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Original Article

Microbial bioburden of inpatient and outpatient areas beyond patient hospital rooms

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Abstract

Objective: To investigate the frequency of environmental contamination in hospital areas outside patient rooms and in outpatient healthcare facilities.

Design: Culture survey.

Setting: This study was conducted across 4 hospitals, 4 outpatient clinics, and 1 surgery center.

Methods: We conducted 3 point-prevalence culture surveys for methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Clostridioides difficile*, *Candida* spp, and gram-negative bacilli including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* in each facility. In hospitals, high-touch surfaces were sampled from radiology, physical therapy, and mobile equipment and in emergency departments, waiting rooms, clinics, and endoscopy facilities. In outpatient facilities, surfaces were sampled in exam rooms including patient and provider areas, patient bathrooms, and waiting rooms and from portable equipment. Fluorescent markers were placed on high-touch surfaces and removal was assessed 1 day later.

Results: In the hospitals, 110 (9.4%) of 1,195 sites were positive for 1 or more bacterial pathogens (range, 5.3%–13.7% for the 4 hospitals) and 70 (5.9%) were positive for *Candida* spp (range, 3.7%–5.9%). In outpatient facilities, 31 of 485 (6.4%) sites were positive for 1 or more bacterial pathogens (range, 2% to 14.4% for the 5 outpatient facilities) and 50 (10.3%) were positive for *Candida* spp (range, 3.9%–23.3%). Fluorescent markers had been removed from 33% of sites in hospitals (range, 28.4%–39.7%) and 46.3% of sites in outpatient clinics (range, 7.4%–82.8%).

Conclusions: Surfaces in hospitals outside patient rooms and in outpatient facilities are frequently contaminated with healthcare-associated pathogens. Improvements in cleaning and disinfection practices are needed to reduce contamination.

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Many studies have demonstrated that environmental contamination with healthcare-associated pathogens is common in rooms of hospitalized patients and residents of long-term care facilities.[1–4] In contrast, relatively little information is available on contamination of surfaces outside patient rooms.[5–8] Care areas and equipment outside patient rooms in healthcare facilities and in outpatient settings may represent an underappreciated reservoir of pathogen transmission. In contrast to patient rooms, these areas are typically shared by numerous patients each day, including some patients managed with contact precautions while in their hospital room. Moreover, cleaning in these areas may be suboptimal with limited monitoring of cleaning performance.

To develop effective control measures, there is a need for data on the burden of contamination with healthcare-associated pathogens in a variety of settings outside patient rooms in hospitals and in outpatient settings. To address this need, we examined the frequency of environmental contamination in multiple hospitals and outpatient facilities in northeastern Ohio. We also evaluated the thoroughness of cleaning by assessing removal of fluorescent markers applied to high-touch surfaces.

Methods

Study setting

The study was conducted in 4 Cleveland area hospitals and 5 outpatient healthcare facilities. The hospitals included a tertiary-care county hospital, a Veterans' Affairs hospital, a community hospital, and a pediatric hospital. The outpatient facilities included 4 outpatient clinics and a surgery center. The institutional review boards affiliated with each facility approved the study protocol.

Culture collection and assessment of cleaning

We conducted 3 point-prevalence culture surveys in each facility with at least 1 month between each culture collection. In the

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hospitals, cultures were collected from multiple areas including radiology, physical therapy, endoscopy, emergency departments, waiting rooms, portable equipment, and subspecialty clinics. In the pediatric hospital, play areas with shared toys were also included. In outpatient clinics and the surgery center, cultures were collected from exam rooms with separate samples from patient and provider areas, patient bathrooms, waiting rooms, and portable equipment. Samples were collected on weekdays between 8 A.M. and 5 P.M.

In hospitals, ˜100 cultures were collected from high-touch surfaces during each point-prevalence survey. In the outpatient facilities, ˜20–70 cultures were collected during each point-prevalence survey with the number of cultures varying depending on the size of the facilities. For all facilities, 1 sample was also collected from the floor in each of the areas being sampled and another was collected from a sink in the patient care area.

The high-touch surface and floor cultures were collected from standardized 20 × 20-cm surface areas using cellulose sponges (Sponge Stick with neutralizing buffer, 3M, New Ulm, MN). The sink drains were sampled using BBL culture swabs (Becton Dickinson, Sparks, MD) inserted 2.5 cm below the strainer.[9]

To assess thoroughness of cleaning, fluorescent solution (Tide laundry detergent) was applied using a cotton-tipped swab to high-touch surfaces as previously described.[10] The solution was allowed to air dry and research personnel returned 1 day later and used a black light to assess whether the fluorescent marker has been removed.

