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Contamination of the Clinical Microbiology Laboratory with Vancomycin-Resistant Enterococci and Multidrug-Resistant *Enterobacteriaceae*: Implications for Hospital and Laboratory Workers

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We surveyed environmental surfaces in our clinical microbiology laboratory to determine the prevalence of vancomycin-resistant enterococci (VRE) and multidrug-resistant *Enterobacteriaceae* (MDRE) during a routine working day. From a total of 193 surfaces, VRE were present on 20 (10%) and MDRE were present on 4 (2%) of the surfaces tested. In a subsequent survey after routine cleaning, all of the 24 prior positive surfaces were found to be negative. Thus, those in the laboratory should recognize that many surfaces may be contaminated by resistant organisms during routine processing of patient specimens.

Multidrug-resistant organisms have become increasingly prevalent in acute care hospitals, as well as in long-term care facilities (National Nosocomial Infections Surveillance System 1999 NNIS ICU Surveillance Report, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention [www.cdc.gov/ncidod/hip/NNIS/ar_surv99.pdf]). A point prevalence study of inpatients in our large, university-affiliated hospital conducted during 1999 demonstrated patient colonization rates of 8.8% for vancomycin-resistant enterococci VRE) and 4.5% for multidrug-resistant *Enterobacteriaceae* (MDRE) (T. Zembower et al., Abstr. 4th Decennial Int. Conf. Nosocomial Healthcare-Associated Infect. 2000, abstr. P-T1-24, 2000). In addition, our active surveillance program that frequently detects these organisms raised the concern for potential contamination of the clinical microbiology laboratory (9). Since these organisms persist on surfaces (7, 8), and experts in the field "believe there is sufficient evidence to state that inanimate surfaces likely play a role in transmission of VRE (15)," we investigated the prevalence of VRE and MDRE in our clinical microbiology laboratory to assess the potential presence of these organisms on environmental work surfaces and adjacent clean areas.

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We used the RODAC imprint technique to collect the surveillance specimens (3). The RODAC contact plates (Simport, Inc., Montreal, Quebec, Canada) were prepared in our laboratory. Each contained tryptic soy agar with 5% sheep blood, vancomycin (6 μg/ml), ceftazidime (2 μg/ml), amphotericin B (2 μg/ml), and clindamycin (1 μg/ml), which we designated VACC agar. The RODAC plate medium (VACC medium) also was formulated in the Northwestern Memorial Hospital clinical microbiology laboratory as previously described (T. Zembower, D. Peters, D. Dressel, G. Noskin, R. Thompson, and L. Peterson, Abstr. 35th Annu. Meet. Infect. Dis. Soc. Am. 1997, abstr. 743, 1997). This selective medium was developed to facilitate the detection of VRE and MDRE from surveillance cultures.

Two separate surveillance samplings were performed. The first occurred during the middle of a normal working day with cultures from 193 distinct environmental surfaces. The 193 sites represented 160 high-use surfaces in the microbiology and molecular typing laboratories. Thirty-three surfaces were also cultured in clean areas housing administrative support functions adjacent to, but outside of, the laboratory. Nine surfaces were inoculated with known densities of the target organisms as controls. High-use surfaces cultured were defined as those commonly contacted by the technologists during a routine working day: bench tops, telephones, keyboards, door handles, biohazard waste containers, chairs, pipettors, gloves, and gowns. One technologist's shoes and the laboratory floor also were sampled. Clean area surfaces included desks, telephones, and computer keyboards, as well as restroom surfaces. Once the data was analyzed, another surveillance study was done that cultured the 24 initially positive areas. The second surveillance study was conducted at the end of the day after routine laboratory cleaning by using MediGuard surface decontaminant cleaner (Metrex Research Corp., Parker, Colo.). Surfaces were sprayed until wet and wiped clean with a paper towel.

