ORIGINAL ARTICLE

Assessment of the Overall and Multidrug-Resistant Organism Bioburden on Environmental Surfaces in Healthcare Facilities

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OBJECTIVE.   To determine the typical microbial bioburden (overall bacterial and multidrug-resistant organisms [MDROs]) on high-touch healthcare environmental surfaces after routine or terminal cleaning.

DESIGN.   Prospective 2.5-year microbiological survey of large surface areas (>1,000 cm^2).

SETTING.   MDRO contact-precaution rooms from 9 acute-care hospitals and 2 long-term care facilities in 4 states.

PARTICIPANTS.   Samples from 166 rooms (113 routine cleaned and 53 terminal cleaned rooms).

METHODS.   Using a standard sponge-wipe sampling protocol, 2 composite samples were collected from each room; a third sample was collected from each *Clostridium difficile* room. Composite 1 included the TV remote, telephone, call button, and bed rails. Composite 2 included the room door handle, IV pole, and overbed table. Composite 3 included toileting surfaces. Total bacteria and MDROs (ie, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci [VRE], *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *C. difficile*) were quantified, confirmed, and tested for drug resistance.

RESULTS.   The mean microbial bioburden and range from routine cleaned room composites were higher (2,700 colony-forming units [CFU]/100 cm^2; ≤1–130,000 CFU/100 cm^2) than from terminal cleaned room composites (353 CFU/100 cm^2; ≤1–4,300 CFU/100 cm^2). MDROs were recovered from 34% of routine cleaned room composites (range ≤1–13,000 CFU/100 cm^2) and 17% of terminal cleaned room composites (≤1–524 CFU/100 cm^2). MDROs were recovered from 40% of rooms; VRE was the most common (19%).

CONCLUSIONS.   This multicenter bioburden summary provides a first step to determining microbial bioburden on healthcare surfaces, which may help provide a basis for developing standards to evaluate cleaning and disinfection as well as a framework for studies using an evidentiary hierarchy for environmental infection control.

Healthcare-associated infections (HAIs) are a significant cause of morbidity and mortality in the United States. A 2011 multistate point-prevalence survey recorded an estimated 722,000 HAIs in 648,000 hospitalized patients resulting in ~75,000 patient deaths.[1] Prevention efforts have recently focused on the role of the physical environment in the transmission of pathogens causing HAIs. High-touch, noncritical hospital surfaces (eg, bed rails, overbed tables, and IV poles) are commonly contaminated with pathogens, and many have been linked to outbreaks in hospitals and long-term care facilities (LTCFs).[2–12]

Most published studies assessing microbial contamination on environmental surfaces in healthcare facilities have been qualitative, reporting only the proportion of positive samples.[3–5,9,10,13] The few studies reporting quantitative results focus either on the amount of overall contamination or contamination by specific pathogens, usually not both.[14–21] Microbial bioburden (MB), when reported, is only from individual surfaces, such as bed rails, or is reported in relation to cleaning method or product efficacy testing.[21–26] These studies can be difficult to compare because they often use different sampling methodologies or reporting units.[15,17,19–22,27]

The lack of consistent quantitative contamination data makes it difficult to correlate bioburden reductions when assessing cleaning procedures or disinfection products. Current CDC guidelines contain no recommended standard MB levels.[28] More than a decade ago, benchmark standards were

proposed for high-touch surfaces in UK hospitals to correlate cleanliness and MB.[29,30] These standards of <5.0 colony-forming units (CFU)/cm^2 or <2.5 CFU/cm^2 are of limited use because the dip-slide sampling method must be used, which limits the sampling to flat surfaces of <12 cm^2.[12,29–33] Without knowing the true MB on hospital environmental surfaces, it can be difficult to assess achievable bioburden concentrations that will impact patient safety. The main objectives of our study were to use standardized, large-area (>1,000 cm^2), sponge-wipe sampling and laboratory processing procedures to determine typical MB (overall bacterial and specific multidrug-resistant organisms [MDROs]) on high-touch, noncritical, healthcare environmental surfaces after routine or terminal room cleaning across a number of facilities.

METHODS

Study Design

This prospective study was conducted between January 2011 and July 2013 in 9 acute-care hospitals and 2 LTCFs in 4 states. Rooms of patients on contact precautions for MDROs (eg, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Clostridium difficile*) were sampled after either routine or terminal cleaning.

