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# Microbial Contents of Vacuum Cleaner Bag Dust and Emitted Bioaerosols and Their Implications for Human Exposure Indoors

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**Vacuum cleaners can release large concentrations of particles, both in their exhaust air and from resuspension of settled dust. However, the size, variability, and microbial diversity of these emissions are unknown, despite evidence to suggest they may contribute to allergic responses and infection transmission indoors. This study aimed to evaluate bioaerosol emission from various vacuum cleaners. We sampled the air in an experimental flow tunnel where vacuum cleaners were run, and their airborne emissions were sampled with closed-face cassettes. Dust samples were also collected from the dust bag. Total bacteria, total archaea, *Penicillium*/*Aspergillus*, and total *Clostridium* cluster 1 were quantified with specific quantitative PCR protocols, and emission rates were calculated. *Clostridium botulinum* and antibiotic resistance genes were detected in each sample using endpoint PCR. Bacterial diversity was also analyzed using denaturing gradient gel electrophoresis (DGGE), image analysis, and band sequencing. We demonstrated that emission of bacteria and molds (*Penicillium*/*Aspergillus*) can reach values as high as 1E5 cell equivalents/min and that those emissions are not related to each other. The bag dust bacterial and mold content was also consistent across the vacuums we assessed, reaching up to 1E7 bacterial or mold cell equivalents/g. Antibiotic resistance genes were detected in several samples. No archaea or *C. botulinum* was detected in any air samples. Diversity analyses showed that most bacteria are from human sources, in keeping with other recent results. These results highlight the potential capability of vacuum cleaners to disseminate appreciable quantities of molds and human-associated bacteria indoors and their role as a source of exposure to bioaerosols.**

People are constantly exposed to various levels of biological (bioaerosols) and nonbiological particles. The nature and magnitude of these exposures depend strongly on their sources. While nonbiological particles from vehicle emissions, industrial processes, and natural sources are comparatively well characterized, bioaerosols are less well understood, despite their potentially significant role as a cause of infectious and allergenic adverse health effects (1). This is especially true of indoor bioaerosols, which are particularly relevant, as most people spend the vast majority (~90%) of their time indoors. The use of high-quality vacuum cleaners and bags is often recommended in order to reduce indoor allergen exposure of asthmatic and allergic people (2). However, it has been shown that vacuuming can also promote the release of large concentrations of antigens by mechanical disturbance of settled dust and release from the vacuum cleaner itself (3).

Household dust can contain a wide range of microbial content, including endotoxins and molds (4). Vacuum bags can be an important reservoir of bacteria, molds, endotoxins, and allergens. The emission and aerosolization of dust during vacuuming can potentially spread *Salmonella* spp. (5, 6) and other bacteria, including *Clostridium botulinum* (7). Environmental dust could be a source of gastrointestinal infection in the home environment, and the causative microbes collected during vacuum cleaning can remain viable in vacuum dust bags or chambers over extended periods (8).

Archaea are microorganisms commonly found in the environment, including in the animal and human gut, with a strong immunogenic potential (9). Since those organisms can be found in high concentrations in animal care facilities (10, 11), they could be found in vacuum dust from homes with animals or more crowded environments. We have previously shown that emission rates of bacteria and fine and ultrafine particles in the exhaust air of various vacuum cleaners are highly variable (12). In addition to releasing allergens and antigens from the surfaces vacuumed, our previous work also suggested that vacuum bag dust and exhaust air may be a source of bioaerosol exposure through bioaerosol emission during vacuuming (12). Measuring the release of bioaerosols from vacuum cleaners requires a specific approach distinct from techniques used to measure dust resuspension from carpets during vacuuming. Perhaps due to this, however, we are unaware of any other data that have quantified vacuum emissions in terms of their microbial diversity or magnitude, despite their potential role in terms of infection transmission or allergic sensitization.

