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MINIREVIEW

Rapid Detection of Antimicrobial-Resistant Organism Carriage: an Unmet Clinical Need

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ANTIMICROBIAL RESISTANCE IN THE HEALTH CARE SETTING

The Centers for Disease Control and Prevention estimates that up to 2 million people in the United States suffer health care-associated (nosocomial) infections each year and that up to 90,000 patients die as a result of their infections (4). In addition, nosocomial infections are becoming increasingly difficult to treat because more than 70% of the bacterial pathogens that cause them are resistant to one or more of the antimicrobials commonly used for treatment (8). Indeed, the rate of antimicrobial resistance among nosocomial pathogens is steadily increasing: present surveillance reveals increasing rates of resistance to oxacillin among *Staphylococcus aureus* isolates and to vancomycin among *Enterococcus* spp. (5, 13, 14, 21). Methicillin (oxacillin)-resistant *S. aureus* (MRSA) strains are now responsible for more than half of all hospital-acquired *S. aureus* infections, and vancomycin-resistant enterococci (VRE) are responsible for more than one-quarter of all hospital-acquired enterococcal infections (8, 21). Moreover, MRSA and VRE have recently been identified by the Society for Healthcare Epidemiology of America (SHEA) as the two antimicrobial-resistant pathogens that are "most out of control" in U.S. hospitals (29). As infections caused by both pathogens increase in frequency, so too have worries about the potential transfer of vancomycin resistance from VRE to MRSA (30). Thus, the recent isolation of vancomycin-resistant *S. aureus* strains from patients in Michigan and Pennsylvania lent new urgency to efforts to prevent and control infections caused by antimicrobial-resistant organisms, particularly MRSA and VRE (6, 7).

PREVENTION AND CONTROL MEASURES

Much has been written and published about the prevention and control of MRSA and VRE, and this literature is well summarized in a recently published guideline from SHEA (29). In simple terms, antimicrobial resistance rates can increase in one of two ways: by emergence of resistance in a previously susceptible organism under pressure of antimicrobial use or by transmission of an already resistant pathogen from one person to another. Prevention and control measures can be categorized similarly: into measures that control antimicrobial use and measures that prevent transmission of already resistant pathogens (23, 40). While decreasing inappropriate antimicrobial use is a critical measure that can be used to control antimicrobial resistance, most health care facilities in the United States have not aggressively implemented antimicrobial use controls (13, 26, 44). By contrast, hospitals have placed more emphasis on preventing transmission of resistant pathogens, such as MRSA and VRE. The bedrock of transmission prevention is hand hygiene, and aggressive hand hygiene campaigns that encourage the use of alcohol-based hand rubs have been associated with reductions in the incidence of both nosocomial infections and resistant organism carriage and infection (35). In addition to hand hygiene, the Centers for Disease Control and Prevention's Hospital Infection Control Practices Advisory Committee recommends the use of contact precautions (also referred to as contact isolation) to prevent the spread of MRSA and VRE in the health care setting (22). Contact precautions require the use of barriers (gowns, gloves, and sometimes masks) to prevent transmission of antimicrobial-resistant bacteria and have been demonstrated to be effective for control of both MRSA and VRE transmission (22, 29).

However, most hospitals institute contact precautions only when culture of a specimen obtained for another reason reveals the presence of MRSA or VRE. Since many patients are colonized with MRSA (in the anterior nares or wounds) and VRE (in the gastrointestinal tract) without symptoms or signs of infection, a large number of unidentified colonized patients may serve as a reservoir for MRSA and VRE transmission in hospitals (29). In the face of steadily increasing rates of MRSA and VRE colonization and infection, many have argued that current control measures are inadequate and that control of MRSA and VRE will be impossible without seeking out the reservoir of colonized patients to prevent the unrecognized spread of resistance (3, 19, 37). For this reason, the new SHEA guidelines for prevention and control of MRSA and VRE recommend that all hospitals institute an active program for surveillance for colonization with resistant organisms (29).

