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Major article

Evaluation of bacterial contaminants found on unused paper towels and possible postcontamination after handwashing: A pilot study

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Background: Bacterial contamination is a concern in the pulp and paper industry. Not only is the machinery contaminated but also can be the end-paper products. Bacterial transmission from unused paper towels to hands and surfaces is not well documented.

Methods: The culturable bacterial community of 6 different unused paper towel brands was determined by culture methods and by sequencing the 16S ribosomal DNA of bacterial contaminants. Next, we investigated the possible airborne and direct contact transmissions of these bacterial contaminants during hand drying after washing.

Results: Between 10^2 and 10^5 colony-forming units per gram of unused paper towels were isolated from the different paper towel brands. Bacteria belonging to the *Bacillus* genus were by far the most abundant microorganisms found (83.0%), followed by *Paenibacillus* (15.6%), *Exiguobacterium* (1.6%), and *Clostridium* (0.01%). Paper towels made from recycled fibers harbored between 100- to 1,000-fold more bacteria than the virgin wood pulp brand. Bacteria were easily transferred to disposable nitrile gloves when drying hands with paper towels. However, no evidence of bacterial airborne transmission was observed during paper towel dispensing.

Conclusion: This pilot study demonstrated that a large community of culturable bacteria, including toxin producers, can be isolated from unused paper towels and that they may be transferred to individuals after handwashing. This may have implications in some industrial and clinical settings as well as in immunocompromised individuals.

Paper towels are made from raw and recycled materials containing cellulose, lignin, and other nutrients suitable for the growth of microbes present in papermaking environments. Bacteria are frequently isolated from paper and board mills worldwide, including India,[1] Canada,[2] United States,[3] New Zealand,[4] Finland,[5-8] France,[8] Germany,[8] and Spain.[7,8] Strains from the genera *Aeromonas, Bacillus, Enterobacter, Microbacterium, Pseudomonas*, and *Staphylococcus* have been isolated from papermaking machinery.[2,9] These bacteria likely contribute to slime (biofilm) formation in the machinery and, thus, diminish the production efficiency by causing corrosion and sheet defects.[10] The main sources of microbiological contamination in paper mills are the recycled waters, the raw materials used, parts of the machinery, and the factory environment.[11] The extreme temperatures in the mill dryers probably kill most, if not all, microbial cells, but heat-resistant spores would likely survive and thus contaminate the machinery as well as the finished paper products.

Studies previously established that the main microbial contaminants in paper products belong to the spore-forming genera *Bacillus* and *Paenibacillus*.[8,12,13] Indeed, because of their high resistance to a wide range of chemical and physical agents,[14] *Bacillus* spores may survive the various procedures encountered in the papermaking process. It is worth mentioning that harmful toxin-producing *Bacillus* species were also detected in paper mills.[6]

Recently, some of the authors of this study supervised a handwashing experiment in a large (>100) undergraduate microbiology

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Table 1
Test paper specifications and concentrations of the culturable bacteria found on test paper

Test paper	Manufacturer	Type	Dimensions per sheet (cm × cm)	Mass per sheet (±0.1 g)	CFU g^{-1}
A	1	Single fold, 100% recycled fibers, unbleached	25.8 × 23.3	2.2	$4.4 \pm 0.2 \times 10^5$
B	2	Multifold, 100% recycled fibers, unbleached	24.0 × 23.5	1.9	$8.6 \pm 1.4 \times 10^4$
C	1	One-quarter fold, 100% recycled fibers, unbleached	33.0 × 30.7	2.1	$3.1 \pm 0.6 \times 10^4$
D	1	Single fold, 100% recycled fibers, H_2O_2 bleached	25.5 × 23.8	2.3	$1.1 \pm 0.1 \times 10^5$
E	3	Nonperforated roll, 60% recycled fiber, H_2O_2 bleached	22.3 × 19.3*	1.5	$1.3 \pm 0.3 \times 10^3$
F	4	Perforated roll, 100% virgin wood pulp, chlorine bleached	28.0 × 15.4	1.9	$1.2 \pm 0.1 \times 10^2$

CFU, colony-forming unit.
*The length for 1 sheet of the nonperforated roll was determined by dividing the total square centimeter of the perforated roll test paper F (431 cm^2) by the width of test paper E (19.3 cm).

laboratory. It was found that all students who had washed their hands with water, regular soap, or antibacterial soap had more bacteria on their hands after washing than before. The only exception was students who had used an alcohol-based hand sanitizer solution as a disinfection process. The students with more bacteria on their hands had dried their hands with paper towels, whereas the hands of students who used the alcohol-based hand solution were rubbed until dried. This experiment suggested that further investigations were needed to verify the possible bacterial transmission from paper towels during handwashing.