In order to better understand the characteristics of vacuum dust microbiology and indoor bioaerosol sources, the objectives of this study were to evaluate the microbial load in vacuum bag dust and emitted air samples during vacuum operation. Specifically, we focused on total bacteria, bacterial diversity, total archaea (10), *Penicillium*/*Aspergillus* genera, and *Salmonella* spp., as well as *Clostridium* cluster 1 concentrations in dust and air. The presence of antibiotic resistance genes and the *Clostridium botulinum* toxin

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gene was also investigated. We focused specifically on the latter 3 microbes due to their potential to cause adverse health effects following inhalation.

## MATERIALS AND METHODS

**Vacuum cleaner characteristics.** We sourced 21 vacuums from staff and students at the Queensland University of Technology. Three of these were commercial models used by professional cleaners. The owner-reported age ranged from 6 months to 22 years, and they were priced from AUD $75 to $800. Specific details of the vacuums tested are described in Knibbs et al. (12). As we aimed to test vacuums representative of real-world use, we did not remove existing dust prior to testing, and all vacuums were tested as supplied by the owner. The dust content in the bag or collection chamber (%) was estimated visually and ranged from 0 to 90%.

**Instrumentation.** Airborne samples were taken using 37-mm closed-face cassettes loaded with polytetrafluoroethylene (PTFE) filters (SKC Inc., Pennsylvania, USA). A fixed flow rate of 11 liters $min^{-1}$ was used, and sampling duration ranged from 4.5 to 10 min. The collected samples, in addition to blanks, were used for DNA extraction. Dust samples were collected from the bag or chamber and stored in 15-ml Falcon tubes prior to analysis.

**Measurements.** A 0.5-m-diameter clean airflow tunnel custom built for investigation of aerosol sources was employed to measure bioaerosol emissions from the vacuum cleaners. The system and our experimental setup have been described previously (12). In brief, HEPA-filtered air was introduced upstream of the vacuum cleaner, and its emissions were carried 2 m downstream by the filtered airflow to the tunnel exit. Approximate uniformity of air mixing at the tunnel exit was confirmed by smoke visualization, and all sampling probes and inlets were located there. The velocity and temperature of air at the tunnel exit were measured by a TSI 9535 VelociCalc hot-wire anemometer (TSI Inc., St. Paul, MN) and TSI 8554 QTrak Plus (TSI Inc., St. Paul, MN), respectively. The tunnel was maintained at a slightly higher pressure than the surrounding environment to prevent intrusion of room air.

The air velocity at the tunnel exit was set to 0.7 m $s^{-1}$ but varied between tests as a result of the individual vacuum being measured. It was therefore not possible to achieve isokinetic sampling, which is of relevance to particles in the typical size range of airborne bacteria. Accordingly, we calculated the deviation from isokinetic sampling (i.e., 100% aspiration efficiency) of the closed-face filter used for bacterial sampling. Aspiration efficiency ranged from >90% for particles <4 μm to 50% for 12-μm particles (12).

**Experimental protocol.** Our experimental approach is presented in detail in the report by Knibbs et al. (12). In brief, we took approximately 25 g of dust from each vacuum's dust bag or chamber, where available. Each vacuum was switched on and run for 10 to 15 min in the HEPA-filtered flow tunnel with no hoses or attachments connected. Due to an absence of *a priori* knowledge regarding the determinants of emission, we assessed two different running conditions to investigate the role of vacuum temperature: (i) cold start (sample a) and (ii) warm start (sample b). For cold starts, the vacuum cleaner had not been used previously on the test day. To mimic normal usage, a warm-start test directly followed the cold-start test (i.e., representing the vacuum being turned off, immediately moved to another room, and turned on again). Mean emission rates of bacteria and molds during each test were calculated using the method described by Knibbs et al. (12).

**DNA extraction from air and dust samples.** Each filter was placed in a 15-ml Falcon-type tube (Corning) that was eluted in 1.5 ml of phosphate-buffered saline (PBS) and 0.05% Tween 20. Samples were centrifuged at 21,000 × *g* for 10 min to pellet the bacterial cells. Supernatant was discarded, and the pellet was used for DNA extraction using a Qiagen DNeasy (Qiagen, Mississauga, Ontario, Canada) column as described by the manufacturer. DNA was eluted in a total of 50 μl of Tris-EDTA (TE) buffer and kept at −20°C for a few weeks until PCR analysis.