OBSTACLES TO ACTIVE SURVEILLANCE FOR MRSA AND VRE CONTROL

Several obstacles have limited the success of MRSA and VRE control measures and stand in the way of implementing

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more aggressive active surveillance strategies. First, the screening techniques used at present require culture, which has a limited sensitivity for the detection of VRE (12) and which requires 48 to 72 h or more to perform. During the time that it takes to return a result, patients must be placed in isolation (unnecessarily, if the result is negative) or may serve as reservoirs for transmission if they are not isolated and are found to be carriers of MRSA or VRE. Second, screening of large numbers of patients requires substantial resources, primarily in time, costs of cultures (particularly if the hospital outsources laboratory services), and costs of isolation (24). Finally, contact isolation itself has negative consequences for patients, including reduced contact with health care workers (24)—which increases the risk for other adverse events (41)—and untoward psychological effects (25).

Many of these obstacles could be overcome with the availability and implementation of rapid, sensitive, and inexpensive screening assays for detection of VRE and MRSA in clinical specimens. Tests that could be performed directly with patient samples (i.e., bacterial growth in culture would not be required) and in a matter of hours would greatly advance efforts to rapidly isolate VRE and MRSA carriers—or conversely, would decrease the unnecessary use of patient isolation by quickly excluding MRSA and VRE carriage. Efforts could then be focused on improving the care and monitoring of the patient in isolation, so that other adverse events do not occur at increased frequencies in this population.

Rapid detection of MRSA and VRE (and other microorganisms) may be useful not only for a more focused and effective use of isolation but also for the use of preventive therapies as part of an overall strategy to reduce nosocomial infections (e.g., the use of mupirocin to prevent *S. aureus* infections [33]).

Many approaches to the more rapid detection of MRSA and VRE are described in the literature, including several commercial assays (1, 38, 42, 45). However, most of these approaches still require bacterial growth in culture prior to detection and therefore require 24 h or more to complete. This review focuses on very rapid, real-time detection of MRSA and VRE directly from patient samples, an area for which there is an important clinical need and relatively little published literature.

DETECTION OF METHICILLIN (OXACILLIN) RESISTANCE IN *S. AUREUS* DIRECTLY FROM PATIENT SAMPLES

Methicillin (oxacillin) resistance in *S. aureus* is mediated by the production of an altered penicillin binding protein called PBP 2a (9, 10). This protein is encoded by the *mecA* gene and confers resistance to all beta-lactam antibiotics. Because clinically and epidemiologically significant resistance to methicillin is always mediated by the *mecA* gene, *mecA* detection has become the "gold standard" for detection or confirmation of methicillin resistance among staphylococci, including *S. aureus* (10). Unfortunately, the *mecA* gene found in *S. aureus* is highly conserved in all species of staphylococci and is homologous to that carried by coagulase-negative staphylococci (CoNS), up to 80% of strains of which are methicillin resistant (14). Since CoNS are common commensals found in clinical samples from nonsterile sites (e.g., nares swabs), detection of the *mecA* gene alone is not sufficient to discriminate between MRSA and methicillin-resistant CoNS in a clinical sample with a mixed flora.

Published experience with rapid detection of MRSA directly from clinical samples therefore focuses upon methods that can detect not only the *mecA* gene but also a gene (e.g., *coa* or *femA*) that can distinguish the presence of *S. aureus* from the presence of CoNS. Even this does not overcome the false-positive results that could be obtained if a patient swab with a mixed flora contains both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant CoNS. Methods have therefore been developed to enrich samples for MRSA.

Methods that use oxacillin enrichment broth to suppress the growth of MSSA require an incubation step that prolongs the time to detection. For example, Levi et al. (27) used a novel method of isothermal signal amplification for detection of both the *mecA* and *coa* genes using a colorimetric detection system. Application of the assay to 100 patient screening swab specimens revealed a sensitivity of 58% and a specificity of 99% compared to the results of a *mecA-femB* PCR. However, because this assay requires an oxacillin broth enrichment step, the turnaround time was approximately 18 h (27).

Other rapid MRSA detection assays that use multiplex PCR are in development. During sample incubation these assays use a shorter (as short as 1 to 2 h) enrichment step, which is done in broth containing 6 μg of oxacillin per ml (to suppress MSSA) and 4% sodium chloride (to suppress CoNS). This method uses the observation that *S. aureus*, unlike CoNS, grows in high-salt conditions.

Francois et al. (20) used a rapid one-step immunomagnetic enrichment technique, using antibody to protein A, to enrich patient samples for *S. aureus*, followed by triplex quantitative PCR for the *mecA*, *S. aureus femA*, and CoNS *femA* genes. Application of this assay to 48 consecutive clinical samples (nares, inguinal, and wound swab specimens) revealed a sensitivity of 100% but a specificity of 64% (nine false-positive results) compared to the results of culture for MRSA detection. The turnaround time for this assay was less than 6 h (20).

