Baseline data for microbial enumeration from control clothing items indicated low numbers of isolated bacteria, relative to the numbers measured for worn items (reported below). No subtraction of baseline counts was performed in the bacterial count data provided below for worn clothing items.

### *3.2. Bacterial Viable Counts Recovered from Worn Clothing Items*

The results obtained for viable bacterial load are displayed in Figure 1. The highest bacterial counts were recovered from socks and underwear (average recovery: $8.2 \times 10^6$ cfu/sample and $1.2 \times 10^7$ cfu/sample, respectively), while recovered bacterial load from T-shirts was approximately 2-log-fold lower (average recovery: $8.3 \times 10^4$ cfu/sample). From baby onesies, an average recovery of $2.3 \times 10^6$ cfu/sample was obtained. High variability in determined bacterial loads from the various clothing items was observed (Figure 1). A sub-analysis of recovered bacterial load from clothing by wearers' gender did not disclose significant differences (Figure 1).

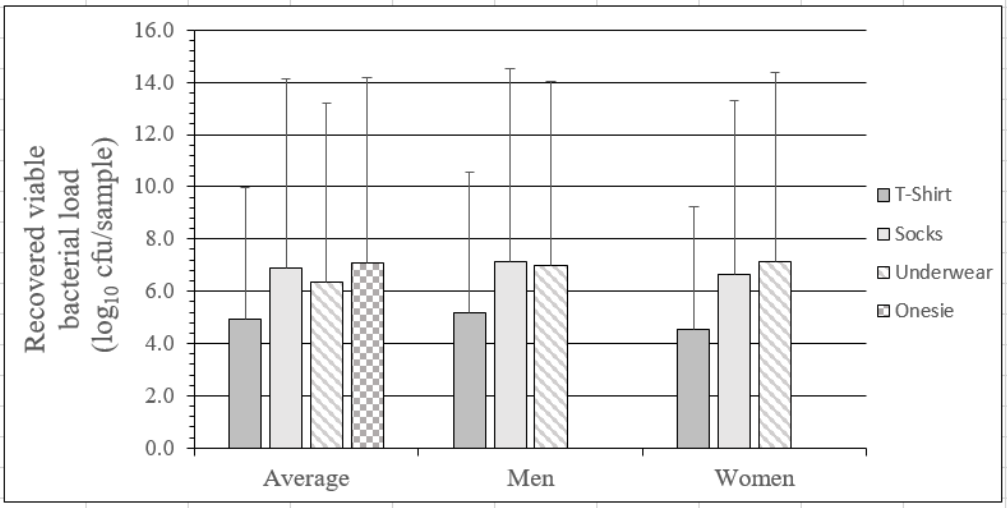


**Figure 1.** Bacterial load (mean ± SD; $n$ = 10 per gender for socks and T-shirts; $n$ = 5 per gender for underwear, and $n$ = 9 for baby onesies) in cfu/sample recovered from clothing items after being worn by volunteers for ≥12 h. No statistical differences were noted in cfu/sample between clothing items worn by men vs. women. Average values reflect pooled counts for items worn by male and female volunteers. Abbreviations used: cfu, colony-forming units; SD, standard deviation.

### *3.3. Bacterial Identities Determined by Sequencing*

Bacterial 16S rDNA sequencing was used to determine the species composition recovered from the various clothing item types (Figure 2). Phylogenetic clustering analysis revealed high levels of *Staphylococcus* species, *Propionibacterium* species, and *Streptococcus* species on most clothing samples (Figures 2 and 3). While those bacteria were recovered from all clothing types, a cluster of oral *Streptococci* species was noted for baby onesie samples (blue box, Figures 2 and 3) and, to a lesser degree, on T-shirts. A cluster of *Corynebacterium* species (purple box, Figure 2) was recovered uniquely from underwear samples. Recovered bacteria from control (washed but not worn) clothing items also were identified by sequencing (Figures S1 and S2). The most abundant sequences were for *Staphyloccus*, *Corynebacterium*, and *Propionibacterium* species.