Dust collected from the bags or chambers was sieved, and only the fine portion of dust was kept for nucleic acid extraction. One hundred milligrams of sieved dust was resuspended in 3 ml of PBS-0.05% Tween 20 and then homogenized using a multipulse vortexer (Glas-Col; Terre-Haute, IN, USA) for 1 min. Samples were left on the bench for a few minutes to let bigger particles settle. One milliliter of the supernatant was used for DNA extraction using the same protocol as that used for filters. DNA was eluted in a final volume of 50 μl of TE buffer and kept at −20°C for a few weeks until PCR analysis, representing DNA from 33 mg of dust.

**Quantitative real-time PCR.** Real-time quantitative PCR (qPCR) was performed on a DNA Engine Opticon 2 (Bio-Rad, Mississauga, Canada). Data were acquired using the Opticon monitor software (Bio-Rad; version 2.02.24). The threshold was determined by the software, and the standard deviation was set to 1.

qPCR for the archaeal 16S rRNA gene optimized for archaeon-only amplification without bias for any archaeal phylum (10) was performed using 0.5 μM $liter^{-1}$ of A751F and A976R primers (13, 14), 12.5 μl of iQ SYBR green supermix (Bio-Rad Laboratories, Hercules, CA, USA), and 2 μl DNA template in a 25-μl reaction mixture. The thermoprotocol involved one hold at 94°C for 5 min and then 35 cycles of 94°C for 10 s, 55.5°C for 20 s, and 72°C for 25. Cycles were followed by one hold at 72°C for 10 min. The plate read was done during the 55°C annealing step. A melting curve program was run to detect amplicon specificity, using the following program: 40°C to 94°C, read every 0.2 s, hold for 1 s. Samples were considered positive for archaeal 16S DNA when the melting temperature was around 88°C. Tenfold serial dilutions of methanogenic archaeon *Methanosarcina mazei* DNA (ATCC BAA-159D) were used for a standard curve. Data were acquired using the Opticon monitor software (Bio-Rad; version 2.02.24).

In the total bacterial qPCR, 16S rRNA gene fragments were amplified using primers EUBf and EUBr with dual-labeled probe EUBp (15). The 25-μl PCR mix contained 12.5 μl of IQ supermix (Bio-Rad), 1 μM $liter^{-1}$ of each primer, 0.5 μM $liter^{-1}$ of probe, and 2 μl of DNA extract or serial dilutions of TOPO-TA cloning vector (Invitrogen, Carlsbad, CA, USA) carrying an *Escherichia coli* ATCC 25922 16S rRNA gene fragment (1,320 bp) as the standard (from $10^2$ to $10^7$ copies per reaction). The thermoprotocol involved an initial denaturation step of 3 min at 94°C, followed by 40 cycles at 94°C for 15 s and 62°C for 2 min before fluorescence reading. Following this protocol, we calculated the number of 16S rRNA gene fragments in the initial samples. The results are expressed in total number of bacterial cell equivalents, assuming one 16S per bacterial genome.

For total *Penicillium* and *Aspergillus* quantification, total *Penicillium*/*Aspergillus* 18S rRNA gene fragments were amplified using the PenAsp assay (www.epa.gov/microbes/moldtech.htm). The 25-μl PCR mix contained 12.5 μl IQ supermix (Bio-Rad), 1 μM $liter^{-1}$ of each primer, 0.5 μM $liter^{-1}$ of probe, and 2 μl of DNA extract or serial dilutions of DNA of *Penicillium chrysogenum* isolated from air in a sawmill industry as the standard (from $10^1$ to $10^7$ copies per reaction). The thermoprotocol involved an initial denaturation step of 3 min at 94°C, followed by 40 cycles of 94°C for 15 s and 60°C for 1 min before fluorescence reading. Following this protocol, the number of 18S rRNA gene fragments in the initial samples was calculated. To simplify the presentation of results, they are expressed as *Penicillium*/*Aspergillus*, assuming one 18S per mold genome.