Environmental Sampling

Samples were collected using environmental sponge-wipes (3M Sponge-Stick with neutralizing buffer; 3M, St. Paul, MN). To best evaluate contamination of the room as a whole, a preliminary study was performed to identify high-touch sites that could be combined as composite samples. Up to 12 high-touch sites (surface area ≤1 m^2) were sampled in patient rooms from 9 healthcare facilities. Each site included IV pump and/or pole, television remote, overbed table, telephone, door handles, call bell, bed rails, supply cart, bathroom hand rail, and toilet handle.

Based on the preliminary data, we developed 3 composites that included items common in the patient rooms of all participant hospitals and LTCFs. The sites sampled as part of each composite were assigned based on a maximum total sampling surface area of 2,258.06 cm^2 per composite and were composed of 1 large surface-area site (bed rails or overbed table) and 2–3 smaller sites. One sponge wipe was used for each composite. Sites were assigned as follows: composite 1 (C1) included bed rails, television remote, call button, and telephone; composite 2 (C2) included overbed table, IV pole, and inside room door handle; and composite 3 (C3) included either the portable commode (grab bars and seat), bedpan (bathroom door handle, toilet flush handle, rinse spout handle, and seat), or bathroom (door handle, flush handle, and grab bar) in rooms of patients with *C. difficile*. Bathroom sites were included for *C. difficile* contact precaution rooms to increase the chance of recovering *C. difficile*.

The healthcare facilities notified samplers when MDRO contact precaution rooms occupied by patients diagnosed with the specified MDR infections were available for sampling. Samples were collected as soon as possible after routine or terminal cleaning and rooms were of similar design on the same unit or ward. "Routine" was defined as the daily cleaning procedure for patient rooms, and samples were collected when patients were out of the rooms. "Terminal" referred to the cleaning and disinfection procedures that occurred after patients were discharged. Routine and terminal cleaning procedures varied by facility. The exact surface area sampled was recorded for each composite, along with cleaning products used, cleaning and sampling times, and specific patient clinical factors (ie, acute diarrhea, presence of catheters, open wounds, dialysis, etc). For each facility, up to 20 routinely cleaned and 10 terminally cleaned rooms were sampled. Field blank samples were collected before and after sampling each room. Samples were shipped to the Centers for Disease Control and Prevention (CDC) for processing on the day of collection when possible.

Sample Processing and Culture Methods

All samples were processed immediately upon receipt at the CDC. Sponge wipes were expressed in 90 mL phosphate-buffered saline containing 0.02% Tween 80 (PBST) using a stomacher. The eluate was then concentrated by centrifugation. The pellets were resuspended in PBST and cultured. To recover and quantify target MDROs, aliquots were cultured on CHROMagar VRE (CHROMagar, Becton Dickinson, San Jose, CA) for VRE, mannitol salt agar (Becton Dickinson) for *S. aureus*, MacConkey agar (Becton Dickinson) for *K. pneumoniae*, MDR-Acinetobacter agar (Hardy Diagnostics, Santa Maria, CA) for *A. baumannii*, and cycloserine cefoxitin fructose agar with horse blood and taurocholate (Anaerobe Systems, Morgan Hill, CA) for *C. difficile*. Total aerobic (tryptic soy agar with 5% sheep's blood; Becton Dickinson) and total anaerobic counts (anaerobic blood agar; PathCon Laboratories, Norcross, GA) were determined. All media were inoculated in duplicate and incubated as appropriate. Broth enrichment was used to recover low levels of target MDROs; tryptic soy broth (Becton Dickinson) was used for Gram-negative bacteria; tryptic soy broth with 6.5% NaCl (Becton Dickinson) was used for MRSA and VRE; and cycloserine cefoxitin fructose broth was used for *C. difficile*.[34] Broth cultures were subsequently subcultured on selective media as described above.