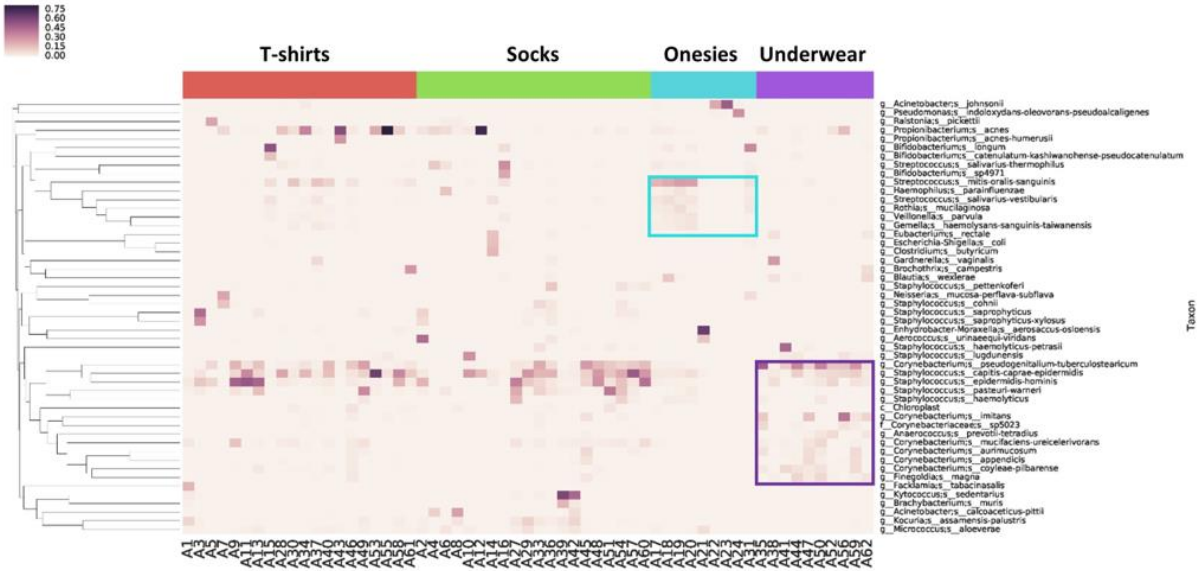


**Figure 2.** Identification and phylogenetic clustering of bacteria identified from clothing items worn by volunteers for ≥12 h. All samples were dominated by *Staphylococcus* species. Unique organism clusters are highlighted in blue and purple boxes for baby onesies and underwear, respectively.

