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Major Article

How clean is "hygienically clean": Quantitative microbial levels from samples of clean health care textiles across the United States

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Background: In the United States, the laundry industry has not reliably measured microbial levels on hygienically clean textiles. The aim of this study was to quantitatively measure the microbial levels found on a sample of hygienically clean textiles.

Methods: Forty-eight health care textile samples were collected from hygienically clean linen scheduled to be used on 3 different patient care units. Samples were taken at 2 separate points in time representing laundry facility processing practices and hospital linen management practices. United States Pharmacopeia 61 testing was completed using a pour plate culturing method, producing a total aerobic microbial count and a total yeast and mold count.

Results: Of the samples, only 27% had a total aerobic microbial count below the expected 100 colony-forming unit level (range, 9-40,000) versus 81% (range, 9-1,000) for total yeast and mold count. Median microbial counts for the 2 separate time points across the 3 different patient care units were also higher than expected.

Conclusions: As far as we know, this study is a first step by the laundry industry to understand what quantitative microbial levels are currently found on hygienically clean health care textiles. These types of data can assist the industry in establishing appropriate outcome targets for process improvement initiatives.

Today, there is a significant focus on the reduction and elimination of health care–associated infections (HAIs). This has become a national priority for the Department of Health and Human Services, the Centers for Disease Control and Prevention (CDC), the Centers for Medicare and Medicaid Services, and other professional and regulatory agencies. In the last several years, there has been an increased focus on the role of the environment and the potential for patient and employee exposures to infectious agents. Health care textiles, such as bed linens, blankets, towels, washcloths, curtains, patient gowns, and even employee uniforms (eg, scrubs and lab coats), can become heavily contaminated with microorganisms. This contamination can occur from body substances (eg, blood, fluid, skin, stool, urine, and vomitus) during patient use or contact.[1,2] Microbiological research has noted that when textiles are heavily contaminated, they can contain bacterial loads as high as 1-100 million colony-forming units (CFU) per 100 cm^2 of fabric,[2] making textiles potential environmental vehicles for the indirect transmission of disease if not processed appropriately. Some of the organisms that can live on health care textiles—such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Clostridium difficile*, and *Acinetobacter baumannii*, which are common HAI-causing organisms—are of particular concern in the health care environment.[2] Multiple studies have demonstrated that microorganisms can bind to and survive for extended periods of time (21-90 days) on textiles.[3,4] Takashima et al[5] found that organisms such as methicillin-resistant *Staphylococcus aureus* and *Pseudomonas* can have high levels (80%-90%) of binding ability to cloth material, especially textiles composed of acrylic, polyester, and wool (cotton was found to have lower levels of binding).

HAI outbreaks

According to Dr Lynne Sehulster,[6] a CDC expert, around 12 reported outbreaks associated with health care textiles have occurred over the past 43 years, with at least 350 patients being

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infected worldwide. Of the 12 reported outbreaks, 7 (58%) were the result of contamination from *Bacillus cereus*, a common environmental spore-forming microorganism. Towels were the most common textile noted as being contaminated with *B cereus* in 4 (57%) of these 7 events. Dr. Sehulster further reported that the root problems associated with the *B cereus* outbreaks included (1) dust contamination of clean textiles, (2) inappropriate wash and rinse water temperatures, and (3) storage conditions that promoted microbial growth. Other known organisms associated with some of the other outbreaks include *Aspergillus, Acinetobacter,* mucormycetes, and *Clostridium difficile*. Further system process problems noted in the 12 outbreaks included 4 hospitals (33%) reporting problems with laundered textile storage; 7 (58%) reporting contaminated washing equipment, inappropriate wash cycle or water temperature settings, or recycled water issues; and 1 (8%) attributing the outbreak to inadvertent contamination occurring during transit and delivery from the laundry to the hospital. This clearly demonstrates that system process errors can lead to unfortunate outcomes.