Bacterial Identification and Antibiotic Resistance Testing

Identification of suspect bacteria was confirmed using conventional biochemical methods (Vitek 2; bioMeriuex, Marcy-l'Étoile, France). *C. difficile* identification and presence of toxin genes were confirmed by polymerase chain reaction.[35] Antimicrobial susceptibility testing was performed on confirmed isolates using standard protocols (disk diffusion or

broth microdilution).[36] MDR-*A. baumannii* isolates were defined using the 3-class-resistant definition.[37] *K. pneumoniae* isolates were considered resistant if they were positive for extended-spectrum β-lactamase (ESBL) by broth microdilution, New Delhi metallo-β-lactamase (bla_{NDM-1}), or carbapenemase (bla_{KPC}) by polymerase chain reaction.[36,38]

Data Analysis

For the preliminary study, the mean, standard deviation, and distribution (median and empirical distribution function (EDF) using nonparametric Brown-Mood and Kuiper tests) of overall aerobic MB per area sampled (CFU/100 cm^2) for each individual site were compared to develop the composite sampling strategy.

Descriptive statistics for overall MB per area sampled and each MDRO were calculated for the main sampling study composites and rooms using univariate analysis. Samples that were only broth positive were assigned a value of 1 CFU in order to be included in the analysis. Overall MB for each sample was calculated from the maximum aerobic or anaerobic colony counts, and total room MB was determined by summing C1 and C2 MB; C3 was not included due to the low number of samples collected. Total room MB was log normalized and compared using the Student t test ($P \leq .05$). SAS statistical software, version 9.3 (SAS Institute, Cary NC) was utilized.

RESULTS

Preliminary Study

Data from the preliminary study of 102 samples from 13 routine cleaned rooms revealed that surfaces with the highest mean bacteria were the room door handles (7,546 CFU/100 cm^2), telephone (2,350 CFU/100 cm^2), and remote/call button (1, 353 CFU/100 cm^2; Online Supplementary Table S1). There was a ~50-fold variation in overall mean aerobic bacteria across the sites, and although MDROs were recovered from several sites, the sites with the greatest bioburden (door handles) yielded no MDROs. The overbed table was the most common MDRO-positive site (53.9%). The number of MDRO-positive sites ranged from 0.0 to 80.0% per room (Online Supplementary Table S2); however, the number of sites sampled per room varied from 4 to 9 sites.

Main Study

During the main study, 375 composite samples were received. After excluding 15 samples due to insufficient information, 360 samples were analyzed. More composites were received from routine cleaned rooms (C1s, 113; C2s, 113; C3s, 16; total = 242) than from terminal cleaned rooms (C1s, 53; C2s, 53; C3s, 12; total = 118). The MB mean and range of composites from routine cleaned rooms (2,700 CFU/100 cm^2; ≤1–130,000 CFU/100 cm^2) was notably higher than that of composites from terminal cleaned rooms (353 CFU/100 cm^2; ≤1–4,300 CFU/100 cm^2; Table 1). C1s from routine cleaned rooms had the highest mean MB (3,800 CFU/100 cm^2), while C2s from terminal cleaned rooms had the lowest bioburden (244 CFU/100 cm^2; Fig. 1).

For each MDRO, recovery from routine cleaned room composites was markedly higher than from terminal cleaned room composites (Table 2). When assessing only the MDRO-positive samples, the highest mean MDRO bioburden was *K. pneumoniae* from routine cleaned room composites (1,284 CFU/100 cm^2) and the lowest was *A. baumannii* (0.66 CFU/100 cm^2) from terminal cleaned room composites (Table 2).

MDROs were recovered from 33.9% of composites from routine cleaned rooms with the highest recovery for any MDRO from C3 (43.8%) followed by C1 (34.5%) and C2 (31.9%; Table 3). VRE was the most frequently recovered
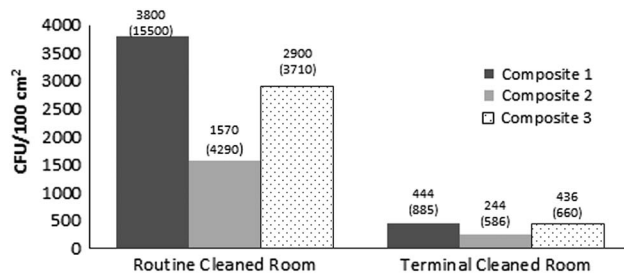

FIGURE 1. Microbial bioburden by composite type from routine and terminal cleaned rooms; mean (standard deviation), routine cleaned composites C1 and C2 (n = 113 each), composite 3 (n = 16); terminal cleaned composites C1 and C2 (n = 53 each) and composite C3 (n = 12); CFU, colony-forming units.

