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Major article

Microbial contamination of hospital reusable cleaning towels

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Background: Hospital cleaning practices are critical to the prevention of nosocomial infection transmission. To this end, cloth towels soaked in disinfectants are commonly used to clean and disinfect hospital surfaces. Cloth cleaning towels have been linked to an outbreak of *Bacillus cereus* and have been shown to reduce the effectiveness of commonly used quaternary ammonium disinfectants. Thus, it is important to determine whether the reuse of cloth towels increases the risk of pathogen transmission in hospitals.

Methods: The goal of this project was to determine the effects of laundry and cleaning practices commonly used in hospitals for washing, storage, and disinfection of cloth cleaning towels on their microbial loads.

Results: Our results indicate that cloth towels used for cleaning hospital rooms contained high numbers of microbial contaminants.

Conclusions: In this case, hospital laundering practices appear insufficient to remove microbial contaminants and may even add contaminants to the towels. Furthermore, it has been previously reported that towels can interfere with the action of common hospital disinfectants. Either independently or in combination, these 2 factors may increase the risk for transmission of pathogens in hospitals. These observations indicate the need to critically reevaluate current hospital cleaning practices associated with reuse of cloth towels.

Hospital housekeeping staff routinely use cloth towels soaked in a hospital disinfectant to clean patient rooms (including terminal cleaning) and other areas of the hospital. These cloth towels are soaked in a bucket containing hospital disinfectants until use, wrung out, and used to clean surfaces inside patient rooms. The towels are then either washed in-house or sent out to a central laundering facility, and the clean towels are stored and then reused in the same manner. A previous report linked the reuse of laundered cleaning cloths to an outbreak of *Bacillus cereus* in a Japanese hospital.[1] Studies of microbial survival in towels have indicated that the more absorbent a cloth towel, the longer the microorganisms can survive, as was previously reported in the case of *Staphylococcus aureus*.[2] Several studies have found that *Staphylococcus* can survive for 19-21 days in cotton cloths.[3] Methicillin-resistant *S aureus* (MRSA) strains capable of causing serious life-threatening infections have been isolated from reused cloth hospital towels.[4]

The goal of this project was to examine the effects of laundry and cleaning practices commonly used in hospitals for washing, storing, and disinfecting cloth towels on the microbial loads in the towels. Ten hospitals were surveyed regarding their cleaning procedures and use of disinfectants for sanitizing rooms after terminal discharge. Clean towels intended for cleaning purposes were collected in triplicate from each participating institution to evaluate both the towels' ability to harbor possible infectious agents and the effectiveness of the laundering practices in removing microorganisms. Swab samples were also collected from the inside surfaces of the buckets in which the towels were soaked in disinfectant. The towels and swabs were cultured for the presence of colony-forming units (CFU) of aerobic spore-forming bacteria, *Clostridium difficile*, molds, heterotrophic bacteria, *S aureus* (including MRSA), total coliforms, and *Escherichia coli*.

METHODS

Ten major hospitals in Arizona, selected at random, were invited and agreed to participate in the study. A survey of cleaning

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Table 1
Culture methods used for all microbial isolation

Organism	Culture method	Incubation conditions	Volume assayed	Further analysis
Heterotrophic bacteria	Spread plating on R2A medium (BD Diagnostics, Sparks, MD)	24°C for 5 days	0.1 mL	
Coliforms and *E coli*	Assayed using the Colilert method (IDEXX; Westbrook, ME)	35°C for 24 h	100 mL	
C difficile	Incubation for 7 days in 0.1% sodium taurocholate and cycloserine-cefoxitin fructose broth	Anaerobic conditions at 37°C for up to 5 days	0.1 mL	A 2 mL aliquot of culture was mixed with an equal amount of absolute ethanol. Bacteria were concentrated by centrifugation, and pellets were used to inoculate cycloserine-cefoxitin fructose agar.
MRSA	TSA amended with 5% sheep's blood, 10 mg/L colistin, and 15 mg/L naladixic acid using the spread-plate method	35°C for 24-48 h	0.1 mL	β-hemolytic colonies were isolated and subcultured on TSA plates with no amendments and incubated at 35°C for 24-48 h.
Molds	Spread-plating 1 mL of eluent on Sabouraud dextrose agar with chloramphenicol (Neogen, Lansing, MI)	24°C for 2-7 days	1 mL	
Aerobic spore-formers	Heat-shocking samples in a water bath at 80°C for 10 minutes, followed by spread-plating on TSA media (BD Diagnostics)	35°C for 24 h	0.1 mL	