Microbiology and molecular typing

Sponges were processed as previously described using sterile phosphate-buffered saline with 0.02% Tween 80.[2] Then 200-µL aliquots of eluate were plated on CHROMagar *Staph aureus* with 6 µg/mL cefoxitin for methicillin-resistant *Staphylococcus aureus* (MRSA), Enterococcosel agar with 20 µg/mL vancomycin for vancomycin-resistant enterococci (VRE), MacConkey agar for gram-negative bacilli, and Sabouraud dextrose agar for *Candida* spp.[2,11] Bacterial and *Candida* spp isolates were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF; Bruker Biotyper CA System, Bellerica, MA). Isolates of Enterobacteriaciae were screened for ceftazidime by plating on MacConkey agar containing 10 µg/mL ceftazidime. Isolates of Enterobacteriaciae, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia* were screened for carbapenem resistance by plating on MacConkey agar containing 1 µg/mL meropenem. Isolates recovered from selective media plates were subjected to susceptibility testing in accordance with Clinical Laboratory Standards Institute guidelines.[12]

For recovery of toxin-producing *Clostridioides difficile* strains, broth enrichment cultures were performed by inoculating 200 µL eluate into *C. difficile* brucella broth with thioglycolic acid and L-cystine (CDBB-TC) broth as previously described.[13] The cultures were incubated at 37°C for 72 hours. All specimens that underwent a color change from red to yellow were plated onto pre-reduced *C. difficile* brucella agar incubated anaerobically for 72 hours. Yellow colonies with the typical appearance were streaked for isolation onto blood plates and were confirmed to be *C. difficile* based on the typical odor and appearance of the colonies and by a positive reaction using a *C. difficile* latex agglutination assay (Microgen Bioproducts, Camberley, UK). Isolates identified as *C. difficile* were grown for 72 hours in CDBB-TC, and the culture medium was tested for production of toxins A and B using the Alere *C. difficile* Tox A/B II ELISA (TechLab, Blacksburg, VA, USA). Only toxin-producing strains of *C. difficile* were included in the analysis. Fluorescent polymerase chain reaction (PCR) ribotyping was performed as previously described.[14] For MRSA isolates, *spa* typing was performed using previously described methods.[15]

Data analysis

The percentages of positive cultures were calculated by facility and area sampled for each pathogen. The mean colony-forming units (CFU) of the pathogens recovered was calculated for each area sampled. For high-touch surfaces, the percentage of sites positive for 1 or more of the bacterial pathogens including *C. difficile*, MRSA, VRE, and GNB was calculated. The thoroughness of cleaning was calculated as the percentage of sites with removal of the fluorescent marker 1 day after culture collection and marker placement.

Results

Table 1 shows the overall percentage of positive cultures of high-touch surfaces for the different pathogens in each study hospital and the percentage of fluorescent marker removal. The overall percentage of contamination with the composite of *C. difficile*, MRSA, VRE, and GNB was 9.1%, with a range of 5.0% to 15.5% for the 4 hospitals. *Candida* spp were recovered from 4.0% of surfaces, with a range of 2.9% to 5.9% for the 4 hospitals. Based on fluorescent marker removal, <40% of sites assessed had been cleaned.

Table 2 shows the percentage of environmental contamination in the 4 hospitals stratified by the areas sampled. For the composite of bacterial pathogens, the waiting rooms had the highest frequency of contamination, and the clinics were the least contaminated (16.4% and 3.2%, respectively). The endoscopy units had the highest frequency of contamination with *Candida* spp (6.2%). The percentage of fluorescent marker removal ranged from 23.4% to 45.8%.

Table 3 shows the overall percentage of positive cultures of high-touch surfaces in the outpatient clinics and surgery center and the percentage of fluorescent marker removal. The percentage of contaminated surfaces for the composite of bacterial pathogens was lowest for clinic 4 and the surgery center (1.9% to 2%); the percentage of contamination with *Candida* spp was lowest for the surgery center (3.9%). The percentage of fluorescent marker removal was substantially higher in the surgery center than in the clinics (82.8% vs 7.4% to 63.6%, respectively).

Table 4 shows the percentage of environmental contamination in the clinics stratified by the areas sampled. For the composite of bacterial pathogens, the bathrooms had the highest percentage of contamination; the percentage of fluorescent marker removal in bathrooms was 100%. In examination rooms, the patient area had more frequent contamination with the bacterial pathogens than the provider area. Fluorescent marker removal ranged from 32% to 100%.

