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Original Article

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Assessment of Environmental Contamination with Pathogenic Bacteria at a Hospital Laundry Facility

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Abstract

Little is known about exposure to pathogenic bacteria among industrial laundry workers who work with soiled clinical linen. To study worker exposures, an assessment of surface contamination was performed at an industrial laundry facility serving hospitals in Seattle, WA, USA. Surface swab samples (n = 240) from the environment were collected during four site visits at 3-month intervals. These samples were cultured for *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant enterococci (VRE). Voluntary participation of 23 employees consisted of nasal swabs for detection of MRSA, observations during work, and questionnaires. Contamination with all three pathogens was observed in both dirty (laundry handling prior to washing) and clean areas (subsequent to washing). The dirty area had higher odds of overall contamination (≥1 pathogen) than the clean area (odds ratio, OR = 18.0, 95% confidence interval 8.9–36.5, $P < 0.001$). The odds of contamination were high for each individual pathogen: *C. difficile*, OR = 15.5; MRSA, OR = 14.8; and VRE, OR = 12.6 (each, $P < 0.001$). The highest odds of finding surface contamination occurred in the primary and secondary sort areas where soiled linens were manually sorted by employees (OR = 63.0, $P < 0.001$). The study substantiates that the laundry facility environment can become contaminated by soiled linens. Workers who handle soiled linen may have a higher risk of exposure to *C. difficile*, MRSA, and VRE than those who handle clean linens. Improved protocols for prevention and reduction of environmental contamination were implemented because of this study.

Keywords: *Clostridium difficile*; environmental contamination; exposure assessment; laundry; linen; methicillin-resistant *Staphylococcus aureus*; MRSA; nasal colonization; occupational health; vancomycin-resistant enterococci; VRE

Introduction

In the last 30 years, it has become clear that the environment is an important reservoir and transmitter of pathogens including viruses, bacteria, and antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Boyce, 2007; Dancer, 2009; Boone and Gerba, 2007). *Clostridium difficile*, MRSA, and vancomycin-resistant enterococci (VRE) are pathogens that cause nosocomial infections and are able to survive for extended time in dust and on surfaces (Dancer, 2009; Otter *et al.*, 2011). The Centers for Disease Control and Prevention (CDC) defines healthcare-associated, or nosocomial, infections as "infections patients can get while receiving medical treatment" (CDC, 2016). Studies have suggested that the clinical environment plays a role as a source or reservoir for these microbes (Manian and Ponzillo, 2007). Examples of this include two *Acinetobacter baumannii* outbreaks, one from a burn unit and one from an ICU, both linked to environmental contamination; the similarity of MRSA isolates from patients to those isolated from hospital rooms; and the extended outbreak of carbapenem-resistant *Klebsiella pneumoniae* (KPC) due to contamination in the air ducts of the National Institutes of Health (NIH) hospital (Aygün *et al.*, 2002; Simor *et al.*, 2002; Sexton *et al.*, 2006; Snitkin *et al.*, 2012).

Studies have shown that surfaces and objects in the immediate vicinity of patients are most likely to be contaminated with patients' microbes, including pathogens (Dancer, 2014). These fomites are important reservoirs for *C. difficile*, MRSA, and VRE in healthcare environments (Hayden *et al.*, 2006; Boyce *et al.*, 2008; Dancer, 2009). Contact with fomites may promote the spread of microbes and human disease (Boyce, 2007). *C. difficile*, MRSA, and VRE are able to survive on surfaces for weeks to months and may be difficult to remove from the environment by standard cleaning and disinfection protocols (Dancer, 2009; Otter *et al.*, 2011).

Hospitalized patients colonized or infected with *C. difficile*, MRSA, or VRE are identified as 'high precaution' patients. This means that barrier precautions including the use of gloves, gowns, and hand-hygiene practices are required for everyone who enters the room and interacts with these patients (Chang *et al.*, 2010). Posted signs make visitors and hospital staff aware of the additional health hazards associated with these patients. In addition to these mandatory procedures, enhanced terminal room disinfection is now being performed in an increasing number of hospitals (Boyce *et al.*, 2011). Terminal room disinfection consists of a thorough cleaning of the whole room after a patient is discharged to reduce the risk of the next patient acquiring the high precaution pathogen (Carling, 2013). In contrast, the soiled linens from these rooms do not routinely receive special treatment (Mr. J. Dale, Director of Housekeeping Harborview Medical Center, personal communication, 2013; Ms. C. O'Hara, Manager of Program Operations at UW Laundry Facility, personal communication, 2013).