TABLE 1. Overall Microbial and Individual MDRO Bioburden Detected in Composite Samples From Routine Cleaned (n = 242) and Terminal Cleaned Rooms (n = 118)

	Overall MB, CFU/100 cm^2		MRSA, CFU/100 cm^2		VRE, CFU/100 cm^2		MDR-*A. baumannii*, CFU/100 cm^2		*K. pneumoniae*, CFU/100 cm^2		*C. difficile*, CFU/100 cm^2	
	Routine	Terminal	Routine	Terminal	Routine	Terminal	Routine	Terminal	Routine	Terminal	Routine	Terminal
Mean	2,700	353	79	0.12	42	1	6	0.01	27	4	1	0.57
SD	11,042	742	852	0.84	204	7	69	0.06	208	48	6	2.9
Range	≤1–30,000	≤1–4,300	≤1–13,000	≤1–8	≤1–1,680	≤1–59	≤1–1,061	0.0–0.7	≤1–2,051	≤1–524	≤1–65	≤1–22

NOTE. MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; MB, microbial burden; VRE, vancomycin-resistant enterococci; MDR, multidrug resistant; CFU, colony-forming units; SD, standard deviation.

TABLE 2.   Individual MDRO Bioburden Detected in MDRO-Positive Samples from Routine Cleaned and Terminal Cleaned Rooms

	MRSA, CFU/100 cm^2		VRE, CFU/100 cm^2		MDR-*A. baumannii*, CFU/100 cm^2		*K. pneumoniae*, CFU/100 cm^2		*C. difficile*, CFU/100 cm^2	
	Routine	Terminal	Routine	Terminal	Routine	Terminal	Routine	Terminal	Routine	Terminal
n	22	5	45	7	6	1	5[a]	2[b]	24	9
Mean	873	3	223	18	222	0.66	1,284	262	11	7
SD	2,760	3	433	21	416	…	765	370	15	8

NOTE. MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MDR, multidrug resistant; CFU, colony-forming units; n; number of composites positive for MDRO; SD, standard deviation.
[a]All were extended-spectrum β-lactamase positive (ESBL+).
[b]One sample was carbapenemase (*bla*$_{KPC}$) positive and 1 was ESBL+.

TABLE 3.   Percent of Composites Positive for Given MDRO from Routine Cleaned and Terminal Cleaned Rooms

MDRO	Composite 1, % (No.)		Composite 2, % (No.)		Composite 3, % (No.)	
	Routine	Terminal	Routine	Terminal	Routine	Terminal
MRSA	10.6 (12)	7.5 (4)	7.1 (8)	1.9 (1)	12.5 (2)	0
VRE	18.6 (21)	5.7 (3)	16.8 (19)	5.7 (3)	31.3 (5)	8.3 (1)
MDR-*A. baumannii*	2.7 (3)	1.9 (1)	2.7 (3)	0	0	0
K. pneumoniae	2.7 (3)	3.8 (2)	1.8 (2)	0	0	0
C. difficile	8.0 (9)	7.5 (4)	11.5 (13)	7.5 (4)	12.5 (2)	8.3 (1)
Any MDRO	34.5 (39)	20.8 (11)	31.9 (36)	15.1 (8)	43.8 (7)	16.7 (2)
Total	113	53	113	53	16	12

NOTE. MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MDR, multidrug resistant.

MDRO from all 3 composites from routine cleaned rooms (18.6% of C1s; 16.8% of C2s; 31.3% of C3s). All 5 target MDROs were recovered from C1s and C2s, but only MRSA, VRE and *C. difficile* were recovered from C3s. In total, 24 isolates of *C. difficile* were recovered; however, 50% were recovered from non–*C. difficile* rooms. Most *C. difficile* isolates were recovered from C1s (38%) or C2s (54%), very few were recovered from C3s (8%). The 2 isolates recovered from C3s were from *C. difficile* isolation rooms. In total, 6 *A. baumannii* isolates were MDR and 6 *K. pneumoniae* isolates were ESBL producers.