For *Clostridium* cluster 1 quantification, 16S rRNA gene fragments were amplified using primers and probe as described by Nakamura (16). The 25-μl PCR mix contained 1× IQ supermix (Bio-Rad), 1 μM $liter^{-1}$ of each primer, 0.5 μM $liter^{-1}$ of probe, and 2 μl of DNA extract or serial dilutions of DNA from *Clostridium perfringens* (ATCC 13124) as the standard (from $10^1$ to $10^7$ copies per reaction). The thermoprotocol involved an initial denaturation step of 3 min at 94°C, followed by 40 cycles of 94°C for 15 s and 60°C for 1 min before fluorescence reading. Following this protocol, the number of 16S rRNA gene fragments in the initial samples was calculated. To simplify the results presentation, the results are expressed in total *Clostridium*, assuming one 16S per cell genome.

**Endpoint PCR for detection of *Salmonella*.** PCR primers targeting all *Salmonella* species were used to amplify DNA from both dust and filter

**TABLE 1** Antibiotic resistance genes targeted by endpoint PCR

| Antibiotic | Target gene(s) | Reference |
|---|---|---|
| Erythromycin | *ermA*, *ermB*, *ermF* | Chen et al. (30) |
| Vancomycin | *vanA* | Dutka-Malen et al. (31) |
| Virginiamycin | *vatD* | Thibodeau et al. (32) |
| Tetracycline | *tetA*, *tetC*, *tetG* | Yu et al. (33) |
| Tetracycline | RPP genes (*tetM*, *tetO*, *tetP*, *tetQ*, *tetS*, *tetT*, *tetW*) | Yu et al. (33) |

samples (17). For each amplification, the PCR mixture (50 μl) contained 1× GoTaq PCR buffer, 0.5 μM of each primer, 2.5 mM of $MgCl_2$, 200 μM of each deoxynucleoside triphosphate (dNTP), 1.25 U of Promega GoTaq polymerase, and 2.5 μl of the DNA extract. The thermoprotocol was taken from the primer reference. DNA from *Salmonella choleraesuis* ATCC 13076 was used as a positive control. Positive amplification was evaluated by migrating a 5-μl subsample on a 2% (wt/vol) agarose gel, at 60 V for 100 min. Amplicon length was estimated by comparison to an EZ Load precision molecular mass ruler (Bio-Rad). Gels were stained with SybrSafe (Invitrogen, Mississauga, Canada) using final concentration of 1× and visualized under UV light on a Chemigenius 2xe (Syngene, Frederick, MD, USA) photo documentation system.

**Endpoint PCR for the detection of antibiotic resistance genes.** Samples from dust and filters were tested for multiple antibiotic resistance genes using endpoint PCR (Table 1). All PCRs were done with primers (see the references shown in Table 1). For each amplification, the PCR mixture (50 μl) contained 1× GoTaq PCR buffer, 0.5 μM of each primer, 2.5 mM of $MgCl_2$, 200 μM of each dNTP, 1.25 U of Promega GoTaq polymerase, and 2.5 μl of the DNA extract. Positive amplification was evaluated by migrating a 5-μl subsample on a 1% (wt/vol) agarose gel, at 60 V for 60 min. Amplicon length was estimated by comparison to an EZ Load precision molecular mass ruler (Bio-Rad). Gels were stained as described above.

**Detection of the *Clostridium botulinum* toxin gene.** A multiplex PCR assay was done to detect and identify the presence of *Clostridium botulinum* types A, B, E, and F. Experimental conditions were the same as those described previously (18).