A 2013 CDC report identified three 'threat' classifications for 18 antibiotic resistant and related pathogens; urgent, serious, and concerning, based on the severity of the disease, cost, and difficulty of treatment. That list included three pathogens addressed in this study. *C. difficile* was classified as an 'immediate public health threat that requires urgent and aggressive action'. MRSA and VRE were classified as serious threats which 'require prompt and sustained action to ensure that the problems do not grow' (CDC, 2013).

Laundry facilities that process soiled clinical linens have been considered an extension of the healthcare environment even when the facility is not physically located on site (Sepkowitz, 1996; Belkin, 2009). However, very few studies have assessed the potential risk of laundry workers who handle soiled hospital linens. Twelve cases of hepatitis A and eight cases of *Salmonella* gastroenteritis related to exposures to soiled linens have been reported (Standaert *et al.*, 1994; Borg and Portelli, 1999; Mitchell *et al.*, 2015). Other reports among laundry workers included *S. aureus* skin infections and viral gastroenteritis that was potentially norovirus (Gellert *et al.*, 1990; Fijan *et al.*, 2008). In Taiwan, a hospital laundry worker was suspected of being the index case in a SARS viral epidemic within the community (Lee *et al.*, 2003). Laundry workers are also at physical risk of cuts and abrasions due to sharps and medical devices left in and among the linens potentially increasing the infection risk (Cohen, 2001).

Three previous studies characterized surface contamination with three clinical pathogens, *C. difficile*, MRSA, and VRE, in an industrial laundry facility (Michael *et al.*, 2016a, 2016b, 2017) while the aim of this new study was to assess global contamination in regard to the occupational risk of exposure in an industrial laundry facility that handles soiled linens.

Methods

Surface samples

To determine the level of surface contamination, surface sampling sites were chosen by identifying high touch areas throughout the laundry facility. This was done by completing a walk-through of the facility with a Certified Industrial Hygienist. The definition used to determine high versus low touch areas came from a report

used by the CDC which differentiates between surfaces with minimal hand-contact, such as floors and ceilings, and surfaces with frequent hand-contact such as door knobs, phones, and tools used on the job (Sehulster *et al.*, 2004). We identified areas within the laundry that were likely to have the highest exposure levels. Sampling these locations increases the chances of correctly identifying overexposures when they are occurring in an occupational setting (European Chemicals Bureau, 2003). By including the locations with the highest and lowest expected frequency of contamination, we were able to determine a range of surface contamination levels that the employees face.

Samples from the same surfaces were collected in January, April, July, and October of 2015 using either 5 ml Sanicult™ Sterile Neutralizing Buffer Swabs (Starplex® Scientific, Etobicoke, Ontario, Canada) or 10 ml 3M™ Sterile Neutralizing Buffer Sponge-Sticks (3M Co. St. Paul, MN, USA) (Michael *et al.*, 2016a). The swabs were used to sample smaller surfaces (~10 cm^2), and the Sponge-Sticks were used to sample larger surface areas (~30 cm^2). These surface sizes were chosen based on an unpublished pilot study and requirements for isolation of the three organisms in the study. There were a total of 240 samples collected over the course of the study, with 30 samples each collected in the dirty and clean laundry areas at every sampling time as previously described (Michael *et al.*, 2016a). Within 4 h of collection, the samples were processed in three parallel enrichment processes that independently targeted one of the three pathogens (*C. difficile*, MRSA, and VRE) from each surface sample.

Subject recruitment

All 110 employees, from both the clean and dirty areas of the laundry facility, were invited to volunteer for the study on each of the four visits. Participants were asked to sign a consent form at each sampling. The procedures and objectives of the research study were then outlined and explained. Participation throughout the study was entirely voluntary. Subject recruitment, study design, and data handling procedures were all approved by the Institutional Review Board of University of Washington (Human Subjects Application #47732).