Composites from terminal cleaned rooms were less frequently MDRO positive (17.8%), with the highest recovery from C1s (20.8%), then C3s (16.7%) and C2s (15.1%; Table 3). The individual MDRO recovery was low (<8.3%), and no individual MDRO was consistently recovered. All 5 MDROs were recovered from C1s, but *A. baumannii* and *K. pneumoniae* were not recovered from C2s, along with MRSA from C3s. In total, 9 *C. difficile* isolates were recovered, and 50% were recovered from *C. difficile* isolation rooms. Only 1 room was positive for *C. difficile* from a C3 alone; 1 *A. baumannii* isolate was MDR, 1 *K. pneumoniae* isolate was an ESBL producer; and another was a carbapenemase producer.

For room-level evaluations of MB and MDRO bioburden, 166 rooms were assessed (113 routine cleaned rooms and 53 terminal cleaned rooms). In routine cleaned rooms, the mean MB (5,373 CFU/100 cm^2) and mean MDRO bioburden (302 CFU/100 cm^2) were almost 8 and 24 times higher,
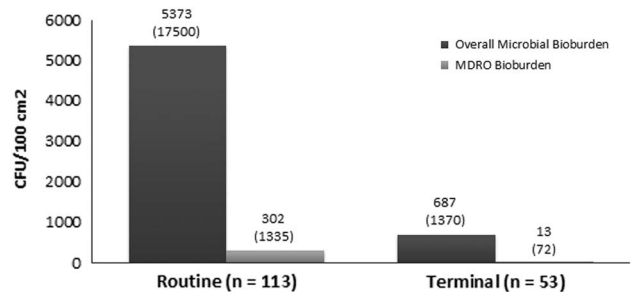

FIGURE 2.   Overall microbial bioburden and multidrug-resistant organism (MDRO) bioburden for routine and terminal cleaned rooms (composites 1 and 2 summed); mean (standard deviation); CFU, colony-forming units.

respectively, than in terminal cleaned rooms (687 and 13 CFU/100 cm^2; Figure 2). There was a significant difference between routine and terminal cleaned rooms for recovery of $\log_{10}$ MB ($P = .0002$).

MDROs were recovered from 39.8% of rooms (75.8% routine; 24.2% terminal) (Fig. 3). Almost 45% of routine cleaned rooms and 30% of terminal cleaned rooms were positive for an MDRO. MRSA was the most common contact precaution-room type sampled (79%); however, VRE was the predominantly recovered MDRO from all rooms (19.3%) and from routine cleaned rooms (23.9%); *C. difficile* was the

predominantly recovered MDRO from terminal cleaned rooms (11.3%). For all MDROs, except MRSA, more rooms were positive for a discordant MDRO than concordant contact precaution MDRO (Table 4). VRE was recovered more often from discordant contact precaution rooms (n = 20) than from concordant VRE contact precaution rooms (n = 12), and all 5 rooms positive for *K. pneumoniae* were discordant contact precaution rooms. Multiple MDRO types were recovered from 11 rooms.

DISCUSSION

We developed a composite sponge-wipe, large-surface-area sampling plan to determine typical bacterial bioburden levels in MDRO contact precaution rooms after routine or terminal cleaning. We found that the mean MB collected from surfaces was 2,700 CFU/100 cm^2 in routine cleaned rooms and 353 CFU/100 cm^2 in terminal cleaned rooms. These results present

FIGURE 3. Percent recovery of each target multidrug-resistant organism (MDRO) and all MDROs from routine cleaned, terminal cleaned, and all rooms.

a broad but not representative cross section of current levels of contamination across these facilities.

When we assessed bioburden by composite type, we found that surfaces sampled as part of C1 (ie, bed rails, TV remote, call button, and telephone), which were usually closest to the patient, were often the most contaminated, which is consistent with other studies.[12,19,22–24] Similar MB was reported by Schmidt et al[23] on plastic bed rails after cleaning (1,112–5,198 CFU/100 cm^2). However, while C1s had the highest MB in samples from routine and terminal cleaned rooms, C2s and C3s were within the same magnitude (log$_{10}$).