**Bacterial biodiversity using the PCR denaturing gradient gel electrophoresis (DGGE) approach and cluster analyses.** The variable V3 regions of 16S rRNA gene sequences were amplified (177 bp) by PCR using GC-341f and 518r primers (19). DNA extracts from dust and filters were amplified using a PCR mixture (50 μl) containing 1× GoTaq PCR buffer, 0.5 μM of each primer, 2.5 mM of $MgCl_2$, 200 μM of each dNTP, 2% dimethyl sulfoxide (DMSO), 1.25 U of Promega GoTaq polymerase, and 2 μl of the DNA extract. Amplification was realized using a denaturation step at 94°C for 5 min followed by 10 cycles of 1 min at 94°C, 1 min at 56°C, and 30 s at 72°C, with a 0.5°C touchdown every second cycle during annealing, followed by 20 cycles with an annealing temperature of 51°C and a final cycle of 5 min at 72°C.

After gel electrophoresis (1.5% [wt/vol] agarose gel) of 5-μl subsamples of the PCR products, the amount of amplified DNA was quantified by comparing band intensities to standard curves obtained with an EZ Load precision molecular mass ruler (Bio-Rad). Gels were stained as described above.

Band intensities were measured with Gene Tools analysis software (SynGen, Cambridge, United Kingdom). Profiles of the amplified 16S rRNA gene sequences were produced by DGGE as described by Muyzer et al. (19) using the Dcode (Bio-Rad). PCR products (100 ng) were loaded onto an 8% polyacrylamide gel in 0.5× Tris-acetate-EDTA (TAE) buffer (Bio-Rad) with a 30 to 60% denaturant gradient (100% denaturant was 7 mol urea and 40% [vol/vol] deionized formamide). The electrophoresis was carried out in 0.5× TAE buffer at 60 V for 16.5 h at 60°C. The DNA fragments were stained for 15 min in 0.5× TAE buffer with SYBR Gold (Molecular Probes, Eugene, OR, USA). Gels were washed twice in 0.5× TAE buffer for 15 min. Images of the gels were acquired using the imaging system Chemi-Genius 2 (SynGen) and the imaging software GeneSnap (SynGen). DNA from bands was carefully picked using a sterile tip and transferred into a 50-μl PCR mixture. Components in the PCR mix were the same as for the PCR DGGE, except for primer 341f that was without a GC clamp. The thermoprotocol was similar to the one described earlier. PCR products were visualized by agarose gel electrophoresis and sequenced on ABI 3730xl (Applied Biosystems, USA) by the CHUL Research Center (CRCHUL) sequencing and genotyping team.

GelCompar II version 6.5 (Applied Maths, Belgium) was used to normalize and compare all the DGGE profiles using hierarchical clustering to join similar profiles into groups (20). For this purpose, all the images of DGGE gels were matched using bands present in all samples. A tolerance in the band position of 1% was applied. The similarity among profiles was calculated with the Jaccard similarity coefficient, and the clustering was performed with the unweighted pair group method using arithmetic averages (UPGMA). Each DNA sequence obtained was compared to sequences available in databases, using BLASTN (21) from the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/).

## RESULTS

Emission rates (14 cold and 13 warm tests) and bag dust data (14 samples) were obtained from the majority of vacuums in our test group. In some cases, operational issues precluded airborne sampling. In other cases, absence of dust in the bag or reservoir did not allow the collection of the dust sample. Nevertheless, we collected sufficient data to address the study aims.

As shown in Table 2, bacterial emission rates from cold and warm vacuums ranged from below the detection limit (8a) to a maximum of $7.40 \times 10^5$ (3b) bacterial cell equivalents $min^{-1}$, with a mean of $1.21 \times 10^5$ and a median at $4.07 \times 10^4$. Mold emission rates showed more variability, ranging from below the detection limit for many bags (16 out of 30) to $6.50 \times 10^6$ (13b) mold cell equivalents $min^{-1}$, with a mean value of $3.32 \times 10^5$ and a median value of 0. There was no correlation between bacterial and mold emission rates. There was no archaea detected in any air or dust samples.