Testing done for the nine controls in the initial study consisted of inoculating flat surfaces with various densities of a vancomycin-resistant *Enterococcus faecalis* (six samples) and an extended spectrum β-lactamase (ESBL) producing *Escherichia coli* (three samples). To avoid contaminating actual work

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TABLE 1. Recovery of vancomycin-resistant *E. faecium* and multidrug-resistant *E. coli* from control surfaces

Study	No. of colonies at the indicated density								
	VRE set 1 at:			VRE set 2 at:			MDRE set at:		
	10^8 CFU/ml	10^6 CFU/ml	10^5 CFU/ml	10^8 CFU/ml	10^6 CFU/ml	10^5 CFU/ml	10^8 CFU/ml	10^6 CFU/ml	10^5 CFU/ml
Initial study	>100	50	2	>100	18	NG	>100	NG	NG
Repeat study (precleaning)	>25	NT	NT	NT	NT	NT	>25	NT	NT
Repeat study (postcleaning)	NG						NG		

[a] Values for RODAC plate growth are indicated in boldface (in CFU/plate). NT, not tested; NG, no growth.

surfaces, each control strain was swabbed (0.01 to 0.1 ml delivered volume) onto the inside surface of empty, 150-mm-diameter, sterile plastic petri dish and allowed to air dry for ca. 2 min before sampling. Sampling of the above controls was repeated for the second set of surveillance cultures with a single density of vancomycin-resistant *E. faecalis* (one sample) and ESBL-producing *E. coli* (one sample). Here, surveillance samples were obtained from the inside surface of the petri dishes both prior to and after cleaning with MediGuard. Thus, in total there were seven VRE and four MDRE controls.

For each sampled area, ca. 10 cm^2 was touched three to five times with the RODAC plate to ensure that the entire area was sampled. The RODAC plates were then incubated for 48 h in CO_2 at 35°C. All catalase-negative, gram-positive cocci found were identified to the species level by traditional manual biochemical methods (5). Agar dilution susceptibility testing was performed according to the NCCLS guideline (6). Organisms that were identified as *E. faecalis* or *E. faecium* and defined as resistant to vancomycin (MIC > 6 μg/ml) were classified as VRE. Similarly, all gram-negative bacteria growing on the plates that were oxidase-negative, glucose-fermenting organisms were identified, and an agar dilution susceptibility test was performed. Organisms that were identified as a member of the family *Enterobacteriaceae* and that were resistant to aztreonam (MIC > 16 μg/ml) and/or ceftazidime (MIC > 16 μg/ml) or were determined to be an ESBL producer by exhibiting a clavulanate effect with ceftazidime (6) were classified as MDRE. Molecular genetic typing was done using restriction endonuclease analysis (REA) as previously described (2, 12). Organisms with a similarity index of >90% were considered sufficiently related to each other for the purposes of epidemiologic linkage.

Of the 193 surfaces initially tested, 20 were positive for VRE (10%) and 4 were positive for MDRE (2%). Additionally, VRE were detected on six of seven control surfaces, with MDRE recovered on two of four control surfaces (Table 1), predominantly from the highest-density inocula. All environmental VRE were identified as *E. faecium*, and all environmental MDRE were identified as *Enterobacter cloacae*. Two of these MDRE were ESBL (ceftazidime MIC = 64 μg/ml; ceftazidime-clavulanate MIC ≤ 1 μg/ml) producers (Table 2, genotype III). All sites positive for VRE or MDRE were high-use microbiology or molecular typing laboratory surfaces (Table 2). Notably, VRE or MDRE were not recovered from any surfaces in the clean areas tested. Genotyping results of the VRE environmental strains showed that there were six different genotypes, and all were related to isolates from hospitalized patients recovered by the laboratory within the previous month (Table 2). Three distinct genotypes of *E. cloacae* were recovered; and two of these types were related to isolates from hospitalized patients recovered within the previous month. However, the third genotype that was isolated from 2 surfaces (type III) was not genetically similar to any recent isolate from a hospitalized patient. Despite an extensive search of our typing database, the source of this ESBL *E. cloacae* is unknown.

Of the 24 surfaces initially positive for VRE or MDRE that were recultured in the second surveillance study after cleaning at the end of a normal working day, none were positive, indicating successful decontamination.

Environmental contamination with VRE appears common in our clinical microbiology laboratory during the workday. A presentation by Willey and colleagues also demonstrated that recovery of VRE from their laboratory environment is common, confirming our findings (B. M. Willey, D. E. Low, and A. J. McGeer, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2106, 1999). Seven (25%) of the 28 surfaces these researchers surveyed contained VRE (five *E. faecalis* and two *E. faecium* isolates). All VRE had identical genotypes to strains previously worked on in that laboratory. This second

TABLE 2. Recovery of VRE and MDRE from microbiology laboratory surfaces.