When we combined C1s and C2s to determine overall room bioburden, routine cleaned rooms were ~8 times more contaminated than terminal cleaned rooms. This difference is significant and was expected because terminal cleaning procedures are often more thorough and use more efficacious cleaning/disinfection products (eg, bleach or hydrogen peroxide vapor) than routine cleaning procedures. In addition, previous research on cleaned bed rails has shown that bacterial counts can rebound within a few hours of cleaning during ongoing patient care.[24] In our study, the time between cleaning and sampling varied for both routine and terminal cleaned rooms.

In this study, we also attempted to determine the bioburden levels of 5 target MDROs. We found that ~40% of the rooms sampled were positive for any MDRO, the majority of which were routine cleaned. Overall, mean MDRO bioburden was low for each target organism; however, even the presence of low amounts of any MDRO on surfaces in patient rooms is cause for concern. While we cannot directly compare MDRO recovery from this study to other published research because facilities and regions have their own unique microbiome, these results do show that MRSA, VRE, and *C. difficile* are more often found on these heathcare surfaces than *A. baumannii* and *K. pneumoniae*. We also found that recovered MDROs often were discordant with the MDRO requiring contact precautions and that many rooms were positive for MDROs different than
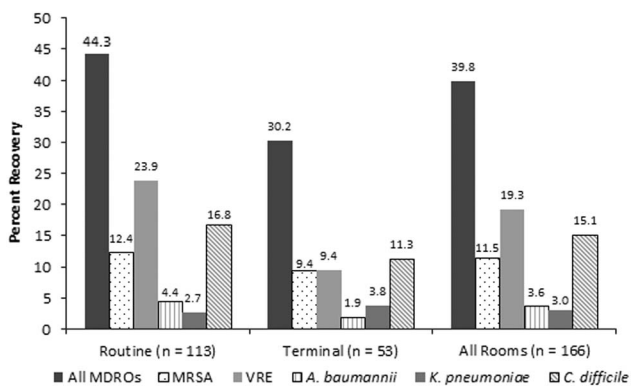
TABLE 4. Summary of MDRO Recovery From Contact Precaution Rooms

MDRO	Rooms Positive for MDRO Recovery	Rooms Positive for Concordant[a] Contact Precaution MDRO, No. (total contact precaution rooms sampled)[b]	Rooms Positive for Discordant[c] Contact Precaution MDRO
MRSA	19	16 (92)	3
VRE	32	12 (51)	20
MDR-*A. baumannii*	6	1 (3)	5
K. pneumoniae	5	0 (4)	5
C. difficile	25	10 (30)	15

NOTE. MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MDR, multidrug resistant.
[a]MDRO(s) recovered by sampling that matched the patient's diagnosis which required contact precautions.
[b]In total, 23 patients were on contact precautions for multiple MDROs.
[c]MDRO(s) recovered by sampling from room that did not match the MDRO causing the patient to be on contact precautions.

those for which patients were isolated or for additional MDROs (Table 4). The presence of MDROs other than those for which patients were isolated may be due to undetected carriage of the current patient, contamination carried in by healthcare workers, visitors, etc, or residual contamination from prior occupants.[39] Many HAI-pathogens, such as, MRSA,VRE, *C. difficile*, and *A. baumannii*, can persist in a viable infectious state on environmental surfaces for days to months.[2,40]

This study has some limitations. Our methods may underestimate total room MB and MDRO bioburden density in the rooms due to the variability of surface characteristics, microorganisms, and sampling efficiency. Additional sources of variablilty that we were unable to measure include the heterogeneity of surface contamination, and differing cleaning methods and cleaning intensities among facilities. Despite the increased labor in processing, we chose to use a large-surface-area sampling strategy to overcome the lack of surface homogeneity and to increase our sensitivity for detecting MDROs, especially in the terminal cleaned rooms. Although the relationships among sampled area, the distribution of sampled area across different sites, and sensitivity of MDRO detection are not fully known, sampling multiple sites did result in increased MDRO detection, as seen in the preliminary study (S1 and S2). Also, during the main study, collecting 2 composite samples increased not only the amount of bioburden recovered but also the percentage of MDRO-positive rooms compared to that expected if we had limited sampling to C1 or C2 sites (data not shown). In addition, we do not know what exact site the MDROs were recovered from due to the composite sampling strategy, and these results only provide a snapshot of room bioburden because the rooms were only sampled once. Another limitation is that we did not assess cleaning protocols or adherence to them in the rooms, so we cannot know whether these items/rooms were cleaned properly or thoroughly. However, with the large number of rooms sampled, the effect of a few badly cleaned rooms would be minimized on the overall mean MB.