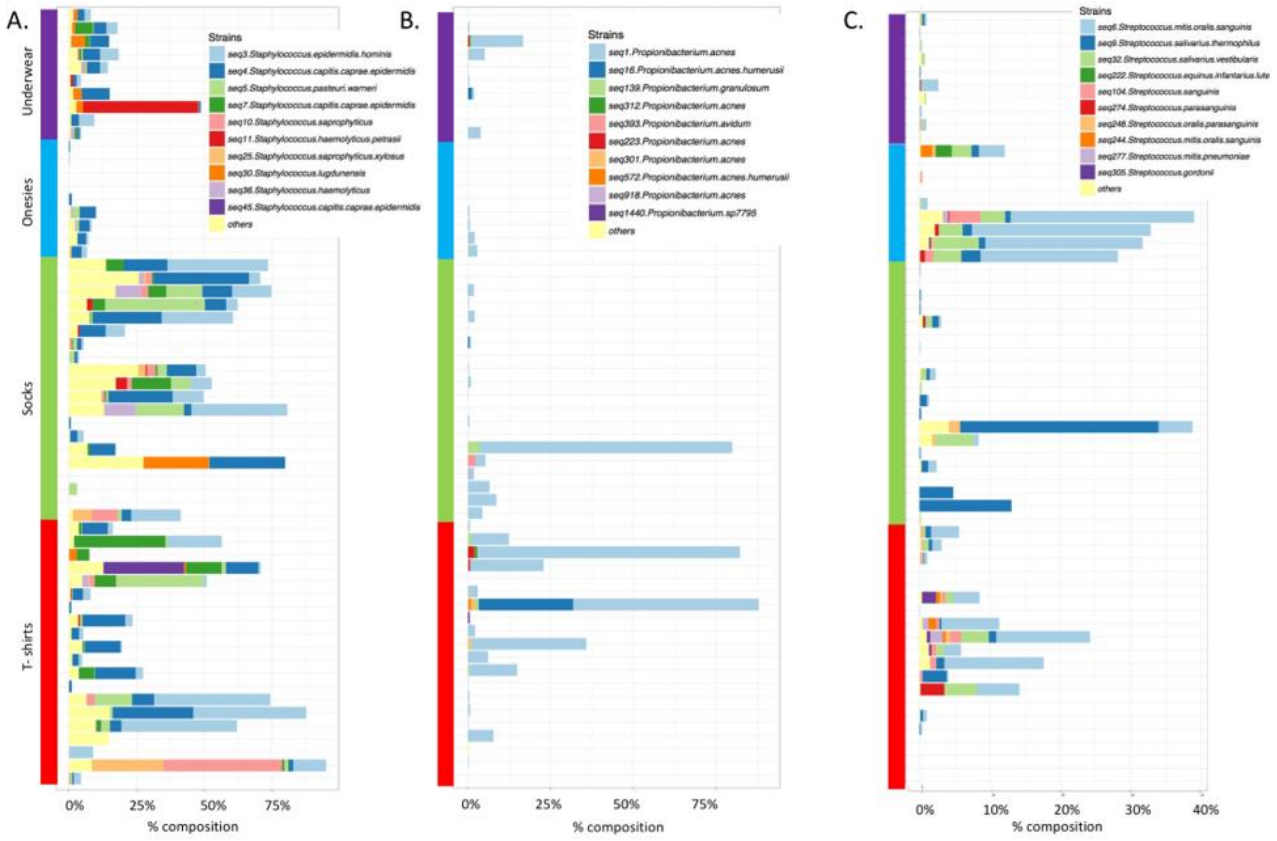


**Figure 3.** Abundance of dominant bacterial species recovered from clothing items worn by volunteers for ≥12 h. The most abundant genus of bacteria (on all clothing item types) was *Staphylococcus* (**A**). *Propionibacterium* species were variable in abundance among individual samples and were found either abundantly or were absent on T-shirts and socks (**B**). Significant levels of *Streptococcus* species, particularly those typically found in the oral environment, were recovered from T-shirts, socks, and baby onesies (**C**).

### *3.4. Fungal Viable Counts Recovered from Clothing Items*

See Table 1 for the fungal colony counts obtained from control clothing items. These generally were below the limit of detection of the assay. No subtraction of baseline counts was performed in the fungal count data provided below for worn clothing items.

Viable fungal counts from worn clothing items (Table 2) were several orders of magnitude lower than bacterial counts from the same clothing types. A low count (30 cfu/sample) was obtained for baby onesies in particular, while the highest counts were recovered from underwear (780 cfu/sample). Fungal counts recovered from socks and T-shirts were 569 cfu/sample and 291 cfu/sample, respectively.

### *3.5. Fungal Identities Determined by Sequencing*

Fungal ITS sequence analysis of worn clothing items revealed an abundance of fungal genera (Table 2), most of which were non-pathogenic commensal fungi. However, sequences corresponding to *Candida parapsilosis*, an opportunistic pathogen, were recovered from 10 of 20 T-shirt samples, 12 of 20 sock samples, and all (10/10) underwear samples. *Candida albicans* sequences were recovered from five T-shirt samples, and *Aspergillus fumigatus* sequences were recovered from five sock samples. In addition, sequences for *Clavispora*, *Acremonium*, and *Cryptococcus* were recovered from T-shirt samples. Sequences for *Ajellomyces-Histoplasma*, *Acremonium*, and *Cryptococcus* were recovered from sock samples, and sequences for *Ajellomyces-Histoplasma* and *Cryptococcus* were recovered from underwear samples. Sequences from *Acremonium* and *Cryptococcus* were recovered from

baby onesie samples (Table 2). These results suggest that opportunistic human pathogens may be present on clothing worn by volunteers for ≥12 h.