Organism and location	Genotype
VRE	
Blood bench surface	I
Blood bench keyboard	I
Blood bench telephone	I
Blood bench hood	I
Blood bench biohazard waste container	I
Urine bench biohazard waste container	I
Molecular typing laboratory floor	I
Molecular typing laboratory pipettor	I
Molecular typing laboratory procedure manual	I
Molecular typing laboratory sleeves of lab coat	I
Surveillance bench floor	I
Molecular typing laboratory shoes	I
Specimen processing sleeves of lab coat	I
Workcard review room telephone	I
Molecular typing laboratory bench surface	II
Molecular typing laboratory pipettor	III
Molecular typing laboratory chairs	III
Surveillance bench sleeves of lab coat	IV
AFB bench biohazard waste container	V
Respiratory bench biohazard waste container	VI
MDRE	
Walk-in refrigerator floor	I
Surveillance bench floor	II
Respiratory bench biohazard waste container	III
Virology bench surface	III

laboratory reporting similar findings strongly suggests our results are not unique. Additionally, VRE contamination of the outpatient clinic environment has been reported in areas caring for patients colonized with this organism Smith et al. (11). These authors found that environmental contamination occurred in 29% of visits. Taken together, the data indicate that the inpatient areas of the hospital are not the only concern for environmental contamination and the potential spread of VRE.

A recent report from England found that infections acquired in laboratories were infrequent, but one of the most common groups implicated were employees of microbiology laboratories (14). Environmental contamination has been implicated in patient-to-patient transmission of VRE (4). Colonization of healthy hospital employees and their households with VRE also has been recently documented and was limited to individual households likely having VRE contact within the hospital (J. Baran, Jr., J. Ramanathan, K. M. Riederer, and R. Khatib, Abstr. 39th ICAAC, abstr. 2017, 1999). Therefore, our results raise the possibility that transmission to workers or visitors in the clinical microbiology laboratory may occur.

Recovery of MDRE from the environment was less common. At our institution, the prevalence of MDRE colonization or infection is less frequent than that of VRE among patients, thus with a lower number of positive cultures this results in fewer opportunities for environmental contamination in the laboratory.

We used the RODAC imprint technique for sampling because it is less labor-intensive than the swab methods and because it can successfully recover multidrug-resistant organisms from the environment (3). Our controls indicate that we were able to detect contamination from an initial inoculum (e.g., spill) of 10^3 to 10^4 CFU/ml for VRE and at a somewhat higher level ($\geq 10^4$ CFU/ml) for MDRE, assuming ca. 0.01 to 0.1 ml was placed on to the control surfaces. Since 10^{10} to 10^{11} CFU of bacteria can readily occur on an agar plate after 24 to 48 h of incubation, such high densities of organism in the clinical laboratory are to be expected.

Importantly, all surfaces that were found to be positive on the initial survey were negative on repeat testing at a later time, after routine laboratory cleaning. This is also expected, since Saurina et al. (10) demonstrated that many commonly used disinfectants, including isopropyl alcohol, sodium hypochlorite, and phenolic and quaternary ammonium compounds were all highly effective at removing VRE from surfaces when used as recommended. Laboratory surfaces must be disinfected at the completion of work and after accidental spills (13). Our routine practice at the end of each work shift is to spray work surfaces (including computer keyboards with plastic covers) with MediGuard until wet and then to wipe the surface clean with a paper towel. This practice appears to be effective for removing both VRE and MDRE.

Frequent environmental contamination within the microbiology laboratory poses three major risks for healthcare workers and patients. First, laboratory workers may become colonized with these organisms and inadvertently carry them to other parts of the hospital or to the community. Second, cross-contamination of specimens can occur so that false infection or colonization of patients is reported from the laboratory (Willey et al., Abstr. 39th ICAAC). Third, medical personnel visiting the laboratory for consultation or during teaching rounds may unknowingly contact a surface with VRE or MDRE and carry these organisms elsewhere within the medical center.

In order to minimize the potential acquisition of antimicrobial agent-resistant bacteria, similar practices should occur in the laboratory as are recommended when contact with a patient or environmental surface contaminated with VRE is anticipated (1). We recommend that disposable lab coats and well-fitting gloves are worn at all times and for all work functions and that these be removed when personnel exit the microbiology laboratory. Additionally, strict daily cleaning must be done (13), since it will adequately decontaminate the environmental surfaces in the microbiology laboratory. Everyone entering the laboratory should use good hand hygiene when leaving so that any transiently acquired organisms are removed from their hands before returning to patient care areas. Such measures should be considered as a routine practice for microbiology laboratories that frequently recover multidrug-resistant pathogens from the clinical specimens that they process.

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