It will be important to determine how these levels of microbial contamination relate to the risk of the patient acquiring an MDRO. These data provide a first step in determining the MB of common hospital surfaces, which may help to develop standards for adequacy of cleaning and disinfection methods on healthcare surfaces and provides a framework for studies using the evidentiary hierarchy for environmental infection control to increase patient safety by cleaning and disinfection.[39] These results could be used to help parameterize models describing the role of environmental surface contamination in transmission. Future areas of investigation include the significance of cleaning type, cleaning products, and other variables on MB and risk of MDRO recovery. In addition, future studies should evaluate whether other sampling methods that are less labor intensive yield comparable bioburden or MDRO recovery as the sponge-wipe method.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/ice.2016.198.

REFERENCES

1. Magill SS, Edwards JR, Bamberg W, et al. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med* 2014;370:1198–1208.
2. Boyce JM. Environmental contamination makes an important contribution to hospital infection. *J Hosp Infect* 2007;65:50–54.
3. Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of *Clostridium difficile* by hospitalized patients: evidence for colonized new admissions as a source of infection. *J Infect Dis* 1992;166:561–567.
4. Simor AE, Lee M, Vearncombe M, et al. An outbreak due to multiresistant *Acinetobacter baumannii* in a burn unit: risk factors for acquisition and management. *Infect Control Hosp Epidemiol* 2002;23:261–267.
5. Aygun G, Demirkiran O, Utku T, et al. Environmental contamination during a carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit. *J Hosp Infect* 2002;52:259–262.
6. Cimolai N. MRSA and the environment: implications for comprehensive control measures. *Eur J Clin Microbiol Infect Dis* 2008;27:481–493.
7. Carling PC, Briggs JL, Perkins J, Highlander D. Improved cleaning of patient rooms using a new targeting method. *Clin Infect Dis* 2006;42:385–388.
8. Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 2004;39:1182–1189.
9. Dubberke ER, Reske KA, Noble-Wang J, et al. Prevalence of *Clostridium difficile* environmental contamination and strain

variability in multiple health care facilities. *Am J Infect Control* 2007;35:315–318.

10. Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H. Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. *J Hosp Infect* 2006;62: 187–194.

11. Verity P, Wilcox MH, Fawley W, Parnell P. Prospective evaluation of environmental contamination by *Clostridium difficile* in isolation side rooms. *J Hosp Infect* 2001;49:204–209.

12. Dancer S, White L, Robertson C. Monitoring environmental cleanliness on two surgical wards. *Int J Environment Health Res* 2008;18:357–364.

13. Carling PC, Beheren SV, Kimc P, Woods C. Intensive care unit environmental cleaning: an evaluation in sixteen hospitals using a novel assessment tool. *J Hosp Infect* 2008;68:39–44.

14. Pfeiffer EH, Wittig JR, Dunkelberg H, Werner HP. Hygienic and bacteriological comparative studies in 50 hospitals V. bacterial contamination of hospital surfaces. *Zbl Bakt Hyg* 1978;167:11–21.

15. Frabetti A, Vandini A, Balboni P, Triolo F, Mazzacane S. Experimental evaluation of the efficacy of sanitation procedures in operating rooms. *Am J Infect Control* 2009;37:658–664.

16. Boyce JM, Havill NL, Otter JA, Adams NMT. Widespread environmental contamination associated with patients with diarrhea and methicillin-resistant *Staphylococcus aureus* colonization of the gastrointestinal tract. *Infect Control Hosp Epidemiol* 2007;28:1142–1147.

17. Oie S, Kamiya A. Contamination of environmental surfaces by methicillin-resistant *Staphylococcus aureus* (MRSA). *Biomed Lett* 1998;57:115–119.

18. Oie S, Hosokawa I, Kamiya A. Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2002;51:140–143.

19. Moore GP, Muzslay M, Wilson AP. The type, level, and distribution of microorganisms within the ward environment: a zonal analysis of an intensive care unit and a gastrointestinal surgical ward. *Infect Control Hosp Epidemiol* 2013;34:500–506.