Two recent outbreaks further demonstrate that health care textiles can become recontaminated if proper processes are not followed. Duffy et al[7] reported on a 2009 outbreak that occurred in New Orleans and resulted in the deaths of 5 pediatric patients (100% case fatality rate). An epidemiologic investigation was conducted and found that the only exposure common to all 5 patients—who had cutaneous mucormycosis (*Rhizopus delemar*) infections—were the health care textiles. On testing, the textiles were found to be contaminated with the same genetic deoxyribonucleic acid (DNA) *Rhizopus* species as the patients. Further investigation revealed no specific point source for the *Rhizopus* within the hospital. The conclusions of the investigation indicated the *Rhizopus* was most likely brought into the hospital on the linens and carts that were exposed to the environment and nearby construction. Several mucormycetes—although not the same species found among the patients—were cultured from the clean textile area of the laundry facility, a delivery truck, and the loading dock, indicating that these places can harbor mold.

Cheng et al[8] investigated a 2015 outbreak in a university-affiliated teaching hospital in Hong Kong. During a 2-month period, 6 cases of pulmonary and cutaneous spore-forming mucormycosis (previously called zygomycosis) infections—predominantly *Rhizopus microsporus* —were found among a cluster of immunosuppressed patients. Of these cases, 50% (3 patients) died. A case-control study was conducted that determined the common source of exposure was contaminated linens supplied by the hospital's designated laundry (no samples of mucormycosis were found on textiles processed at the control laundry facilities). On inspection of the offending laundry, major deficiencies were found in the facility's physical environment, including ambient temperature, lighting, ventilation, and dust levels on the equipment. A discrepancy between the wash cycle temperature, measured by an infrared thermometer, and the preset temperature in the washing machines was also found. Finally, freshly processed linens were packaged while still damp. All of these factors could have led to the propagation of the implicated mucormycetes.

Hygienically clean health care textiles

It is estimated that over 10 billion pounds of health care textiles are laundered annually.[9] Most health care professionals and patients assume that health care laundry is clean and safe. The linen may in fact look clean, but it is not intended to be sterile. Research shows that how health care laundry is processed, handled, transported, and stored can impact the microbial level or contamination of the textiles, which, in turn, can lead to an increased risk of transmission to high-risk patients. Today, the most common term used to denote a safe health care textile product is *hygienically clean*. According to the Association for the Advancement of Medical Instrumentation, *hygienically clean* means "free of pathogens in sufficient numbers to cause human illness."[10] However, the Association for the Advancement of Medical Instrumentation does not quantitatively define what the term *sufficient numbers* means. Even the CDC's evidence-based "Guidelines for environmental infection control in health-care facilities" document—that includes guidelines for processing health care textiles—does not include a quantitative value associated with the term *hygienically clean*. The guideline does state that an appropriate 3-4 log10 reduction (approximately 99%) in microorganism contamination should occur if known wash process components of appropriate time, temperature, chemical use, and agitation are followed.[2,11,12] According to Sehulster,[9] based on this expected 3-4 log10 reduction, the standard processes should create a hygienically clean textile with a potential microbial level of 10-100 CFU/cm^2. Dr. Sehulster further notes that this potential postprocess microbial range is dependent on the initial colony count of the contaminated textile, the type of textile, or the type of organism present. Additionally, how the textiles are handled, transported, and stored after washing can also impact the microbial level on the hygienically clean textile. If any of these processes fail, the expected microbial level may be higher.

Microbial testing

Because we know that contaminated health care textiles can (although rarely) lead to HAIs, health care organizations can benefit from the availability of quantitative tools to monitor and benchmark laundry and linen process outcomes. Although the CDC does not recommend routine microbial testing of textiles (only during outbreaks where the textiles are suspected), having an industry benchmark can be beneficial for process improvement efforts.

Outside the United States, some countries have established guidelines that include microbial testing of textiles to determine safe microbial levels. The Certification Association for Professional Textile Services Administration, which has 400 members in 15 European countries, Japan, China, and the United Arab Emirates, has established criteria for determining safe microbial levels for "hygienically clean" laundered textiles.[13] The United States has not set such a standard, as there is no current evidence of what the microbial level should be. Before the United States can consider adopting the European microbial level, further comparison of the differences between laundry process standards and laboratory testing methods in the United States and Europe will need to occur.