Observation and survey

Employees who consented to inclusion into the study received a temporary identification number that was taped to their gown for the remainder of their shift. If their gown was taken off or replaced, the number was applied to their new gown. This number was used to identify and link their survey data and observations upon return to the laboratory for that particular observational and sampling date only. Each subject was observed for 20 consecutive minutes during each site visit. Observations were done one subject at a time. Touches of the nose, eye, and face were recorded. The face was defined as everything except the nose and eye. Subjects knew that observations occurred during the shift, but not necessarily when they were being observed individually.

A survey was given to the subjects at the beginning of the first and third sampling visit. Names were needed to link each visit because subjects may have had different numbers for each observation time point. The survey included age, smoking status, where they normally work in the laundry, job title, job task, length of employment, frequency and type of personal protective equipment (PPE) used, 6-month antibiotic history, and rashes or other skin disorders.

Laundry facility

All samples were collected at an industrial laundry facility as previously described (Michael *et al.*, 2016a). Soiled linens were kept on the second floor, separate from the clean linens on the first floor of the facility. There were two main processing lines. The first line was the standard laundry owned linens which represents ~98% of all the linens. These were sorted into carts and then sent down chutes to the first floor to be laundered. The second line was for customer-owned goods (COG), which represent ~2% of the linens. These were sorted in a separate area and washed in smaller batches in an independent process. The COG linens were manually placed into washers on the first floor. All clean linens were dried, sorted, folded, and packaged for delivery exclusively in the first floor 'clean area'. Gowns and gloves were mandatory in all locations within the dirty area but voluntary in the clean area with the exception of the clean room and the COG washer area.

Participants from the dirty areas worked in all four locations; the receiving, primary sort, secondary sort, and the COG area. The tasks workers were responsible for in the receiving included unloading the trucks that delivered the soiled linens, cataloguing, and recording information about where the linens came from and what they consisted of, weighing the bins of soiled linen and placing bins in the correct sorting area. Workers in the primary sort area were responsible for unloading the bins and opening the plastic bags that held the soiled linens, once the linens were on the conveyor belt. Linens were manually sorted into similar types of linen into empty bins, other workers replaced these sorted bins with empty bins when they filled up. Workers in the primary sort area were responsible for sorting out

non-linen items that were mistakenly thrown in with the linens. Once the linens were sorted, the full bins were placed into the secondary sort area where workers placed the linens in small batches on a conveyor belt that dropped them into the tunnel washers on the first floor. Workers in the COG area sorted smaller loads of linen, re-bagged them into similar linen types and then dropped them through a chute into a waiting area on the first floor.

Participants from the clean area represented all four of the areas where surface samples were collected as well as from the clean room. Workers in the COG washers placed bags of soiled linens into washing machines and then dryers. Once the linens were dry, they were sent to the workers in the folding area. In the folding area, workers either folded small linens by hand or larger items on machines. This is where linen was ironed if needed. Once folded, linens were taken to shelves where workers in the processing area collected clean, folded linens to be sent back out to customers. Workers in the clean room made small, custom packages that were sent to operating rooms, ICUs, and pediatric units. There was also one maintenance worker and one management level worker. Both employees worked primarily on the clean side of the laundry facility, but did go onto the dirty area when needed.

Surfaces in the same eight areas within the laundry facility were sampled at each sampling visit. The receiving area included the loading dock, the weigh in area, incoming carts, and the glove/gown area. The primary sort area was the conveyor belt where linens were manually sorted. The secondary sort area consisted of carts with sorted linens, the wash deck where linens were dropped down to the COG washer area and the two chutes where linens were dropped into tunnel washers. The COG area consisted of surfaces where COG linens were sorted. On the clean side of the laundry, the COG washer area was sampled. The folding area consisted of areas where hand folding, machine-assisted folding, and ironing occurred. The processing area consisted of carts with clean linens, steam tunnels, and the garment repair areas. The break area consisted of the locker rooms, the eating area, and the front office area (Michael *et al.* 2016a).