**Table 2.** Fungal counts recovered from worn clothing items, and identification using internal transcribed spacer (ITS) DNA sequencing.

| Clothing Item | Mean Viable Fungal Count (cfu/Sample) | Genera Identified |
|---|---|---|
| T-Shirt | 291 ± 845 | *Lasiodiplodia*, *Cladosporium*, *Alternaria*, *Aspergillus* [1], *Penicillium*, *Clavispora* [1], *Candida* [2], *Acremonium* [1], *Fusarium*, *Chaetomium*, *Rhodotorula*, *Cryptococcus* [2], *Naganishia*, *Cutaneotrichosporon*, *Trichosporon*, *Blakeslea*, *Poitrasia*, *Mucor* |
| Socks | 569 ± 1127 | *Lasiodiplodia*, *Cladosporium*, *Aureobasidium*, *Alternaria*, *Aspergillus* [1], *Penicillium*, *Ajellomyces-Histoplasma* [1], *Candida* [2], *Acremonium*, *Fusarium*, *Chaetomium*, *Rhodotorula*, *Cryptococcus*, *Naganishia*, *Trichosporon*, *Mucor*, *Pilobolus* |
| Underwear | 780 ± 556 | *Cladosporium*, *Alternaria*, *Aspergillus* [1], *Penicillium*, *Candida* [3], *Fusarium*, *Rhodotorula*, *Cryptococcus*, *Naganishia*, *Ajellomyces Histoplasma* [1], *Cutaneotrichosporon*, *Tricosporon*, *Mucor*, *Phycomyces*, *Rhizopus* [1] |
| Baby Onesie | 30 ± 0 | *Cladosporium*, *Aspergillus* [1], *Penicillium*, *Clavispora*, *Candida* [1], *Acremonium*, *Fusarium*, *Rhodotorula*, *Cryptococcus*, *Tricosporon*, *Mucor* |

[1] Denotes an opportunistic pathogenic species. [2] Denotes an opportunistic pathogenic species found in ≥50% of samples.[3] Denotes an opportunistic pathogenic species found in all samples from the given clothing type. Abbreviations used: cfu, colony-forming units.

## 4. Discussion

The potential for clothing items to serve as reservoirs for commensal and pathogenic bacteria and fungi has received attention in the past two decades [5–11,14,19–23]. This topic is relevant to infection prevention and control, since microbial transfer is possible in both directions, i.e., from the wearer to the clothing [19,21–25], and from the clothing to the wearer [23]. Re-aerosolization of pathogens, including bacteria and fungi, from contaminated clothing back to the indoor environment, may also occur [26,27]. In addition, there is a known potential for transfer of bacteria and fungi from clothing to washing machine surfaces (contributing to a washing machine microbiome) [21,24], and from the washing machine to clothing being laundered [8–11]. Knowledge of the abundances and species of microbes being transferred in these processes is needed in order to determine any potential health implications, as well as to determine strategies for mitigating any potential risk. In the present study, our intent was to evaluate the transfer of bacteria and fungi from volunteers to specific clothing items (T-shirts, socks, underwear, and baby onesies) during a short duration of wearing (≥12 h) through viable microbial enumeration techniques and sequencing-based identification techniques. Different clothing items were evaluated, since these items contact differing anatomical niches, each having unique characteristics (i.e., moisture levels, types of glandular secretions, etc.; see discussion below).