TSA, trypticase soy agar.

practices was conducted at each hospital, and 3 clean towels were obtained from each location. Swab samples were also collected from the inside surface of a bucket used to soak the towels in disinfectant at each hospital. The survey of cleaning practices included questions about the protocols used for cleaning rooms, towel use, and laundry procedures. Other questions involved the disinfectant(s) used, whether the towels were soaked or sprayed in the disinfectant, exposure time, frequency of disinfectant changes, fabric content of the cleaning towels, towel washing and drying practices, and towel storage conditions.

The average surface area of the cleaning towels from all participating hospitals was calculated as approximately 1040 ± 284 cm^2. Because of the substantial variability in towel sizes, all bacterial analyses were conducted on a per-towel basis. Each towel was placed into a Stomacher bag with 300 mL of buffered peptone water (EMD, Gibbstown, NJ), based on towel size and absorbance, to ensure complete saturation of the towel. Each towel was manually kneaded until the liquid was completely absorbed, after which the peptone broth was extracted from the towel by wringing. The extract was assayed using selected media for isolation of the various bacteria.

At each hospital, a disinfectant soaking bucket was swabbed just above the disinfectant liquid line using a sponge stick containing Letheen broth (3M, St Paul, MN). After sampling, the broth was extracted from the sponge stick by manual agitation, and then 4 mL of extracted broth was assayed using selected media for isolation of the various bacteria. Samples from the towels and buckets were cultured for total bacteria (heterotrophic bacteria), coliform bacteria, *E coli, C difficile,* MRSA, molds, and aerobic spore-forming bacteria. Test methods for each organism are presented in Table 1.

Gram-positive cocci and catalase-positive, tube coagulase-positive, slide-coagulase positive, and polymixin B-resistant colonies were then cultured on CHROMagar MRSA (BD Diagnostics, Sparks, MD) to confirm identification as MRSA. Selected coliforms and presumptive *E coli* isolates were identified using API 20E bacterial identification test kits (bioMérieux; Marcy l'Etoile, France). The data were log-transformed, and ANOVA was used to assess relationships between the use of towels and towel characteristics. Completely randomized designs were used to perform the ANOVA, with a rejection region of 5% using the *F* distribution.

RESULTS

In the questionnaires on cleaning and laundry practices, 8 of the 10 hospitals reported using cotton towels, and the other 2 (sites 3 and 6) reported using microfiber towels. Two hospitals (sites 2 and 3) sent their linens to be laundered in a central facility, and the others laundered their towels in-house. All but 1 of the hospitals reported a quaternary ammonium compound as their disinfectant of choice; the lone exception was a rehabilitation hospital (site 9) that reported using bleach for terminal cleaning under all circumstances. In addition, all but 1 of the hospitals (site 6) reported soaking their cleaning towels in a bucket with disinfectant.

Table 2
Frequency of microbial isolation from cleaning towels and buckets

	Viable microbes	Total coliform bacteria	*E coli*	Aerobic spore-forming bacteria	Fungi
Towels	28/30 (93)	7/30 (23)	1/30 (3)	17/30 (56)	4/30 (13)
Soak buckets	6/9 (67)	1/9 (12)	ND	4/9 (44)	ND

ND, not detected.
NOTE. Data are number positive per number sampled (% positive).