Pathogen isolation

To determine if samples contained *C. difficile*, 1 ml aliquots were anaerobically incubated in 5 ml of Cycloserine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (Anaerobe Systems, Morgan Hill, CA, USA) media and incubated for 48 h at 36.5°C. Tubes with a color change from red to yellow/orange were vortexed and 0.1 ml of the solution was inoculated onto a selective CHROMagar *C. difficile* media, (CHROMagar™, Paris, France) and incubated for 24 h at 36.5°C under anaerobic conditions per the manufacturer's directions. Colonies that fluoresced under UV light (360 nm) were Gram stained. Isolates that were Gram-positive rods were saved for further processing (Michael *et al.* 2016b).

Culturing of MRSA used 1 ml of the original sample which was aliquoted into 1.5 ml of Bacto® m Staphylococcus Broth at 1.5× concentration (Difco Laboratories, Sparks, MD, USA) with 75 µg/ml polymyxin B and 0.01% potassium tellurite (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 36.5°C with 5% carbon dioxide. Tubes were examined for growth at 48 h, and samples that were turbid with black precipitate were considered positive. Positive samples were plated onto oxacillin-resistant *S. aureus* base® media (ORSAB; Oxoid Limited, Basingstoke, UK) and incubated at 35°C for 48 h. Dark blue colonies that grew on ORSAB were streaked onto Brucella agar (Difco Laboratories) supplemented with 5% sterile sheep blood and incubated at 36.5°C in 5% CO_2 for 24 h to determine if β-hemolysis was present. *S. aureus* and presumptive MRSA isolates were verified using the Staphaurex® (Remel, Lenexa, KS, USA) and Oxoid penicillin binding protein latex agglutination test® (Oxoid Microbiology P, Basingstoke, UK) according to manufacturer's directions (Michael *et al.* 2016a).

Isolation of VRE consisted of transferring 1 ml of the original surface sample into 1 ml of Brain Heart Infusion broth (BHI; Difco™ Laboratories) supplemented with 20 µg/ml of aztreonam and 6 µg/ml of vancomycin at 41°C for 24–72 h. Turbid samples were transferred onto m-Enterococcus™ agar (mE; Difco Laboratories), containing 6 µg/ml vancomycin (mE_{Van6}), and incubated at 41°C for 24–48 h (Novais *et al.*, 2005). Pink or maroon colonies were isolated and transferred to BHI_{Van18} plates (Difco Laboratories) containing 18 µg/ml vancomycin and incubated at 41°C for 24–48 h. Vancomycin resistance was defined as growth on BHI_{Van18} media as previously described (Michael *et al.*, 2017). A single colony for each of the three pathogens was taken from each positive sample.

Statistical methods

Averages of prevalence of contamination and touching behavior were calculated. STATA 13.1 (Stata Corp. College Station, TX, USA) was used for all analyses. Except for tests of possible seasonality, the surface swabs collected in each site visit were assumed to be independent measurements; even though measurements were repeated at the same approximate location. The 3-month

sampling interval difference was sufficient to justify this assumption. Logistic regression was used to determine the crude odds ratios (ORs) of detecting surface contamination. Tetrachoric correlation, used with binary variables, between the pathogens was also assessed (Edwards and Edwards, 1984). Other between-variable comparisons used the Student *t*-test for continuous variables, chi-square test for categorical variables, Cochran's *Q* test, and the McNemar test to assess seasonal differences and a Wilcoxon rank–sum test to compare touching behaviors. All four areas of the clean area were combined, because of the low number of the three bacteria isolated, into the reference area against which the dirty areas were compared. ORs were determined for finding each pathogen and for ≥1 pathogen in the dirty versus clean area. The odds of finding contamination were also stratified by specific areas and sampling times within the dirty side compared to the reference clean area. Sample size for environmental samples was calculated to have the power to detect the difference between surface contamination between the dirty and clean sides based on pilot data, but not necessarily for comparisons adjusting for other variables. A *P* value of ≤0.05 was considered significant.

Results

Participant characteristics

Of the 110 employees in the facility, 23 volunteered to participate in the study; 14 employees from the clean area and nine employees from the dirty area. The remaining employees declined. Employees from the clean and dirty areas participated between one and four times, out of the four site visits. The employees from the clean area participated on average 2.7 times, while employees from the dirty side participated on average 2.4 times ($P = 0.55$).