PCR quantification of bacteria in dust showed small variation (Table 3). The mean value of bacteria was $4.71 \times 10^6$ bacterial cell equivalents/g of dust (median value, $2.36 \times 10^6$), suggesting homogeneity in bacterial load in vacuum dust and little impact of users, environments, and dust age. The same observation was made for mold concentrations, with a mean value of $2.06 \times 10^7$ mold cell equivalents (median value, $4.62 \times 10^6$). *Clostridium* cells were also quantified, and only 28.6% (4 out of 14) of the sampled bags were positive, with a mean value of $3.25 \times 10^5$ cell equivalents and a median value of $2.45 \times 10^5$. No *C. botulinum* nor *Salmonella* spp. were detected from any air or dust samples.

Antibiotic resistance gene detection was performed for both dust and air samples. No positive samples were observed in dust for ErmA, Erm F, TetG, ribosomal protection protein (RPP), VanA, and VanD genes. The following samples were positive for one or 2 genes: sample 3 (ErmB and TetA/C genes), sample 10 (ErmB gene), sample 17 (ErmB gene), and sample 18 (ErmB and TetA/C genes). Antibiotic resistance genes from air samples were also negative for ErmA, ErmF, RPP, and VanA genes. However, positive air samples were detected: sample 4a (TetA/C genes), sample 6a (TetA/C genes), sample 9b (TetA/C genes), sample 10a (TetA/C genes), sample 13b (TetA/C and VanD genes), sample 14a (ErmB gene), sample 14b (TetA/C genes), sample 17a (TetG

**TABLE 2** Emission rates for bacteria and molds (*Penicillium*/*Aspergillus*) for cold (a) and warm (b) tests

| Test | ER (cell equivalents $min^{-1}$)[a] | |
|---|---|---|
| | Bacteria | Mold |
| 1a | 8.23E4 | BD |
| 1b | 1.21E5 | 3.95E5 |
| 3a | 5.17E4 | BD |
| 3b | 7.40E5 | BD |
| 4a | 9.87E2 | 5.52E4 |
| 4b | 4.58E4 | BD |
| 5a | 1.39E5 | BD |
| 5b | 8.78E3 | 7.08E4 |
| 6a | 7.58E3 | BD |
| 7a | 1.85E4 | 6.43E5 |
| 7b | 7.32E4 | BD |
| 8a | 0.00E0 | BD |
| 8b | 1.72E4 | 8.18E4 |
| 9a | 5.15E3 | 6.90E5 |
| 9b | 8.01E3 | BD |
| 10a | 6.17E4 | 1.02E5 |
| 10b | 3.63E4 | 6.48E5 |
| 12a | 4.07E4 | 1.48E5 |
| 13a | 4.73E5 | BD |
| 13b | 3.36E3 | 6.50E6 |
| 14a | 2.25E5 | 8.49E4 |
| 14b | 4.13E4 | BD |
| 15a | 4.36E3 | 3.76E4 |
| 15b | 3.94E5 | BD |
| 17a | 6.40E5 | 1.70E5 |
| 17b | 1.96E5 | BD |
| 18a | 1.52E4 | BD |
| 19a | 3.88E4 | BD |
| 19b | 6.12E3 | BD |

[a] BD, below detection limit; ER, emission rate.

gene), and sample 19b (TetA/C genes). Blank filters were negative for all antibiotic resistance genes tested.

The Jaccard similarity coefficient showed that there was low similarity between DGGE profiles of vacuum bags included in this study. There was no correlation in the bacterial diversity between corresponding air samples (a and b) and between the air samples and corresponding dust sample. A total of 13 ribotypes were sequenced (Table 4). All four ribotypes present in air samples were also present in some vacuum bags. All ribotypes belonged to a total of six different phyla and classes: *Firmicutes* (5/13), *Deltaproteobacteria* (2/13), *Alphaproteobacteria* (2/13), *Proteobacteria* (2/13), *Actinobacteria* (1/13), and *Bacteroidetes* (1/13).