Table 5 shows the mean CFU of the pathogens recovered per positive high-touch surface. The concentration of GNB on surfaces was higher than the concentration of the other pathogens. The concentrations of the pathogens were similar in the hospital and outpatient clinic sites. For the high-touch surfaces, the 64 gram-negative bacterial species recovered included *S. maltophilia* (N = 24), *Klebsiella pneumoniae* or *K. oxytoca* (N = 15), *P. aeruginosa* (N = 12), *A. baumanii* (N = 7), *Enterobacter cloacae* (N = 4), *Serratia marcescens* (N = 1), and *Proteus mirabilis* (N = 1). Also,

Table 1. Environmental Contamination in 4 Hospitals in Areas Outside Patient Rooms

Organism	Hospital 1 (N=327)	Hospital 2 (N=291)	Hospital 3 (N=300)	Hospital 4 (N=277)	Total Hospitals (N=1,195)
Any MRSA, VRE, *C. difficile*, GNB[a]	36 (11.0)	16 (5.5)	15 (5.0)	42 (15.2)	109 (9.1)
MRSA	15 (4.6)	1 (0.3)	4 (1.3)	10 (3.6)	30 (2.5)
VRE	10 (3.1)	2 (0.7)	2 (0.7)	3 (1.1)	17 (1.4)
C. difficile	5 (1.5)	8 (2.7)	5 (1.7)	5 (1.8)	23 (1.9)
GNB[a]	10 (3.1)	9 (3.1)	5 (1.7)	29 (10.5)	53 (4.4)
Candida spp	17 (5.2)	13 (5.9)	10 (3.3)	8 (2.9)	48 (4.0)
Marker removal, no. removed/no. placed (%)	82/285 (28.4)	87/274 (31.8)	N/A	92/232 (39.7)	261/791 (33.0)

Note. GNB, gram-negative bacilli; MRSA, methicillin-resistant *Staphylococcus aureus*; *C. difficile*, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.
[a]GNB included Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*.

Table 2. Environmental Contamination in 4 Hospitals by Areas Sampled

Organism	Radiology (N=195)	Portable Equipment (N=282)	Emergency Department (N=226)	Physical Therapy (N=81)	Endoscopy (N=113)	Waiting Rooms (N=190)	Clinics (N=127)
Any MRSA, VRE, *C. difficile*, GNB[a]	19 (9.8)	15 (5.3)	18 (8.0)	10 (12.3)	14 (12.4)	27 (14.2)	6 (4.7)
MRSA	4 (2.1)	3 (1.1)	3 (1.3)	6 (7.4)	4 (3.5)	8 (4.2)	2 (1.6)
VRE	2 (1.1)	2 (0.7)	0 (0)	6 (7.4)	2 (1.8)	5 (2.6)	0 (0)
C. difficile	4 (2.1)	4 (1.4)	5 (2.2)	1 (1.2)	3 (2.7)	5 (2.6)	1 (0.8)
GNB[a]	10 (5.1)	8 (2.8)	10 (4.4)	0 (0)	6 (5.3)	15 (7.9)	4 (3.1)
Candida spp	9 (4.6)	6 (2.1)	8 (3.5)	4 (4.9)	7 (6.2)	11 (5.8)	3 (2.4)
Marker removal, no. removed/no. placed (%)	38/116 (31.0)	52/164 (31.3)	60/131 (45.8)	20/69 (30.0)	25/107 (23.4)	39/128 (30.5)	27/76 (34.7)

Note. GNB, gram-negative bacilli; MRSA, methicillin-resistant *Staphylococcus aureus*; *C. difficile*, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.
[a]GNB included Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia*.

Table 3. Environmental Contamination in Outpatient Clinics

Organism	Clinic 1 (N=104)	Clinic 2 (N= 66)	Clinic 3 (N=55)	Clinic 4 (N=55)	Surgery Center (N=205)	Total Samples (N=485)
Any MRSA, VRE, *C. difficile*, GNB	16 (15.4)	4 (6.1)	5 (9.1)	1 (1.9)	4 (2.0)	30 (6.2)
MRSA	3 (2.9)	0 (0)	0 (0)	0 (0)	1 (0.5)	4 (0.8)
VRE	5 (4.8)	0 (0)	1 (1.9)	0 (0)	0 (0)	6 (1.2)
C. difficile	5 (4.8)	0 (0)	2 (3.6)	1 (1.9)	1 (0.5)	9 (1.9)
GNB[a]	3 (2.9)	4 (6.1)	2 (3.6)	0 (0)	2 (1.0)	11 (2.3)
Candida spp	22 (21.2)	5 (7.6)	4 (7.3)	6 (10.9)	8 (3.9)	45 (9.3)
Marker removal (%), no. removed/no. placed (%)	4/54 (7.4)	35/98 (35.7)	21/61 (34.4)	28/44 (63.6)	82/99 (82.8)	170/367 (46.3)