The employees from the clean and dirty areas were statistically significantly different for three factors: time spent in USA, gender, and prevalence of MRSA nasal colonization. The employees from the clean areas lived an average of 31.1 years in the USA, while the employees from the dirty areas lived in the USA an average of 17.1 years ($P = 0.03$, *t*-test). The subjects from the clean area were 78.6% (11/14) female while the dirty area subjects consisted of 22.2% (2/9) females ($P = 0.006$, *t*-test). Employees who worked on the dirty side had higher odds of a positive nasal culture than employees who worked on the clean side (OR = 10.4; confidence interval = 0.9–117.2, $P = 0.06$). The sample size was not sufficient to evaluate whether these differences were influenced by other variables known to differ between clean and dirty areas (gender and years in the USA). Age, household size, smoking behavior, antibiotic use, and current work at a hospital were not significantly different between the two groups ($P > 0.05$). Self-reported hand washing behavior was the same between the employees on the clean and dirty areas with the exception of one employee from the dirty area who reported not washing their hands after removing their gloves (see Supplementary Table S1, available at *Annals of Work Exposures and Health* online).

The total time spent observing the employees was 1200 min (20 h) over the course of the entire study period. There were 38 observations of employees from the clean area and 22 observations from employees in the dirty area. The average number of touches per 20-min observation period, including touches of the nose, eye, and face, observed among employees from both areas was 1.7 (standard deviation [SD] = 2.4). Employees from the clean area showed more touching behaviors on average with 2.1 touches (SD = 2.7) than employees from the dirty area with 1.1 touches (SD = 1.7) but the difference was not statistically different ($P = 0.14$, *t*-test). None of the touching behaviors were significantly different between the dirty and clean areas (Table 1). All other observed touching behaviors were not significantly different using the Wilcoxon rank–sum test or *t*-test, between employees from the clean and dirty areas. Similar results were observed with a chi-square test after dichotomizing the number of touches per 20-min observation period (≥60th percentile as 'high').

To determine if multiple covariates were associated with nasal colonization a logistic mixed model was run using the STATA command *melogit*, initially adjusting for area (dirty versus clean) and touch frequency using an exchangeable covariance matrix, random intercepts,

Table 1. Observed self-touch data (number of touches observed in 20-min observation period).

Mean ± SD, by area of laundry	Overall ($n = 23$)	Dirty ($n = 9$)	Clean ($n = 14$)	*P* value*
Person hours	20.0	7.4	12.7	
Average touch	0.4 ± 0.7	0.3 ± 0.1	0.5 ± 0.1	0.14
Nose	0.5 ± 1.0	0.3 ± 0.8	0.6 ± 1.1	0.27
Eye	0.1 ± 0.4	0.1 ± 0.2	0.1 ± 0.5	0.43
Face	0.7 ± 1.2	0.3 ± 0.8	0.9 ± 1.3	0.08
Hair	0.4 ± 0.8	0.4 ± 0.7	0.4 ± 0.9	0.86
Total touch	1.7 ± 2.4	1.1 ± 1.7	2.1 ± 2.7	0.14

**t*-test comparing dirty versus clean touching behavior.

and random slopes. The data for this model consisted of the 60 observations clustered into 23 groups, or subjects. This was a repeated measures problem as we had multiple measures on the 23 subjects in the two different areas. Due to the small amount of data, the covariate-adjusted mixed model did not converge (as opposed to the unadjusted model which converged without issue), especially as we allowed both random slopes and a random intercept. Even when we altered the type of mixed model, to linear (Jennrich *et al.*, 1986; Louis, 1988; Everitt, 1995; Diggle, 1998), to improve model convergence for small data samples, there was still evidence of issues with estimation when covariates were included in the statistical model, mostly due to the few degrees of freedom available in the dataset once the mixed model was estimated. Much of the issue likely stemmed from only one subject from the clean area having had a MRSA positive nasal sample. While we could model only touch (and not area) or touch only in dirty area, these results were non-significant ($P = 0.7087$). This led us to conclude that, while we could do a valid unadjusted mixed model for estimating associations in the clean versus the non-clean area (less nasal colonization in the clean area, $P = 0.0325$), adding covariates was incompatible with the available data.