**TABLE 3** Bacteria, molds (*Penicillium*/*Aspergillus*), and *Clostridium* cluster I cell equivalents per gram of dust from vacuum cleaner dust bags

| Sample no. | No. of cell equivalents/g of dust[a] | | | | | |
|---|---|---|---|---|---|---|
| | Bacterial content (mean) | SD | Mold content (mean) | SD | *Clostridium* content (mean) | SD |
| 1 | 2.67E6 | 1.85E5 | 2.98E7 | 2.05E7 | NA | |
| 3 | 1.62E6 | 1.08E5 | 4.78E6 | 6.45E5 | BD | |
| 4 | 1.02E6 | 1.98E5 | 8.39E7 | 1.56E7 | NA | |
| 5 | 1.59E7 | 1.30E7 | 4.66E7 | 1.09E7 | NA | |
| 7 | 3.84E6 | 3.76E6 | 4.50E7 | 9.68E5 | 2.91E5 | 8.71E3 |
| 9 | 1.26E7 | 3.34E6 | 1.39E7 | 3.52E6 | NA | |
| 10 | 2.04E6 | 2.82E5 | 3.41E6 | 1.19E6 | NA | |
| 12 | 1.39E6 | 7.20E5 | 4.51E7 | 2.07E7 | 6.19E5 | 1.20E5 |
| 13 | 1.79E6 | 5.67E4 | 2.79E6 | 1.52E6 | NA | |
| 14 | 2.91E4 | 0.00E0 | 1.58E6 | 3.02E5 | NA | |
| 15 | 1.52E6 | 2.30E5 | 4.46E6 | 1.20E6 | NA | |
| 17 | 3.44E6 | 1.52E6 | 2.90E6 | 6.71E5 | 1.98E5 | 1.13E4 |
| 18 | 3.44E6 | 6.30E5 | 3.93E6 | 3.08E6 | 1.91E5 | 6.08E4 |
| 19 | 1.47E7 | 8.75E6 | 0 | | NA | |

[a] BD, below detection limit; NA, not applicable.

**TABLE 4** Closest affiliations of ribotypes from bands in DGGE profiles from dust and air samples

| Ribotype | Frequency of ribotype per: | | | Organism with most similar sequence | % similarity |
|---|---|---|---|---|---|
| | Cold test | Warm test | Dust sample | | |
| 1 | 0 | 0 | 2 | *Staphylococcus epidermidis* | 94 |
| 2 | 0 | 0 | 4 | *Staphylococcus aureus* | 100 |
| 3 | 0 | 0 | 3 | *Staphylococcus epidermidis* | 92 |
| 4 | 3 | 5 | 11 | *Staphylococcus aureus* | 92 |
| 5 | 0 | 0 | 5 | *Staphylococcus hominis* | 97 |
| 6 | 0 | 0 | 5 | *Psychrobacter arcticus* | 92 |
| 7 | 1 | 1 | 12 | *Haloanella gallinarum* | 100 |
| 8 | 0 | 0 | 1 | *Pseudomonas luteola* | 94 |
| 9 | 2 | 1 | 12 | *Corynebacterium tuberculostearicum* | 100 |
| 10 | 0 | 0 | 9 | *Azorhizobium caulinodans* | 98 |
| 11 | 0 | 0 | 3 | *Xanthobacter autotrophicus* | 99 |
| 12 | 0 | 1 | 3 | *Azorhizobium caulinodans* | 99 |
| 13 | 3 | 1 | 3 | *Moraxella osloensis* | 100 |

## DISCUSSION

Understanding exposure to bioaerosols is a complex task. Humans encounter several sources of biological contaminants throughout their activities, and even though some health outcomes can be clearly linked to bioaerosol exposure, others are less clear. This is due partly to a lack of information describing the characteristics of bioaerosols encountered during activities which can lead to exposure. Vacuum cleaning may be one such activity. It has previously been shown to cause resuspension of settled dust and consequent exposure to dust and allergens. In the present study, we have shown that the microbial diversity of vacuum bag dust and bioaerosol emissions can be marked, although the two may bear little similarity in keeping with our previous results for total bacteria in bag dust and emissions (12).