Note. GNB, gram-negative bacilli; MRSA, methicillin-resistant *Staphylococcus aureus*; *C. difficile*, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.
[a]GNB included Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia*.

18 (28%) of the isolates were carbapenem resistant including 7 *S. maltophilia*, 4, *P. aeruginosa*, 4 *A. baumanii*, and 3 *Klebsiella* spp. Two extended-spectrum β-lactamase (ESBL)–producing organisms were recovered, including 1 *E. cloacae* and 1 *S. maltophilia*. The most common *Candida* spp recovered were *C. parapsilosis* (39%), *C. metapsilosis* (11.9%), *C. lusitaniae* (9%), *C. guilliermondii* (7.5%), *C. famata* (7.5%), and *C. albicans* (6%). *Spa* typing was completed for 25 MRSA isolates recovered from high-touch surfaces. The most common *spa* types were *t535* (N = 7), *t002* (healthcare-associated MRSA clone) (N = 5), *t064* (N = 3), and *t024* (N = 2). *Spa* types with 1 isolate per type included *t008* (community-associated MRSA clone), *t334*, *t975*, *t4210*, *t127*, and *t216*.

The overall percentages of positive cultures of floors for the composite of bacterial pathogens in the hospitals and outpatient clinics were 35.7% (30 of 84) and 31.8% (14 of 44), respectively. For hospitals, the percentages of contamination were as follows: MRSA (9.5%), VRE (14.3%), *C. difficile* (9.5%), and GNB (15.5%). For outpatient facilities, the percentages of contamination were as follows: MRSA (9.1%), VRE (4.5%), *C. difficile* (13.6%), and

Table 4. Environmental Contamination in Outpatient Clinics by Areas Sampled

Organism	Exam Room Patient Area (N=101)	Exam Room Provider Area (N=162)	Waiting Room (N=70)	Bathroom (N=13)	Portable Equipment (N=139)
Any MRSA, VRE, *C. difficile*, GNB[a]	11 (10.9)	4 (2.5)	7 (10.0)	2 (15.4)	6 (4.3)
MRSA	2 (2.0)	0 (0)	1 (1.4)	1 (7.7)	0 (0)
VRE	3 (3.0)	1 (0.6)	1 (1.4)	0 (0)	1 (0.7)
C. difficile	5 (5.0)	1 (0.6)	2 (2.9)	0 (0)	1 (0.7)
GNB	1 (1.0)	2 (1.2)	3 (4.3)	1 (7.7)	4 (2.9)
Candida spp.	9 (8.9)	16 (12.9)	8 (11.4)	2 (15.4)	9 (6.5)
Marker removal, no. removed/no. placed (%)	34/99 (39.1)	54/95 (56.8)	18/54 (32.1)	13/13 (100)	51/106 (48.1)

Note. GNB, gram-negative bacilli; MRSA, methicillin-resistant *Staphylococcus aureus*; *C. difficile*, *Clostridioides difficile*; VRE, vancomycin-resistant enterococci.
[a]GNB included Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia*.

Table 5. Mean (Range) Colony-Forming Units of Healthcare-Associated Pathogens Recovered From Culture Sites

Organism	Hospitals	Outpatient Clinics
MRSA	197 (20–2,880)	183 (20–1,200)
VRE	70 (20–420)	20 (20–20)
GNB[a]	1,247 (20–5,000)	650 (20–5,000
Candida spp	210 (20–5,000)	185 (20–3,000)

Note. GNB, gram-negative bacilli; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.
[a]GNB included Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia*.

GNB (20.5%). The overall percentages of positive cultures of floors for *Candida* spp in hospitals and outpatient clinics were 19.0% and 11.4%, respectively. In total, 90 sinks were cultured, including 60 in hospitals and 30 in the outpatient clinics. *Pseudomonas aeruginosa* and other *Pseudomonas* spp were recovered from 62 (66%) of the sinks. No carbapenem-resistant *Pseudomonas aeruginosa* or Enterobacteriaceae were recovered.