The primary aim of this observational study was to quantitatively identify what microbial levels are present on a sample of hygienically clean health care textiles in the United States. This study is not designed to determine what a safe microbial level should be. Although a postprocess level of 10-100 CFU for hygienically clean textiles has been proposed as receivable,[9] it is unclear if this level is achieved today. Over the past decade, changes in the laundry industry have occurred (eg, types of fabric compositions, chemicals, and water temperatures used). These changes could impact the expected 3-4 log10 reduction if not properly balanced with the 4 main elements of the wash process (agitation, time, temperature, and chemicals). Assessing the average microbial (CFU) level of hygienically clean textiles today can provide the health care laundry industry with a quantitative reference to help guide both continuous quality improvement efforts and future research needs. Future publications will address the secondary aims of the study: (1) to compare the microbial counts found using 2 different United States Pharmacopeia (USP) 61 testing techniques (pour plate vs contact plate) and, (2) to identify differences in microbial counts based on facility-level delivery and handling and textile storage practices.

METHODS

Study population

Only US-based laundry facilities and acute care hospitals were considered eligible for the study. To be included, both the laundry facility and 1 of its contracted acute care hospitals had to agree to participate (1:1 grouping). Other laundry facility inclusion criteria comprised the following: (1) facilities were located off-site of the health care facility, (2) facilities used commercial style equipment to process health care textiles, and (3) facilities had an established delivery system with a contracted acute care hospital at least 5 days a week. This daily delivery could include either a specific unit-based 24-hour exchange cart built at the laundry (ie, goes directly up to the unit on delivery) or a cart with bulk linens that were stored in the hospital's central clean storage area and later added to a specific unit-based exchange cart by the hospital.

Additional inclusion criteria for participating hospitals included (1) utilization of a daily linen exchange cart system for 3 specific patient care areas—obstetrics, intensive care, and a medical-surgical floor unit—and (2) linen exchange cart scheduled to be on the patient care unit for a minimum of 24 hours to a maximum of 28 hours (ie, carts exchanged on the patient care unit daily).

The only exclusion criteria—for either the laundry or the hospital—included facilities experiencing a recent (within 6 months) deficiency citation from a regulatory agency for either linen handling or bloodborne pathogens.

Recruitment

Although no human subjects (patients) participated in the study, facility-level participation and data collection were required. Researchers were concerned that laundry and hospital facilities would be hesitant to participate because of worries regarding confidentiality or established internal requirements for research participation. Therefore, institutional review board (IRB) approval was obtained from Eastern Kentucky University in hopes the process of informed consent and confidentiality would remove any barriers. After obtaining IRB approval, laundry facilities that processed health care textiles were identified for recruitment through the use of a membership list from the Association for Linen Management, a nonprofit 501(c)(6) trade association for the commercial laundry industry and funding source for this study. Names of laundry facilities were randomly selected from the membership list and then contacted to request participation December 2016-April 2017. Of the 97 laundry facilities contacted, 25 met the inclusion and exclusion criteria. Of those, 17 agreed to participate. These laundry facilities were encouraged to provide researchers with the name and contact information of a contracted hospital facility they selected. However, many laundry facilities were hesitant to provide this information without first contacting the hospital themselves. Follow-up telephone calls were conducted by the researchers for any hospital requesting more information. Ultimately, only 8 laundry facilities and 8 contracted acute care hospitals (1:1 grouping, 8 laundries and 8 hospitals, 16 total facilities) agreed to participate. Because the recruitment process had already taken 5 months, it was determined to go ahead with those who had agreed to participate. Written consent to participate was obtained separately from both laundry and hospital facilities per IRB-approved study protocol.

Training

Before implementation, synchronous training webinars were conducted by the study investigators using GoToWebinar (LogMeIn, Boston, MA) conferencing technology. Each facility was asked for at least 1 designated staff member to participate in 1 of the scheduled webinar trainings (multiple dates were offered). All eight 1:1 facility pairs signed up for a training session. One of the live trainings was recorded, and a link to the recording was provided to all facilities for review by other staff members.

As part of the study protocol, each hospital was asked to designate an individual familiar with sterile technique, such as a nurse or sterile processing personnel, to collect the specimens. To familiarize the designated staff with the proper technique of collecting sterile textile specimens, a video demonstrating the step-by-step process was developed by a nationally certified infection prevention nurse. A link to the video recording was sent electronically to the designated hospital study contact prior to the implementation date.

Sample procedures

No patients were directly involved in this study. Only facility-level demographic data, textile process information, and textile specimens for culture were collected. Facility-level demographic information was provided by each facility through the completion of a 1-time demographic survey. Three separate days during a 2-week time frame were used for data collection for the eight 1:1 facility groups (16 facilities) during the months of May and July 2017. For each of the 3 data collection cycles, routine facility-level processes (ie, wash, handling, delivery, and storage) and specimen collection information were documented by participating staff using a data collection tool.