As early as in the 1950s, vacuum dust microbial content was analyzed in the context of *Salmonella* infection in infants (5). During an outbreak in a hospital ward, the authors found that the sole source of the infectious agent was the dust bag of the vacuum floor polisher. Despite this interesting report, the literature in this area is very sparse, and no data describing transmission potential are available. Hypotheses have also been formulated regarding the possible role of household dust content in cases of infant botulism (7). The dust found indoors could act as a vehicle for infant botulism infection that can have severe consequences, such as sudden infant death syndrome (7).

House dust microbial content is commonly reported (22, 23) in addition to particle emissions from vacuum cleaners (24–27), but no studies have quantified emissions of microbial content from vacuum cleaners. A recent study used molecular biology to study the bacterial content of house dust collected with a vacuum cleaner (28). They used chemical, culture, and qPCR markers to determine that the average load of total bacteria (qPCR) is around 7E5 cell equivalents/mg of dust. Our study found about 100 times

less (4E6 16S/g of dust). It is important to remember that, in our study, used vacuums were brought to the lab, and we did not collect information about the age of the bags' contents or their provenance. Various sources of dust (e.g., sand, food debris, etc.) are likely to lead to different bacterial content. In the Karkkainen et al. study (28), hundreds of households were enrolled in an environmental exposure assessment, and they were asked to vacuum the whole house during a limited period. Our study approach may better capture real-world situations where vacuum content may vary quite a lot and subsequent exposures will depend on the vacuum type. The bacterial and mold content of our vacuum dust samples was very constant, suggesting little impact of users or vacuum models. The microbial content and diversity were very high, and given the emission rates, the content is likely to become and remain airborne. Vacuum emissions could potentially lead to short and more intense bioaerosol exposures than those due to resuspension of settled dust (12).

Bacterial diversity of the vacuum dust is in accordance with that expected, and several human-related taxa were found. Human skin and hair have been shown to be strong sources of bacteria in floor dust and air indoors, which can be readily resuspended and inhaled (25). Our results show that although vacuum operation is typically brief, vacuum emissions can release appreciable quantities of human-derived bacteria. Such emissions could potentially lead to inhalation of infectious or allergenic aerosols.

The mold emission rate was higher in several cases, and this observation did not correlate with the dust content. Dust containing higher mold concentrations does not necessarily lead to higher emission rates, in keeping with our previous results for bacteria (12). The vacuum characteristics here are likely to be the main predictor of emission, rather than dust content. However, isolating the vacuum-specific determinants of emission was not our focus in this study. The building history of the vacuum cleaner owner's home was not recorded, so we were unable to assess the role of factors such as water damage, mold problems, pets, or other indoor sources of bioaerosol. These results have highlighted the need for further work addressing these issues. The assessment of resuspension and emissions under real-world usage would also be useful in better defining determinant factors.

Vacuums can represent a reservoir for antibiotic resistance genes, and those genes may remain stable and were detectable using a molecular approach in this study, in keeping with previous results for bacteria (8). The possible impact of this reservoir on spreading of drug resistance to humans is unclear. Even though no quantitative data are available for antibiotic resistance gene emission while vacuuming, the observed emission rates for bacteria might suggest that the genetic content of those bacterial cells, including antibiotic resistance genes, may contribute to indoor bioaerosol exposure. Previous research reported a reduction of the viable microbial load in carpets of 87% using UV-C-equipped vacuum cleaners (29). However, reducing the viability of microbes does not interfere with allergenic properties or genetic transfer of antibiotic resistance genes, which indicates possible scope for development of additional technologies to mitigate indoor sources of potentially allergenic material.

In summary, our study demonstrated that vacuum emissions may be a source of bioaerosols that are complex in source, nature, and diversity. This exposure source is underrepresented in indoor aerosol and bioaerosol assessment and should be considered, especially when assessing cases of allergy, asthma, or infectious diseases without known environmental reservoirs for the pathogenic or causative microbe.

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