Pathogen isolation from surfaces

Contamination with all three pathogens was observed in both the dirty and the clean areas of the laundry facility (Table 2). The primary and secondary sort areas were the most contaminated areas within the dirty side for all three pathogens and for ≥1 pathogen. The COG washers were the most contaminated area on the clean side. The dirty area had statistically higher prevalence of contamination than the clean area for contamination with ≥1 pathogen (67% dirty area versus 10% clean area, $P < 0.001$, chi-square test) and when stratified by specific pathogen.

There were significant correlations between pathogens. The spearman correlation coefficients ranged from 0.49 (between *C. difficile* and MRSA) to 0.64 (between VRE and MRSA) and were all statistically significant ($P < 0.001$). Ten samples had all three pathogens and 28 had two pathogens, distributed among various areas of the plant, mostly in sorting areas, and a few in COG washing areas.

Prevalence of surface contamination from different locations within the facility is shown in Fig. 1. The primary and secondary sort areas had the highest levels of contamination for all measures, both overall (>1 pathogen) and for individual pathogens. On the clean side, the COG washer area had the highest contamination for all three pathogens (Fig. 1).

The four sampling times were distant enough in time that they were not considered to be repeated measures. However, to determine if there was a difference in the prevalence of positive surface samples between sampling times a Cochran's *Q* test was run. Surface contamination with ≥1 pathogen did not change over time. Season variation of individual pathogens is described elsewhere (Michael *et al.*, 2016a, 2016b, 2017).

The dirty area had a higher odds of contamination with ≥1 pathogen than the clean area (OR = 18.0, 95% confidence interval = 8.9–36.5, $P < 0.001$; Table 3). The odds of contamination were high for each individual pathogen: *C. difficile*, OR = 15.5; MRSA, OR = 14.8; and VRE, OR = 12.6 (each, $P < 0.001$; Table 3). When stratified by specific location, the OR of find-

Table 2. Prevalence of surface contamination by laundry area and date of sample collection.

	C. difficile[a]		MRSA[b]		VRE[c]		≥1 Pathogen[d]	
	n (%)		*n* (%)		*n* (%)		*n* (%)	
	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean
Jan 2015	3 (10)	1 (3)	6 (20)	0 (0)	16 (53)	3 (10)	20 (67)	4 (13)
April 2015	12 (40)	0 (0)	5 (17)	1 (3)	17 (57)	3 (10)	20 (67)	3 (10)
July 2015	4 (13)	0 (0)	12 (40)	1 (3)	11 (37)	1 (0)	20 (67)	2 (7)
Oct 2015	6 (20)	1 (3)	10 (33)	1 (3)	15 (50)	3 (10)	20 (67)	3 (10)
Total	25 (21)	2 (2)	33 (28)	3 (3)	64 (53)	10 (8)	80 (67)	12 (10)

MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant enterococci.

[a]Data from Michael *et al.*, 2016b.

[b]Data from Michael *et al.*, 2016a.

[c]Data from Michael *et al.*, 2017.

[d]Novel data from the current study.