The first specimen, representing the laundry's wash, handling, and delivery process, was to be collected when the laundry facility delivered the hygienically clean textiles to the hospital. This textile specimen was to be collected on arrival or within 4 hours of delivery to the hospital. Facilities were instructed that the specimen could be collected within the clean central supply room or immediately on delivery of the exchange cart to the patient care unit. The second textile specimen, representing the hospital's handling and storage process, was to be collected after the clean, unused textiles had been stored on an exchange cart within a patient care unit for 24 hours. This specimen could be collected on the patient care unit just before the exchange cart was removed or once the cart was returned to the clean central supply room within the hospital. A 4-hour window was again provided so that specimens were collected between 24 and 28 hours after initial delivery to the unit.

Testing methods

Textile specimens were sent for culturing via United Parcel Service to a national laboratory that used the USP microbiological examination of nonsterile products—microbial enumeration test USP 61. The USP 61 microbial enumeration test was developed for pharmaceutical, cosmetic, and personal care products, including textiles such as face pads, to verify safety before releasing products to the market. This testing method was designed for testing of either products prior to sterilization or nonsterile products expected to have levels of organisms deemed low enough to meet pharmacopeia safety requirements. In recent years, this testing method has also been used for health care linens as part of the laundry facility certification process. Since this method has been introduced into the industry, it was selected for this study.

The USP 61 microbial enumeration test is a quantitative test that determines the total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) present on a test product or sample. Several testing techniques can be used with the USP 61 test, including the pour plate, surface spread, and contact plate methods. For this study, the technique used was the pour plate method. With this technique, a 4×4 textile sample is placed into a sterile solution and spun to release any attached microorganisms on or within its fibers. The fluid

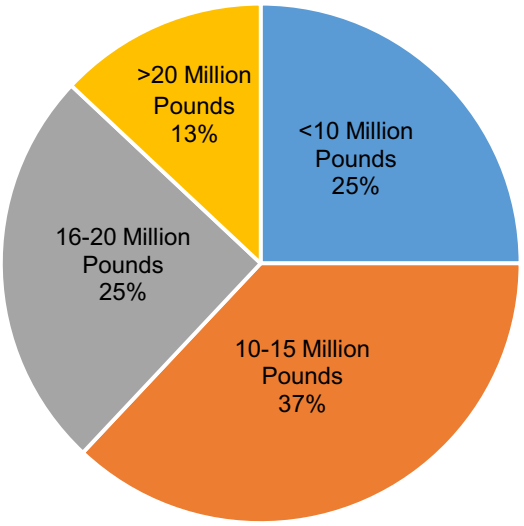

Fig 1. Laundry facility size by pounds per million produced annually.

is then poured onto a testing plate inoculated with soybean-casein digest agar for TAMC and Sabouraud dextrose agar for TYMC. The CFU are then counted after a 48-hour incubation period, and the results are reported using values in CFU/g.[14] No specific organisms were identified in this study, and only colony counts were reported.

RESULTS

Demographics

Of the 16 facilities (8 laundries and 8 hospitals) that participated in the study, 37.5% were located in the Northeastern United States, 25% were from the Southeast, 25% were from the Midwest, and 12.5% were from the West. For the 8 participating laundries, 75% held an industry-established, nonregulatory, facility-level health care textile certification. Fifty percent had an operations manager who held an individual-level, nonregulatory certification. However, only 25% of the 8 laundry facilities held both facility and individual-level certification (50% held 1 or the other, and 25% held none).

Categorizing the size and complexity (use of automation) of laundry facilities within this industry was done by measuring the volume of textiles produced and processed annually. For the 8 laundry facilities that participated in the study, the average volume of textiles processed annually was approximately 14.5 million, with a range of 2-22 million pounds. Figure 1 provides a percentage breakdown of the size of the participating laundry facilities based on pounds per million produced annually. These breakpoints are currently used by the industry to categorize facilities by size and level of automation used to process and move textiles. Facilities producing fewer pounds annually use less automated equipment and more manual processes compared with fully automated facilities that produce millions (>20) of pounds.