Thus, the aims of this study were to determine the extent of bacterial contamination of various paper towel brands as well as to isolate and identify the culturable bacterial community present in these commercial products. We also investigated the possible airborne and direct contact transmission of these bacterial contaminants during paper dispensing and after handwashing, respectively.

MATERIAL AND METHODS

Test paper towels

Six paper towel brands (designated A to F), commercially available in Canada, were tested for bacterial load and diversity. Specifications for each brand are found in Table 1. Test papers A, B, and C were made from 100% recycled fiber without any bleaching process. These 3 brands differed in the way they were folded: (A) single folded, (B) multiple folded, and (C) one-quarter folded. Test paper D was a single folded paper towel made from 100% recycled fiber bleached with hydrogen peroxide (H_2O_2). Test paper E was a nonperforated, H_2O_2-bleached paper towel roll made from 60% recycled fiber and 40% virgin wood pulp. Test paper F was made from 100% virgin wood pulp and was chlorine bleached. Paper towel brands A to E were wrapped into thick paper sleeves with the sheet extremities exposed to the environment, and paper brand F was completely wrapped in plastic.

Bacterial enumeration

Samples were prepared following a modified version of a protocol described elsewhere and under a biologic safety level II cabinet.[15] First, 10 g, approximately 5 sheets of paper (see Table 1), of the desired test paper were selected in the middle of the pile, transferred with sterile forceps in a sterile 42-ounce glass jar blender (Model BL10450H; Black & Decker, New Britain, CT) containing 500 mL of distilled water with 0.025% Tween 20 and shredded for 30 seconds. The mix was then left to rest for 60 minutes before stirring it for 30 seconds. Afterward, serial dilutions of the suspension were spread on tryptic soy agar (TSA) plates (Becton, Dickinson and Company, Sparks, MD). TSA has been previously used to isolate a great number of bacterial species on paper and board machines.[16,17] A set of plates was incubated in aerobic conditions and duplicates were incubated in an anaerobic system (model 1025; Thermo Forma, Marietta, OH) using a gas mix of carbon dioxide (5%), hydrogen (10%), and balanced with nitrogen at 25°C. After 48 hours of incubation, colony-forming units (CFU) were counted on each plate to determine the culturable bacterial load on each test paper brand. Plates were then reincubated 5 more days to confirm colony differentiation. One or 2 colonies from each distinct colonial morphology were picked, restreaked on TSA plates, and reincubated at 25°C for further characterization (see below). The entire experimentation was done twice for each paper towel brand (n = 2; 5 towels per brand per test).

Genotypic characterization

Amplification of the 16S ribosomal RNA gene of the selected isolates was done using the colony polymerase chain reaction (PCR) technique. Briefly, a single colony was transferred to a 50 μL PCR mix containing 1× GoTaq PCR buffer, 0.5 μmol l^{-1} of universal primers 63 forward and 1387 reverse,[18] 200 μmol l^{-1} of each dNTP, 3 mmol l^{-1} of $MgCl_2$, 1.25 U of Promega GoTaq polymerase (Fisher Scientific, Ottawa, ON, Canada). All PCR reactions were performed with a DNA Engine DYAD thermocycler (Bio-Rad, Mississauga, ON, Canada) as described elsewhere[19]: 1 hold at 94°C for 5 minutes, then 30 cycles of 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 90 seconds. A final elongation step at 72°C for 5 minutes was performed at the end. The PCR products were sequenced at the genomic platform of the Centre Hospitalier de l'Université Laval using an ABI Prism 3100 apparatus (Applied Biosystems, Foster City, CA). Each DNA sequence was compared with sequences available in the GenBank database from the National Center of Biotechnology Information (NCBI) using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/). Affiliation of the isolates to known bacterial genus or species was established based on sequence similarity.

Bacterial transfer from paper towels to hands

Under a biologic safety level II cabinet, the hands of a laboratory worker wearing disposable nitrile gloves were sprayed with a 70% ethanol solution. Next, the hands were rubbed for 15 seconds and air-dried for 3 minutes. The hands were submerged in sterile distilled water for 15 seconds before using 3 sheets of test paper towel B, 1 sheet at the time, to dry hands. Finally, the bacterial contamination on the nitrile gloves was estimated as described elsewhere.[20] Briefly, the middle of the left palm as well as the middle of the second, third, and fourth fingers were sampled using 25-cm^2 contact agar plates containing TSA medium. The contact surface on the palm was 25 cm^2 and 10 cm^2 for each of the 3 fingers. Control samples were generated by using a new pair of disposable nitrile gloves and the same wash process described above, replacing only the paper drying step by air-drying for 3 minutes. After 48