Discussion

We examined the frequency of environmental contamination with healthcare-associated pathogens in 4 hospitals focusing on areas outside patient rooms and in 5 outpatient healthcare facilities in northeastern Ohio. In the hospitals, 9.1% of high-touch surfaces sampled were positive for 1 or more bacterial pathogens and 4.0% were positive for *Candida* spp. In outpatient facilities, 6.2% of sites were positive for 1 or more bacterial pathogens and 9.3% were positive for *Candida* spp. The thoroughness of cleaning was suboptimal with fluorescent markers removed from only 33% and 46.3% of high-touch surfaces in hospitals and outpatient facilities, respectively. These findings demonstrate that environmental contamination is common in hospitals outside patient rooms and in outpatient facilities and suggest a need for improved cleaning protocols.

Our findings are consistent with previous studies that have demonstrated substantial contamination with healthcare-associated pathogens in areas outside patient rooms. In a point-prevalence culture survey, Jury et al[7] found that 14% of outpatient clinic and emergency department rooms were contaminated with *C. difficile*. Prior to a cleaning intervention, Hefzy et al[5] recovered MRSA and VRE from ˜20% of surfaces cultures in outpatient clinics affiliated with a hospital in Egypt. It has also been demonstrated that patients colonized or infected with VRE, *C. difficile*, and MRSA may shed these organisms during outpatient clinic visits and physical therapy appointments.[6,7,16,17]

Cleaning and disinfection are challenging in outpatient clinics and in areas outside patient rooms. Multiple patients pass through areas each day with limited time for cleaning between patients. Based on fluorescent marker removal from high-touch surfaces, cleaning procedures were suboptimal in most of the areas studied. Notably, contamination was uncommon in the surgery center which had a high frequency of fluorescent marker removal in comparison to the other areas studied (82.8% marker removal). However, in outpatient clinics, patient bathrooms had a high level of contamination despite 100% marker removal. Sites such as patient bathrooms may require more frequent or intensive cleaning than other areas.

Although contamination with GNB was relatively common, only 30% of isolates were resistant to carbapenems and only 3% were ESBL-producing organisms. Previous studies have suggested that recovery of multidrug-resistant GNB after procedures and patient care activities may be relatively infrequent in comparison to pathogens such as MRSA, possibly because many GNB survive less well on dry surfaces than gram-positive bacteria.[3,4,8] In addition, none of the study facilities reported outbreaks or high endemic rates of infections with multidrug-resistant GNB. Although sink contamination has been linked to transmission of multidrug-resistant GNB,[9] no carbapenem-resistant *P. aeruginosa* or Enterobacteriaceae were recovered from sinks in the current study.

Contaminated environmental surfaces have not traditionally been considered an important source for transmission of *Candida* spp. However, the emerging fungal pathogen *Candida auris* has frequently been recovered from environmental surfaces in rooms of colonized or infected patients,[9] and a recent report of an outbreak of *C. auris* in an intensive care unit linked transmission to shared temperature probes.[18] Some studies have also implicated environmental sources, including contaminated portable equipment, as a source for acquisition of other *Candida* spp.[19–21] For example, Sanchez et al[20] reported that a *Candida parapsilosis* strain causing infections was recovered from inanimate surfaces in a new intensive care unit before patients were admitted. The ability of *C. auris*, *C. parapsilosis*, and *C. glabrata* strains to survive for prolonged periods on dry and moist surfaces may increase the likelihood of transmission from the environment.[9]

The finding that floors were more frequently contaminated that high-touch surfaces is consistent with many previous culture surveys.[21] The significance of floor contamination remains uncertain, but several recent studies have implicated contaminated floors as a potential source for dissemination of pathogens.[15,21–23] For example, contamination of hospital rooms with healthcare-associated pathogens typically progressed from floors to sock bottoms, bedding, and high-touch surfaces.[15]

Our study has several limitations. Although the fluorescent marker results suggest that cleaning was suboptimal, it is possible that some high-touch items (eg, portable equipment) were not cleaned because they were not used. Because 3 separate point-prevalence surveys were collected, we cannot exclude the possibility that culture collection might have had an impact on cleaning processes during the study. However, there were no major differences in results for cultures collected at different time periods.

In summary, we found that environmental contamination was common in hospitals outside patient rooms and in outpatient healthcare facilities. Improvements in cleaning and disinfection practices are needed to reduce contamination.

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