Of the 8 participating hospitals, 75% had >350 beds and 25% had ≤350 beds. Only half (50%) the facilities stated that someone from the hospital, such as the infection preventionist or quality and safety staff, visited the off-site laundry to inspect its processes within the last year or annually. One facility (12.5%) stated that the laundry facility had never been inspected by hospital personnel. Figure 2 depicts the timeline in which the participating hospitals inspected the contracting laundry facilities. Although the majority of the hospitals had inspected the laundries in the past, the majority (62.5%) stated that no contracted laundry supervisor or other laundry personnel had been invited to participate in the hospital's infection control or quality and safety committee, which would provide an avenue for the laundry to collaborate and participate in continuous multidisciplinary quality improvement efforts.

Culture results

In total, there were forty-eight 4 × 4-inch textile samples collected from hygienically clean linens (24 clean textile samples taken at time of delivery and 24 clean, unused textile samples taken after 24 hours on the patient care unit). Based on the data collection tool completed by the facility, all 48 specimens were cut from a clean percale sheet or pillowcase using sterile technique by a registered nurse. Additionally, all specimens were recorded as being collected at the 2 expected time points within the required time frames and tested by the USP 61 method.

As hygienically clean textiles are not intended to be sterile, it was not surprising that no samples demonstrated a CFU count of zero. The distribution of CFU/g counts can be seen in Figure 3. For aerobic bacterial counts (TAMC), the results demonstrated that only 27% of the samples had CFU counts of 100 or less. For yeast and mold counts

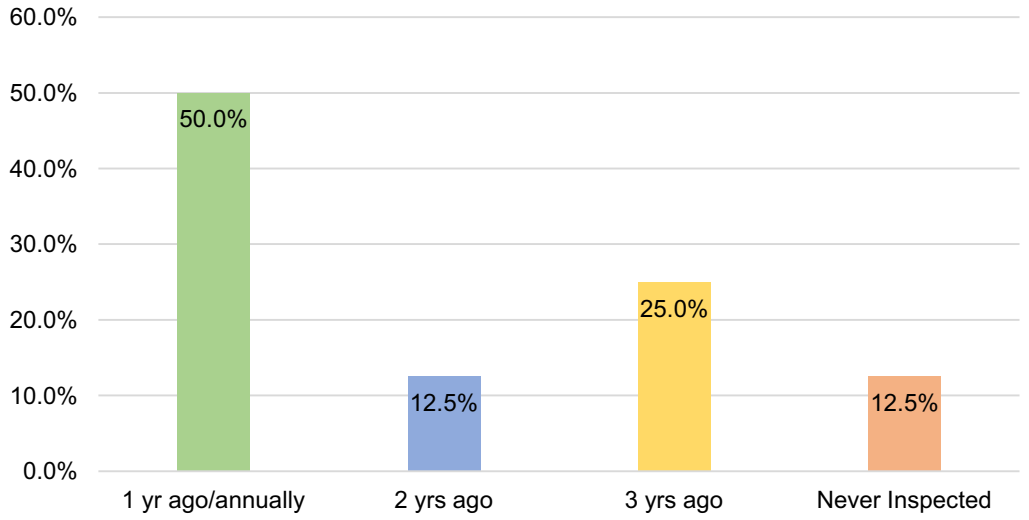

Fig 2. Percent of participating hospitals that inspected contracted laundry.