In terms of viable microbial loads, relatively high variability in obtained average colony counts were observed for each clothing item type evaluated in our study. The highest average bacterial and fungal cell counts were recovered from socks and underwear. Baby onesies also yielded relatively high average bacterial cell counts, but almost no fungal counts. T-shirts yielded lower average colony counts for bacteria and fungi. The differences in average microbial load observed for the various clothing types likely reflect the fact that warmer, more moist environments applicable to socks and underwear are capable of hosting greater microbial growth than the drier, cooler environments afforded by T-shirts. Differences in average colony counts for clothing worn by males or females were not statistically significant, likely due to the high variability in counts determined in the case of both genders. Smith et al. [22] reported that bacterial levels on the surface of garments worn by men were consistently higher than those for garments worn by women. These authors attributed the differences to the relatively higher skin surface areas of males vs.

females. The enumeration methodologies differed between the Smith et al. study and ours. Our studies were conducted by recovering microbes from clothing samples by extracting with PBS, while Smith et al. [22] based their conclusions on data from contact plates applied to the garments at various anatomical locations.

Bacterial 16S rDNA sequencing-based analysis of nucleic acid recovered from worn clothing items suggested that *Staphylococcus* species are universally present on worn clothes, as they are on skin. Although the species identified included members of the expected commensal skin flora, sequences for three species of coagulase-negative *Staphylococci* (*S. haemolyticus*, *S. lugdenensis*, and *S. saphrophyticus*) also were recovered. These opportunistic pathogens can cause skin and soft-tissue infections [28]. Moreover, *S. lugdenensis* and *S. haemolyticus* have been implicated in the production of sweat malodor [29]. Sequences for *Propionibacteria* were recovered primarily from socks and T-shirts, and not from underwear or baby onesies. This genus is able to grow on oily (sebaceous) skin sites, due to the bacteria's ability to produce free fatty acids (including propionic acid) which acidify the skin and prevent colonization by other microorganisms, including pathogens [30]. *Propionibacterium acnes*, the species for which sequences were recovered at the highest abundance, causes a variety of pro-inflammatory reactions including common acne [31].

Sequences for *Streptococcus* species were recovered abundantly from most clothing types sampled, with the exception of underwear. Members of this genus were particularly abundant on T-shirts and baby onesies, likely due to the fact that each of the *Streptococcal* species identified (*S. gordonii*, *S. salivarius*, *S. sanguinis*, *S. mitis*, *S. oralis*, *S. parasanguinis*, and *S. vestibularis*) are constituents of the normal flora found in saliva. These bacteria may have contaminated the baby onesies through drool. Interestingly, several other bacteria which are part of the oral flora were found on T-shirts and onesies, albeit at a lower abundance: for example, *Gemella* is a Gram-positive bacterium found in the oral mucosa, while *Rothia* is also a member of the oral flora, where it cause caries and more rarely, endocarditis in immunosuppressed individuals, and *Veillonella* is often found in symbiosis with *Streptococcal* species [32]. The finding of abundant sequences for this subgroup of bacteria derived from the niche oral environment underscores the ease with which worn clothing may become contaminated from specific body fluids.

Underwear was found to harbor a unique cluster of bacteria, not recovered from other clothing types. Sequences for bacteria belonging primarily to the genus *Corynebacterium*, but also including Gram-positive anaerobic species belonging to the family *Clostridiales* (*Finegoldia magna* and *Anaerococcus prevotii*) were recovered from worn underwear. *Corynebacterium* species are facultatively anaerobic actinobacteria which are commonly isolated from skin [33]. Although these are generally considered non-pathogenic, they may cause opportunistic infections in immunocompromised patients [33]. *Clostridiales* such as *Finegoldia* and *Anaerococcus* are also opportunistic, causing wound infections in diabetics, as well as other nosocomial infections [34].