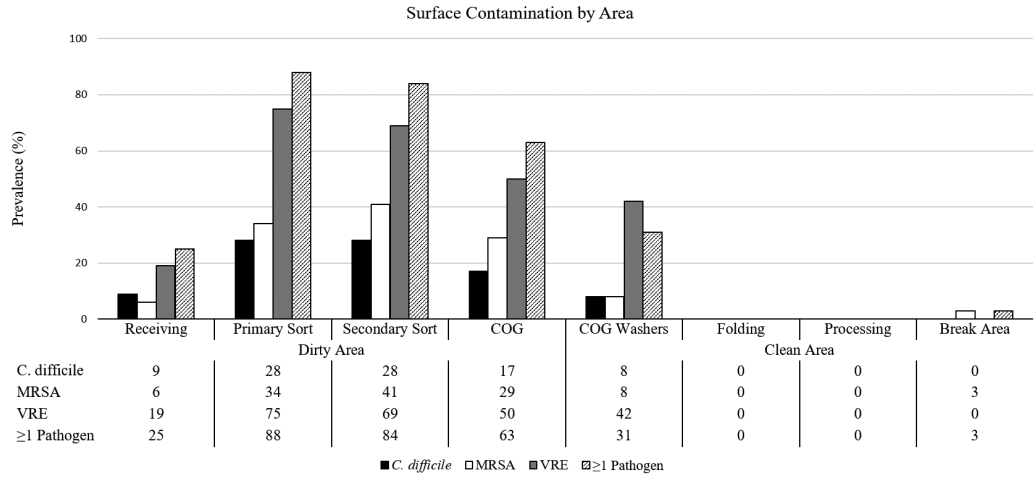

Figure 1. Prevalence of positive surface contamination by location within the laundry facility pooled across all four sampling periods. COG = customer-owned goods area; MRSA = methicillin-resistant Staphylococcus aureus; VRE = vancomycin-resistant enterococci.

Table 3. Odds ratio of contamination in dirty area compared to the clean area.

Location	Any pathogen		*C. difficile*		MRSA		VRE	
	OR	CI	OR	CI	OR	CI	OR	CI
Clean area	1	—	1	—	1	—	1	—
All dirty areas	18.0*	8.9–36.5	15.5*	3.6–67.2	14.8*	4.4–49.8	12.6*	6.0–26.3
Receiving	3.3**	1.2–9.1	6.1	1.0–38.2	2.6	0.4–16.3	2.5	0.8–7.6
Primary sort	69.4*	20.5–234.3	23.1*	4.7–113.9	20.4*	5.3–79.5	33.0*	11.8–92.4
Secondary sort	53.0*	17.1–166.9	23.1*	4.7–113.9	26.7*	6.9–102.5	24.2*	9.0–65.1
COG	16.5*	5.9–46.4	11.8**	2.0–68.7	16.1*	3.8–68.1	11.0*	3.9–30.8

OR = crude odds ratio, calculated by logistic regression, using clean area as reference category, and with no adjustment for other variables. CI = 95% confidence interval; COG = customer-owned goods area; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant enterococci. *$P < 0.001$, **$P < 0.05$.

ing ≥1 pathogen was higher in all locations within the dirty area compared with the clean area including the receiving area (OR = 3.3, $P = 0.02$), the primary sort area (OR = 69.4, $P < 0.001$), the secondary sort area (OR = 53.0, $P < 0.001$), and the COG area (OR = 16.5, $P < 0.001$). As with the prevalence, the highest ORs were observed in the primary and secondary sort areas (Table 3).

Discussion

This study assessed contamination with *C. difficile*, MRSA, and VRE in a dedicated clinical laundry facility servicing bulk hospital linen. The study used self-report, observation, and surface swab collection culture methods. The dirty area was significantly more contaminated than the clean area for ≥1 pathogen (67% dirty area versus 10% clean area, $P < 0.001$, *t*-test). This remained the case when the pathogens were examined separately for dirty versus clean areas (Michael *et al.*, 2016a, 2016b, 2017). The results are not unexpected given that soiled hospital linens have previously been shown to be contaminated with a variety of pathogens (Kehoe, 2015; Cheng *et al.*, 2016). The results correspond to our hypothesis that the dirty area would have higher contamination levels than the clean area. The results are consistent with other studies that assessed peripherally associated healthcare settings such as dental school clinics, fire stations housing first responders, and veterinarian clinics (Wulf *et al.*, 2006; Roberts *et al.*, 2011; Roberts and No, 2014).

The COG washer area, which is located within the clean, first floor area of the laundry facility, had higher contamination levels than the receiving area (45.8%

versus 28.3% for contamination with ≥1 pathogen; Fig. 1). Because of this study, the laundry facility has implemented new protocols which aim to reduce the levels of surface contamination in the COG washer area. These protocols included the use of Environmental Protection Agency-registered disinfectant on the high touch surfaces of the COG washers, guard rails to physically block clean carts from getting underneath soiled linen chutes, color coding of carts (red carts are for soiled linen use only), and providing additional PPE, such as gloves and face shields, available at the point of use and posted PPE donning and doffing guidelines (Mr. C. Curtis, personal communication, 2016).