20. Rutala WA, Katz EBS, Sherertz RJ, Sarubbi FA. Environmental study of a methicillin-resistant *Staphylococcus aureus* epidemic in a burn unit. *J Clin Microbiol* 1983;18:683–688.

21. Claro T, O'Reilly M, Daniels S, Humphreys H. Surface microbial contamination in hospitals: a pilot study on methods of sampling and the use of proposed microbiologic standards. *Am J Infect Control* 2015;43:1000–1002.

22. Schmidt MG, Anderson T, Attaway HH, Fairey S, Kennedy C, Salgado CD. Patient environment microbial burden reduction: a pilot study comparison of 2 terminal cleaning methods. *Am J Infect Control* 2012;40:559–561.

23. Schmidt MG, Attaway HH, Fairey SE, Steed LL, Michels HT, Salgado CD. Copper continuously limits the concentration of bacteria resident on bed rails within the intensive care unit. *Infect Control Hosp Epidemiol* 2013;34:530–533.

24. Attaway HH, Fairey S, Steed LL, Salgado CD, Michels HT, Schmidt MG. Intrinsic bacterial burden associated with intensive care unit hospital beds: effects of disinfection on population recovery and mitigation of potential infection risk. *Am J Infect Control* 2012;40:907–912.

25. Carling P, Perkins J. Ferguson J. Thomasser A. Evaluating a new paradigm for comparing surface disinfection in clinical practice. *Infect Control Hosp Epidemiol* 2014;35:1349–1355.

26. Boyce JM, Havill N, Dumigan D, Golebiewski M, Balogun O, Rizvani R. Monitoring the effectiveness of hospital cleaning practices by use of an adenosine triphosphate bioluminescence assay. *Infect Control Hosp Epidemiol* 2009;30:678–684.

27. Suzuki A, Namba Y, Matsuura M, Horisawa A. Bacterial contamination of floors and other surfaces in operating rooms: a five-year study. *J Hyg Camb* 1984;93:559–566.

28. Sehulster S, Chinn R. Guidelines for environmental infection control in health care facilities: recommendations of CDC and the Healthcare Infection Control Advisory Committee (HICPAC). *MMWR* 2003;52:1–42.

29. Dancer SJ. How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *J Hosp Infect* 2004;56:10–15.

30. Malik R, Cooper RA, Griffith CJ. Use of audit tools to evaluate the efficacy of cleaning systems in hospitals. *Am J of Infect Ctrl* 2003;31:181–187.

31. Dancer SJ. Pitfalls in microbiological sampling of the healthcare environment. A response to "Evaluating a New Paradigm for Comparing Surface Disinfection in Clinical Practice". *Infect Control Hosp Epidemiol* 2015;36:849–850.

32. Mulvey D, Redding P, Robertson C, et al. Finding a benchmark for monitoring hospital cleanliness. *J Hosp Infect* 2011;77:25–30.

33. White LF, Dancer SJ, Robertson C, McDonald J. Are hygiene standards useful in assessing infection risk? *Am J Infect Control* 2008;36:381–384.

34. Nerandzic MM, Donskey CJ. Effective and reduced-cost modified selective medium for isolation of *Clostridium difficile*. *J Clin Microbiol* 2009;47:397–400.

35. Persson S, Torpdahl M, Olsen KEP. New multiplex PCR method for the detection of *Clostridium difficile* toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. *Clin Microbiol Infect* 2008;14: 1057–1064.

36. CLSI. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. *CLSI document M100-S22* Vol 32. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

37. Kallen AJ, Hidron AI, Patel J, Srinivasan A. Multidrug resistance among Gram-negative pathogens that caused healthcare-associated infections reported to the National Healthcare Safety Network, 2006–2008. *Infect Control Hosp Epidemiol* 2010;31: 528–531.

38. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1) genes. Centers for Disease Control and Prevention website. http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html. Published 2011. Accessed January 24, 2012.

39. McDonald LC, Arduino M. Editorial Commentary: climbing the evidentiary hierarchy for environmental infection control. *Clin Infect Dis* 2013;56:36–39.

40. Rutala WA, Weber DJ. The benefits of surface disinfection. *Am J Infect Control* 2004;32:226–231.