A real-time PCR assay for rapid detection of MRSA directly from nares swab specimens is now commercially available (IDI-MRSA; Infectio Diagnostic, Quebec City, Quebec, Canada). This assay, performed on the SmartCycler instrument (Cepheid, Sunnyvale, Calif.), amplifies a target that links the staphylococcal cassette chromosome *mec* and a sequence from the *orfX* gene that is unique to *S. aureus* and uses a molecular beacon to detect the amplicon (23a; IDI-MRSA package insert; www.idi-mrsa.com). Although the test has recently been approved for use in Canada and the United States, published data describing its performance characteristics in detail are not yet available. However, the package insert describes a sensitivity of 92.5% and a specificity of 96.4% from a four-center study with more than 750 nasal swab specimens when the results were compared to those of the reference method, screening on oxacillin agar with and without a broth enrichment step (IDI-MRSA package insert; www.idi-mrsa.com).

DETECTION OF VRE DIRECTLY FROM PATIENT SAMPLES

Vancomycin resistance in enterococci may be mediated by several different genes, including *vanA*, *vanB*, *vanC*, *vanD*, and *vanE*. Of these, *vanA* and *vanB* predominate and are the only two that are of epidemiologic importance due to the transmissibility of the resistance genes (2). The *vanA* and *vanB* genes are found almost exclusively in *Enterococcus faecium* and *E. faecalis*, the enterococcal species that most commonly cause disease in humans, but have been detected in other species of *Enterococcus* (16, 32); and *vanB* has been detected in resident anaerobic flora of the human bowel (41a). *VanA*-mediated resistance is associated with inducible high-level resistance to vancomycin and teicoplanin, while *vanB*-mediated resistance is associated with resistance to vancomycin but retained susceptibility to teicoplanin. Both *vanA* and *vanB* act in concert with several other genes to produce ligase enzymes, which preferentially produce D-Ala–D-Lac over D-Ala–D-Ala in the peptidoglycan layer of the enterococcal cell wall. Because vancomycin acts by binding to the terminal end of the D-Ala–D-Ala pentapeptide molecule to inhibit cross-linking during cell wall synthesis, replacement of D-Ala–D-Ala with D-Ala–D-Lac confers resistance to vancomycin (2).

Because *vanA* and *vanB* are found almost exclusively in enterococci and are always considered epidemiologically significant (unlike *mecA* genes in CoNS), there is no need to design amplification assays to detect additional genes to identify the organism to the species level. Several investigators have developed PCR-based assays for *vanA* and *vanB* detection, with early efforts using gel-based systems and detection of resistance genes from isolated colonies (17). Satake et al. (39) first described the detection of *vanA* and *vanB* directly from fecal specimens using a multiplex PCR in a gel-based assay. Gel-based assays that detect *vanA* and *vanB* directly from fecal specimens or rectal swabs have reported sensitivities of 68 to 87% and specificities approaching 100% (34, 39). However, gel-based assays, while they are capable of providing results within 6 to 8 h from the time of specimen collection, increase the risk of laboratory and sample contamination, require more technician time, and slow the time to detection, in comparison to real-time PCR-based assays.

Palladino et al. (31) have recently reported on the use of real-time PCR to detect *vanA* and *vanB*, both from isolated colonies and directly from patient samples (32). Compared to a composite gold standard that included culture from vancomycin-containing enrichment broth, PCR directly with rectal swabs had a sensitivity of 50%, which was better than that of direct culture from rectal swab specimens but which was nonetheless complicated by a high level of PCR inhibition (55%). The sensitivity could be greatly improved (to 88%) by performing PCR after 24 h of incubation of the sample in vancomycin-containing enrichment broth (32). This step obviously increases the turnaround time by a full day, but it still provides results 2 to 3 days sooner than traditional culture techniques can. The investigators concluded that the high rate of specimen inhibition made real-time PCR detection of *vanA* and *vanB* directly from rectal or fecal swab samples to be unsuitable for routine use (32). Roche Diagnostics (Indianapolis, Ind.) now sells this assay as an analyte-specific reagent kit. This kit-based assay is sold for research purposes only and requires the purchase of a LightCycler instrument (Roche Diagnostics). The kit includes all the PCR reagents, internal control, primers, and hybridization probes specific for *vanA* and *vanB*.