Table 3
Microbial contamination of soak buckets (n = 9)

Parameter	Heterotrophic bacteria	Total coliform bacteria	Aerobic spore-forming bacteria
Mean, CFU/100 cm^2	269	0.15	153
Maximum, CFU/100 cm^2	1,300	1.3	1,320
Minimum, CFU/100 cm^2	ND	ND	ND

ND, not detected.

The microbial load was higher on the clean towels than on the swab samples taken from the buckets containing disinfectant. The overall results for the towels and swabs collected from the 10 hospitals are presented in Tables 2, 3, and 4. The mean total number of bacteria found on the towels was 133 CFU/cm^2, whereas the mean total number of bacteria found on the inside surface of the disinfectant buckets was 0.605 CFU/cm^2. Viable bacteria were detected on 93% of the towels, but on only 67% of the soak buckets. Spore-forming bacteria were isolated from 56% of the towels, coliform bacteria from 23%, *E coli* from 3.3%, and mold from 13%. Spore-forming bacteria were isolated from 44% of the soak buckets; and coliform bacteria from 12% (Table 2).

Neither MRSA nor *C difficile* were isolated from the towels or the soak buckets, but interestingly, total coliforms were recovered from

Table 4
Microbial contamination of reusable cleaning towels (mean ± SD log CFU/towel; n = 3)

Hospital	Heterotrophic bacteria	Total coliform bacteria	Aerobic spore-forming bacteria	Fungi
1	4.1 ± 0.2	0.5 ± 0.5	3.3 ± 0.2	0.9 ± 1.6
2	1.1 ± 1.9	ND	1.7 ± 1.5	ND
3	3.8 ± 0.8	0.3 ± 0.5	1.0 ± 1.7	ND
4	3.9 ± 0.3	ND	1.0 ± 1.7	ND
5	3.5 ± 0.6	ND	1.9 ± 1.6	ND
6	5.0 ± 0.1	1.3 ± 0.5	3.6 ± 0.3	3.3 ± 0.3
7	3.0 ± 0.1	ND	ND	ND
8	3.7 ± 0.5	ND	1.5 ± 1.3	ND
9	3.8 ± 0.1	ND	3.9 ± 0.6	ND
10	2.3 ± 2.0	ND	ND	ND

ND, not detected.

both sources. Bacteria identified from the towels included *Pseudomonas luteola*, *Pantoea* spp, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Serratia plymuthica*, *Pasteurella pneumotropica*, *Aeromonas hydrophilica*, and *Micrococcus luteus.* Molds identified from the towels included *Aspergillus niger*, *Fusarium* spp, and *Cladosporium* spp.

Statistical analyses indicated significant differences in total bacteria, mold, coliform bacteria, and aerobic spore-forming bacteria in the towels (Table 5). Along with the overall differences, classification of the towels into 4 groups for analysis based on their fabric content revealed statistical differences between cotton and microfiber towels for all microbial contaminants (Table 5). The microfiber towels harbored greater numbers of bacteria compared with the cotton towels.

Table 5
Statistical differences in towel materials (mean + SD log CFU/towel; n = 24)

	Cotton		Microfiber		
	n	Mean ± SD	n	Mean ± SD	*P* value
Heterotrophic bacteria	24	3.17 ± 1.29	6	4.39 ± 0.88	.0381
Total coliform	24	0.07 ± 0.23	6	0.78 ± 0.70	.0002
Aerobic spore-forming bacteria	24	1.66 ± 1.63	6	2.28 ± 1.80	.4152
Fungi	24	0.12 ± 0.58	6	1.67 ± 1.84	.0012

DISCUSSION

In the 10 hospitals participating in this study, almost all (93%) sampled cleaning towels contained viable microorganisms even after laundering. There were significant differences among hospitals in terms of the numbers and types of microorganisms recovered. Possible explanations for these findings include the substantial variation in laundering and cleaning practices among the hospitals, as well as variations in methods of disinfectant application, towel materials, and conditions for storage of the cleaning towels, resulting in habitats more or less conducive to microbial proliferation.