Each of the clothing item types evaluated also were found to harbor fungi, including pathogenic fungal species. For instance, sequences for the pathogenic yeast *Candida parapsilosis* were recovered from >50% of T-shirt and sock samples, and 100% of underwear samples. *C. parapsilosis* is notorious for its ability to form biofilms on biotic and abiotic surfaces, and the frequent use of antifungals of the azole class (fluconazole in particular) has led to high rates of antifungal resistance in *C. parapsilosis* worldwide [35]. The frequency with which *C. parapsilosis* sequences were isolated from clothing items worn $\geq$12 h, and the known potential for fungi in general to transfer to sterile sentinel washcloths during clothes-washing processes [11], suggest that transfer of *Candida* between members of a household via the clothes-laundering process is a distinct possibility. Although *Candida* sequences, specifically, were not recovered during our study of potential microbial transfer from public washing machines to sentinel washcloths [11], Döğen et al. [36] isolated *C. parapsilosis* from 25 out of 99 (25%) swabbed domestic washing machines, especially from the rubber door seals. Similar results were obtained by Babič et al. [37], with 20% of sampled domestic washing machines being positive for *C. parapsilosis*.

Not surprisingly, our data demonstrate a relationship between the skin microbiome, the anatomical specificity of gland secretions, and the types of microbes recovered from specific clothing items worn for a short period of time (≥12 h). As the skin microbiome is known to change over time, during aging and in various disease states [12,13,38–41], one might expect the microbial loads recovered from worn clothing items to change accordingly. The recovery of opportunistic skin pathogens likely will reflect any existing disease states impacting the skin microbiome.

A few limitations to our study deserve discussion. One limitation is that the volunteers enlisted to wear the clothing items may not be representative of the greater population of the United States or of the world as a whole. This may bias the conclusions in a manner that underestimates the acquired burden of bacteria and/or fungi to be expected on the worn clothing items. For instance, clothing worn in very humid regions of the world may acquire greater fungal loads relative to those acquired in more temperate and dry regions. Secondly, our enumeration methodology was designed to detect culturable microbes. Microbes that might have been viable, yet unculturable, would not have been detected in the enumeration assays used. We used bacterial 16S ribosomal RNA (rRNA) gene sequencing to identify bacteria present on the clothing items and fungal ITS gene-targeted sequencing to identify fungi. Other investigators have used next-generation sequencing as part of microbiome identification to expand the reach of the analysis. This is especially useful for detecting new microbial species [42].

## 5. Conclusions

The results of this study demonstrate that even a limited duration of time being worn (≥12 h) can lead to accumulation of recoverable bacteria and fungi on clothing items, including T-shirts, socks, underwear, and baby onesies. In addition to the commensal microbes that were expected to be transferred to clothing on the basis of current knowledge of the skin microbiome, genomic sequences for certain opportunistic pathogenic microbes were also recovered from the worn clothing items. This finding extends previous reports describing the transfer of commensal and pathogenic bacteria and fungi during low-temperature washing cycles used during clothes laundering in domestic and public washing machines [2–6,10,11,24]. Specifically, the washing machine microbiome receives contributions from the worn clothing being laundered, as well as the water used [8–11,24]. Given consumer preference for low-temperature, eco-conscious, washing machine detergents [43], the "clothing microbiome" is likely to persist post laundering, to contribute to the machine microbiome, and to potentially transfer to the laundered clothing [10,11,24].

These considerations suggest that disruption of the continuous cycle of contaminating clothing during wear, contaminating the washing machine during laundering of worn clothing, and transfer of microbes from the washing machine to the clothing being washed, may play a role in preventing or mitigating risk of infection dissemination. This disruption may be achieved through use of higher temperature wash/rinse cycles, use of bleach or enzyme-containing detergents, or use of laundry sanitizers during the wash or rinse cycles. Such improvements in home and public laundering practices and detergents intended to minimize environmental impact while optimizing antimicrobial efficacy may help reduce the accumulation of opportunistic pathogens in clothing and therefore mitigate the associated risk to health.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres14030065/s1, Figure S1: Sequence identification to the genus level of genomic material recovered from control (blank) clothing items; Figure S2: Sequence identification to the species level of genomic material recovered from control (blank) clothing items.

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**Informed Consent Statement:** A general release of liability form was executed by each study volunteer and retained by Reckitt.

**Data Availability Statement:** Supporting data for this paper may be requested from the corresponding author.

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