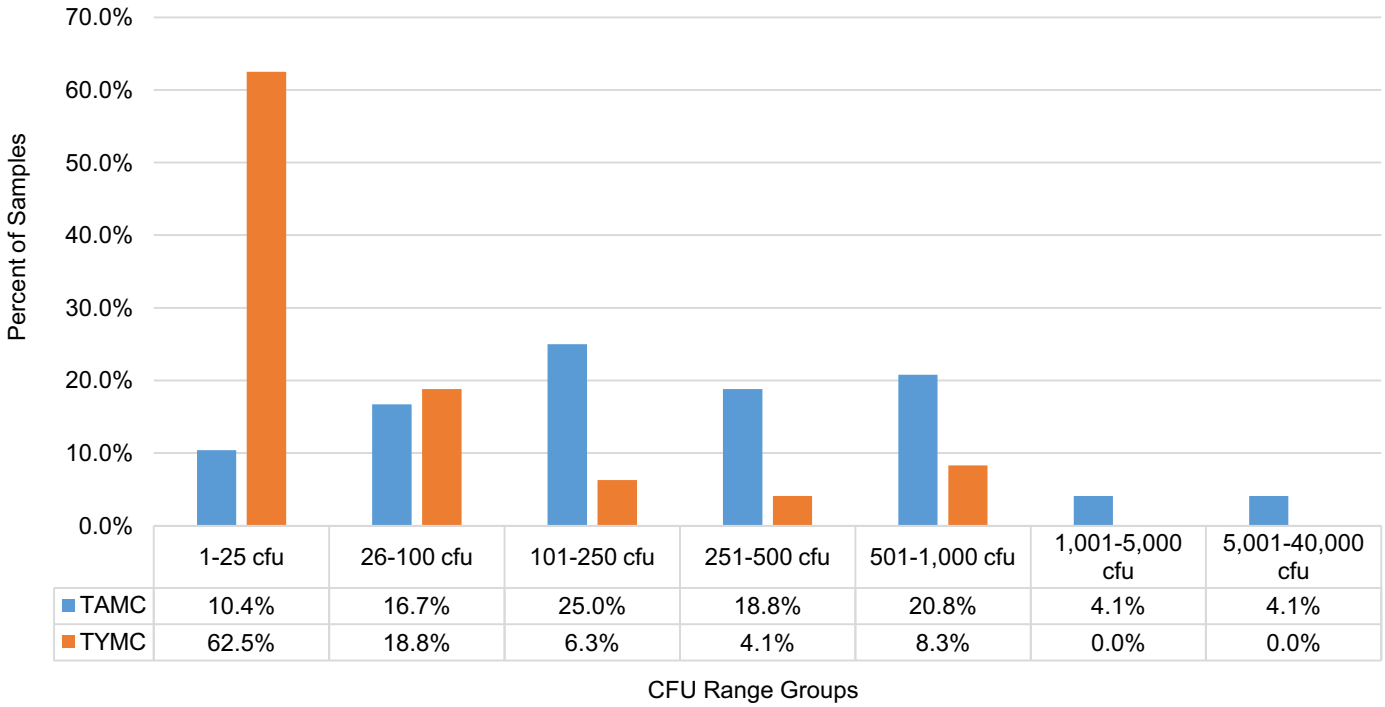

Fig 3. Percent of specimens per CFU ranges for all 48 samples. *CFU*, colony-forming unit; *TAMC*, total aerobic microbial count; *TYMC*, total yeast and mold count.

Table 1
CFU counts for all 48 hygienically clean textile samples

USP 61 results	Mean	Median	Range
TAMC CFU/g	1,386	250	9-40,000
(TAMC outliers removed)	(360)	(245)	(9-1,520)
TYMC CFU/g	108	20	9-1,000

CFU, colony-forming unit; *TAMC*, total aerobic microbial count; *TYMC*, total yeast and mold count; *USP*, United States Pharmacopeia.

(TYMC), however, over 81% had counts of 100 or less, with the majority (62.5%) having a CFU of 25 or less.

The mean, median, and range of all 48 samples are included in Table 1. The large difference in TAMC mean (1,386 CFU) and median (250 CFU) is a result of 2 TAMC sample outliers, 1 with a result of 10,000 CFU/g and the other with a result of 40,000 CFU/g. Both samples came from the same 1:1 facility pairing and data collection cycle (40,000 at time of delivery and 10,000 after 24 hours on patient care unit). If these outliers are removed, the recalculated TAMC mean and median results are 360 CFU/g and 245 CFU/g, respectively (the associated TYMC counts for the 2 outlier samples were zero and were not removed).

In the study, the laboratory results were broken down further by time of delivery, representing the laundry facility processes, and after 24 hours on a patient care unit, representing the hospital facility handling practices. Culture results were also obtained for 3 patient care unit types (obstetrics, intensive care, medical-surgical floor), as handling and storage practices may be different based on the unit's patient population and patient care routines. Table 2 presents the culture results by facility-level and patient care unit types. Variations are noted in CFU counts between facility point of collection levels and unit types.

DISCUSSION

This study was conducted to begin to identify what quantitative microbial levels are currently present on hygienically clean textiles in the United States and whether these levels are within projected levels based on expected log reductions established in the literature. Study results rejected the null hypothesis, as they demonstrated a much higher microbial CFU count range (TAMC = 9-1,520 CFU, excluding outliers, and TYMC = 9-1,000 CFU) than the expected range of 10-100 CFU.