The questionnaire data facilitated comparison of different factors influencing the microbial loads of cleaning towels used in the study hospitals. Significant differences in the presence of bacteria and mold were observed based on the disinfectant application method used. Spraying of towels with a power sprayer was associated with a higher microbial load than soaking, likely because spraying does not completely saturate the towel fibers with disinfectant. But even though soaking resulted in a smaller overall microbial load on the towels, coliforms were still isolated from the disinfectant buckets.

Some of the isolated bacteria are known to have significant involvement in nosocomial infections. *Klebsiella* infections are primarily associated with hospital care,[5] and in this study both *K pneumoniae* and *K oxytoca* were isolated from the hospital towels. *K pneumoniae*, the most significant species, is known to cause respiratory diseases[6] and bloodstream infections.[7] More recently, the extended-spectrum β-lactamase–producing *K pneumoniae* was shown to be highly resistant to antibiotics and a contributor to nosocomial infections.[8] *K oxytoca* has also been implicated in hospital outbreaks, primarily in immunocompromised individuals and frequently involving environmental sources.[9,10] One outbreak attributed to *K oxytoca* occurred at a university hospital in Turkey and involved the spread of bloodstream infections.[5] Extended-spectrum β-lactamase–producing *K oxytoca* infections were recently attributed to contaminated handwashing sinks in the intensive care unit of a hospital.[11] The isolation of *K oxytoca* from cleaning towels in this study suggests a real potential for the towels to serve as a reservoir for this nosocomial pathogen.

P luteola is also a significant nosocomial pathogen that can cause cutaneous abscess and bacteremia.[12] Although *S plymuthica* is identified primarily in plants,[13] it is also encountered in nosocomial infections,[14,15] specifically wound and community-acquired infections.[16] Other bacteria identified in this study, including *Pantoea* spp, are not known to cause nosocomial infections, but were recently associated with hospital outbreaks.[17]

A hydrophilica is involved in nosocomial infections, presenting as necrotizing fasciitis.[18] Another potential source of nosocomial infections isolated from the cleaning towels, *M luteus*, is known to cause pneumonia, septic arthritis, and meningitis.[19] All of the bacterial species isolated from the cleaning towels and soak buckets have reported significance in nosocomial infections. Interestingly, aerobic spore-forming bacteria were isolated more frequently in the towels compared with other bacterial contaminants, indicating that spore-forming bacteria are better able to survive the laundering process, including the washing and drying. In a recent study, *B cereus* present in linens after in-house laundering was a major source of contamination, and was isolated from clean towels, washing machines, and dryers.[1]

A significant difference was observed in the bacterial numbers recovered from cotton and microfiber towels. Bacteria have been shown to adhere more tenaciously to microfiber towels, allowing them to spread or transfer onto different surfaces as the towels are used.[20] In a recent study evaluating the efficacy of reusable towels for decontamination of surfaces, microfiber towels showed superior results when used in new condition, but after reprocessing, the cotton towels more effectively removed bacteria from surfaces. The decontamination efficacy of microfiber towels was reduced after just 20 washing cycles, contrary to the manufacturer's indications of sustained efficacy after 500 washes.[21]

Typical hospital laundering practices are not sufficient to remove all viable microorganisms and spores from towels, regardless of whether they are sent to a central laundering facility or laundered in-house. It is unclear whether bacteria remain trapped in the towel fibers through the laundering process or are reintroduced through subsequent storage or handling. Although hospital disinfectants show efficacy against the organisms found in the towels, these findings suggest that current treatment practices should be reevaluated. Our results indicate that future studies should evaluate the potential role of cloth towels as a reservoir for nosocomial pathogens, along with their possible role in overall cleaning procedures at hospitals, clinics, and long-term care institutions. Furthermore, the development of guidelines for the reuse of cloth towels in health care environments should be considered as part of the larger picture of medical institution cleaning.

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