Finding more than one pathogen in the same location was not unexpected, and the correlation of the presence of one pathogen with the presence of another pathogen could be due to several reasons. One reason is that dirty surfaces contain more total bacteria; thus, it is more likely to have contamination with multiple pathogens. The other potential reason is that VRE and *C. difficile* are both found in the gastrointestinal tract and if contamination with feces is present, there is a higher chance of finding one or both pathogens. In addition, there is evidence of co-infections with *C. difficile* and VRE (Fujitani *et al.*, 2011). This is not surprising because colonization or infection with these two pathogens have some of the same risk factors: antibiotic use, recent surgery, living in close quarters, etc. (CDC, 2013). The reason that there were more samples that contained both VRE and MRSA, compared to both VRE and *C. difficile*, may be because so few samples were positive with *C. difficile* and that VRE was isolated with the highest frequency of all three pathogens.

Correlation between pathogens could be influenced by seasonality of isolation since both *C. difficile* and MRSA have been shown to be seasonal. *C. difficile* rates in general increase in late winter/early spring and are thought to be due to an increase in antibiotic use for respiratory infections which are also seasonal (Gilca *et al.*, 2012). MRSA rates appear to change based on the season, with July–September being the months with the highest prevalence of skin and soft tissue infections (Wang *et al.*, 2013). On the dirty side of the laundry, *C. difficile* had a significantly higher prevalence in April while MRSA had a higher, although not significantly different, prevalence in July, which correlates with what has been previously reported in the literature (Gilca *et al.*, 2012). In contrast, seasonality in VRE infections have not been as well established, and in our previous study VRE rates did not vary by season (Michael *et al.*, 2017). No seasonal differences were observed in the clean side, but due to the low number of positive samples from this area (10/120), we may not have had the resolution to detect seasonality.

This study had several limitations. The first was the low sample size of subjects (n = 23). There were few differences between the two groups of subjects based on both observations and self-reported behaviors and demographics. It is not clear if this was due to the small number of participants or if there were no differences. More robust comparisons of behavior and demographics between the two groups of workers may have been possible with a larger sample size. The small number of positive surface swab samples from the clean areas reduced the resolution of some comparisons as mentioned above. This study assessed global contamination in regard to the occupational risk of exposure and not risk of illness. Determining the risk of illness based on exposure in the laundry was beyond the scope of the study. A known methodological issue in environmental sampling for bacteria is low recovery rates. When sampling, environmental surfaces recovery can be <1% and is dependent on a number of factors including sampling method, environmental surface, environmental conditions, the original concentration, the method of removal from swab/sponge stick (ex: vortexing versus using a stomacher), and the target bacteria sampled (Piepel *et al.*, 2012). Additionally, the sampled surface area was limited to the amount of buffer solution in the swabs and sponge-sticks. Thus, it is likely that our recovery rates underestimated the actual level of contamination for each of the bacteria. The lower prevalence of *C. difficile* in this study, compared to MRSA and VRE, may be due in part to the fact that it is difficult to regrow spores from the environment (Claro *et al.*, 2014). Additionally, this study only assessed contamination based on presence or absence of each organism. Determining the actual concentration of organisms may have provided additional insights. The assumption that the same surfaces at different sampling times were independent allowed us to pool the data and have a larger sample size, but could have introduced error if seasons were truly different and just not observed with the sample size. However, the lack of significant difference between the seasons, other than for *C. difficile* contamination in April, supports this pooling of our data.

Conclusion

This study suggests that laundry facility workers who handle soiled linen may have a higher risk of exposure to surface contamination with *C. difficile*, MRSA, and VRE than those who handle the linens once they are clean. Environmental microbiology collection standards, as well as, defined occupational exposure limits have not

been developed and thus it is difficult to assess microbiological risk in this occupational setting.

Supplementary Data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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Conflict of Interest

The authors declare no conflict of interest. The UW Consolidated Laundry Facility did not participate in the design of the experiment. All data collected over the course of this study did not originate from the laundry facility management and they did not have a conflict of interest.

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