Another result surprising to the investigators involved the findings for the 2 sample collection time points. Many within the industry had predicted that microbial counts would increase over time, as increased handling of textiles can occur on patient care units. However, the findings in Table 2 demonstrate that the overall average microbial level for both TAMC and TYMC results was lower at the second sample collection (after 24 hours on patient care unit) versus the first sample collection (time of delivery). When comparing the data from the 3 different patient care unit types (obstetric, intensive care,

Table 2
Mean/average CFU results by facility level and unit type

	All samples combined*		OB unit*		ICU		MS floor	
Point of sample collection	TAMC CFU/g	TYMC CFU/g	TAMC CFU/g	TYMC CFU/g	TAMC CFU/g	TYMC CFU/g	TAMC CFU/g	TYMC CFU/g
At time of delivery (laundry)	*384 (#23)	118 (#24)	*464 (#7)	86 (#8)	379 (#8)	47 (#8)	319 (#8)	220 (#8)
After 24 h on unit (hospital)	*335 (#23)	99 (#24)	*294 (#7)	72 (#8)	338 (#8)	51 (#8)	369 (#8)	175 (#8)

CFU, colony-forming unit; *ICU*, intensive care unit; *MS*, medical-surgical; *OB*, obstetric; *TAMC*, total aerobic microbial count; *TYMC*, total yeast and mold count.
*Results exclude 2 TAMC outlier samples, both from the OB unit.

and medical-surgical floor), the medical-surgical floor was the only unit that showed an average CFU count increase for TAMC levels after the textiles were stored on the unit for 24 hours (Table 2). Initial examination of selected facility-level process practices (also collected in this study) did not reveal any obvious differences among the 3 units in how textiles were delivered or stored. Further analysis of these data will be completed, and these findings will be published later.

It is not clear why the microbial level results are higher than what was expected. One explanation for this could relate to the storing of slightly damp textiles. For this study, the textile samples were collected from flat sheets or pillowcases. Flat sheets and pillowcases are not placed in a commercial dryer after washing. Instead, they are passed through a flatwork ironer, which uses high heat (325°F-370° F), but the time the linen is exposed to this heat is very short (around 5-10 seconds). According to Blaser et al,[2] exposure to high heat further reduces microbial levels by an additional 1-2 log10. Their findings showed no major differences in reduction levels when either a dryer or ironer was used. However, sheets can still be slightly damp when they come off the ironer and immediately folded and placed in plastic carts for delivery. The storing of damp textiles might then allow low levels of microorganisms to proliferate in a warm, moist environment. This might also explain why microbial levels overall were higher at the time of delivery versus 24 hours after being stored on the patient care units, when the textiles had time to dry. Another reason could be related to small drifts in standard practices over time. Implementing internal quality process improvement activities with appropriate outcome measures (ie, CFU counts) to benchmark against could provide this industry with another important tool.

Limitations

There are several limitations to this study, the first being the low number of facilities that agreed to participate. This impacted the total number of textile specimens available for testing, making the results less generalizable and limiting analysis and interpretation across the 3 patient care unit types. Second, there is no universal industry-based testing method or specimen collection technique for health care textiles. This limits the comparison of our results with other culture data in the literature. Finally, as an observational study, no process practices were controlled across the participating facilities; therefore, there was no guarantee that all facilities were meeting the same standard industry practices. This may be why the range of CFU counts was so large and included 2 significant specimen outliers.

CONCLUSIONS

Improving quality and reducing errors—such as HAIs—continue to be a national priority. It has been estimated that 85% of poor quality outcomes is a result of system or process failures.[15] Although it is less common for health care textiles to be implicated as the transmission source for an HAI outbreak, it does occur. The laundry industry, just like the health care industry, needs to embrace continuous quality improvement strategies and the use of benchmarking to improve process practices. As far as we know, this study is an important first step by the laundry industry in engaging in the research process. Understanding what microbial levels are found on hygienically clean health care textiles produced today in the United States is vital for outcome measurement. These data can assist organizations in evaluating and improving internal practices as part of continuous quality improvement. Further research and data are needed to answer many other unanswered questions within this industry.

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