JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2009, p. 1229–1230 Vol. 47, No. 4
0095-1137/09/$08.00+0 doi:10.1128/JCM.02424-08

Predicting Clearance of Colonization with Vancomycin-Resistant Enterococci and Methicillin-Resistant *Staphylococcus aureus* by Use of Weekly Surveillance Cultures▽

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Received 17 December 2008/Returned for modification 6 February 2009/Accepted 17 February 2009

We analyzed surveillance cultures for vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) collected during a multicenter trial to determine if three negative cultures collected at weekly intervals would predict clearance of VRE or MRSA from colonized patients. Seventy-two percent of VRE-colonized patients and 94% of MRSA-colonized patients were culture negative after three consecutive negative cultures.

Infections with oxacillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) have increased dramatically in recent years, and together, they cause 12% of all health care-associated infections (2, 8, 16). Patients infected with MRSA or VRE, as well as asymptomatically colonized patients, serve as a reservoir for transmission of these bacteria to other hospitalized patients. To prevent spread, national guidelines recommend that health care providers use contact precautions during care of colonized and infected patients until it can be demonstrated that they are no longer colonized (5, 10, 14). Colonization with VRE can be prolonged (1, 3, 7), so the current recommendation by the Hospital Infection Control Practices Advisory Committee (HICPAC) is that isolation precautions should be maintained until VRE-negative results are documented with at least three consecutive negative cultures collected a minimum of 1 week apart (6, 14, 15). Persistent carriage with MRSA is also well documented (11, 13). Criteria for documenting clearance of MRSA from colonized patients are not established, although the standard of three negative weekly cultures is commonly applied to this population.

The National Institute of Allergy and Infectious Diseases (NIAID) supported a large, cluster-randomized trial assessing strategies to reduce transmission of VRE and MRSA in 19 intensive care units (ICUs) (W. C. Huskins, C. M. Huckabee, N. P. O'Grady, P. R. Murray, H. Kopetskie, L. Zimmer, M. E. Walker, R. L. Sinkowitz-Cochran, J. A. Jernigan, M. Samore, D. Wallace, and D. A. Goldmann, submitted for publication). Stool or perianal swabs for VRE and anterior nasal swabs for MRSA were collected at all ICU sites from patients upon admission, weekly thereafter, and on discharge and were processed at a central laboratory. More than 22,000 swabs from approximately 9,900 patients were collected for VRE culture, and a similar number was collected for MRSA, during the 18-month study period. The trial did not include any systematic effort to "decolonize" patients, and topical antimicrobial agents (e.g., mupirocin, chlorhexidine, and vancomycin) were used infrequently in the participating ICUs. The systematic collection of specimens from ICU populations at multiple geographic sites presented an opportunity to evaluate the HICPAC recommendations for VRE and their applicability for MRSA colonization.

Stool or perianal swabs for VRE were inoculated into bile-esculin azide broth supplemented with 8 μg/ml vancomycin and incubated at 35°C for 18 to 24 h. Broths were subcultured onto bile-esculin azide agar plates with 6 μg/ml vancomycin, incubated at 35°C, and inspected at 24 and 48 h of incubation. *Enterococcus* isolates were tested for *vanA*/*vanB* genes, using the LightCycler VRE detection test (Roche Applied Science, Indianapolis, IN). Nasal swabs for MRSA were inoculated into Mueller-Hinton broth supplemented with 7% NaCl and 2 μg/ml oxacillin and incubated at 35°C for 18 to 24 h. The broths were then subcultured onto mannitol salt agar plates with 4 μg/ml oxacillin, incubated at 35°C, and inspected after 24 and 48 h of incubation. Isolates of *S. aureus* were tested for the *mecA* gene by using the LightCycler MRSA detection test (Roche Applied Science, Indianapolis, IN).

We identified all patients with at least one positive surveillance culture for VRE or MRSA, followed by those with a minimum of two additional cultures, the first of which was negative. Of the specimens processed for VRE, slightly more than half (52%) of the cultures were negative after the initial negative culture. After two negative cultures, the next culture was negative in 68% of the culture sets, and 72% were negative after three negative cultures (Table 1). Of the specimens processed for MRSA, the percentages of negative cultures after one, two, and three negative cultures were 70%, 82%, and 94%, respectively.

These data demonstrate that a significant proportion of patients colonized with VRE would not be detected even after three negative weekly cultures. In contrast, the vast majority of previously MRSA-colonized patients were culture negative after three negative cultures.

The results of this study must be viewed with the following caveats. Highly sensitive culture techniques were used, includ-

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▽ Published ahead of print on 25 February 2009.

TABLE 1. Predictive values of negative VRE and MRSA cultures

No. of consecutive negative cultures following a positive	VRE		MRSA	
	No. of culture sets[a]	% (95% CI) sets with next culture negative[b]	No. of culture sets	% (95% CI) sets with next culture negative[b]
1	511	52 (47–56)	345	70 (65–75)
2	148	68 (60–75)	134	82 (75–88)
3	60	72 (59–81)	69	94 (86–98)

[a] Culture sets with one additional culture following the consecutive negative cultures.
[b] CI, confidence interval.

ing selective broth enrichment, selective differential agar media, and prolonged incubation (9, 12). If less-sensitive culture methods were used, then the negative weekly cultures would have underestimated the number of patients who remained colonized with VRE or MRSA. This could be responsible for the findings in this study compared with earlier reports that three negative weekly cultures could be used to exclude colonization with antibiotic-resistant bacteria (3, 4, 5). A single culture site was selected for MRSA (nares) and VRE (rectal or perianal) cultures in this study. Although these are the most commonly colonized sites, inclusion of additional culture sites would have increased the number of positive cultures and decreased the predictive value of sequential negative cultures. In other words, it is likely that additional colonized patients were not identified by the criterion of three consecutive negative cultures, due to restrictions of culture sites. We did not culture patients after ICU discharge, so we did not evaluate whether patients who were culture negative after three cultures remained culture negative long-term. Despite these limitations, this study supports the observation that colonization with VRE, and MRSA to a much lesser extent, may persist despite three initial negative cultures (1, 3, 7, 11, 13). It may be prudent to monitor previously colonized, high-risk patients for the reemergence of antibiotic-resistant bacteria.

The study was conducted through the Bacteriology and Mycology Study Group clinical research network with assistance from the data coordinating center, the Bacteriology and Mycology Statistical and Operations Unit.

This study was supported in part by federal funds from the National Institute of Allergy and Infectious Diseases, NIH, under contract numbers N01 AI-15440 and N01 AI-15441. Additional support for this study was provided by the Mayo Clinic Center for Translational Science Activities (M01-RR-00585 and UL1-RR024150).

We have no potential conflicts of interest.

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