We have also developed real-time PCR-based *vanA* and *vanB* detection assays for application directly to patient samples (perirectal or rectal swab specimens or fecal samples). The full description of our assay and its validation are pending, but the performance characteristics (compared to those of culture) reveal that it has a sensitivity and a specificity that each exceed 90% (K. Dodgson et al., Abstr. 104th Gen. Meet. Am. Soc. Microbiol. abstr. L-001, p. 76, 2004). We have used this assay since July 2003 to provide same-day turnaround for *vanA* and *vanB* detection from perirectal swab specimens. If specimens are received in the laboratory by 9:30 a.m., results are provided by 4 p.m., Monday through Friday. This assay has reduced the mean time to detection of VRE in our patients from 3.4 to 1.3 days and has allowed the earlier isolation of VRE carriers and the earlier discharge of patients to long-term-care and rehabilitation centers (B. Sigurdardottir et al., 14th Annu. Sci. Meet. Soc. Healthcare Epidemiol. Am., abstr. 234, p. 102, 2004). An evaluation of the impact of this test on measures of VRE transmission on our high-risk inpatient units is ongoing.

COST CONSIDERATIONS

Although upfront costs and expertise are required to establish in-house molecular assays such as real-time PCR, the cost of PCR per assay is often less than that of traditional culture techniques for VRE and MRSA detection (32, 39). In addition, if earlier detection allows early isolation and prevents the spread of MRSA and VRE, the cost savings could be enormous, as both MRSA and VRE infections have been associated with higher rates of mortality and higher costs than infections with the susceptible forms of the organisms (and certainly compared to the rate of mortality and the cost from the outright prevention of infection) (11, 18, 28, 29). Early detection of MRSA and VRE may also allow the earlier discharge of patients to long-term-care or rehabilitation facilities (which often require patient testing for VRE and/or MRSA infection or colonization prior to transfer). In our limited experience with the use of the real-time PCR assay for VRE detection, we have decreased the lengths of stay for patients discharged to long-term care by almost 2 days, saving the hospital an estimated $205,000 annually (Sigurdardottir et al., 14th Annu. Sci. Meet. Soc. Healthcare Epidemiol. Am., 2004).

When commercial kits for detection of these organisms become available, their cost will factor heavily into their acceptance into the laboratory.

PITFALLS OF REAL-TIME PCR DETECTION OF MRSA AND VRE DIRECTLY FROM PATIENT SAMPLES

The rapid turnaround times of real-time PCR assays may increase clinical acceptance and lead to significant increases in their use. For example, since we introduced our *vanA-vanB* detection assay, the number of VRE screenings ordered has increased by 17%. As for any laboratory test, there is a potential for overuse—the costs of testing all hospitalized patients, including those at low risk for VRE and MRSA carriage, may

then outweigh the benefits. These assays must therefore be used as part of a well-designed and carefully planned overall strategy for the reduction of antimicrobial resistance in the health care setting. One example of appropriate use would be to perform active surveillance by using rapid assays for patients residing on high-risk units or for patients with established risk factors for MRSA or VRE carriage. Cost-benefit analyses should be performed to evaluate the contribution of the assay to the control of MRSA and VRE.

One reason to focus the use of these assays on the high-risk patient population is the extremely high sensitivity of PCR. For example, while cultures of perirectal swab specimens have a detection limit for VRE of 10^4 CFU/ml (12), our in-house PCR assay can detect *vanA*-positive strains down to 50 to 80 CFU/ml and *vanB*-positive strains down to 8 CFU/ml. Paule et al. (32a) recently found that a gel-based PCR assay for *vanA* and *vanB* detection directly from rectal or perianal swab specimens was more sensitive than culture (18 of 38 specimens found to be positive by screening were PCR positive but culture negative). This assay detects *E. faecium* isolates containing *vanA* or *vanB* down to 20 CFU/ml (32a). However, it is not clear how much risk for VRE transmission accrues to patients who carry extremely low levels of the organism. We believe that detection of low-level VRE carriers who have other risk factors is epidemiologically important, if only because the use of antimicrobial agents in those patients is likely to increase the burden of VRE quite quickly (15). In contrast, detection of low-level carriers among patients who have few or no risk factors and who are expected to have a short hospital stay is less likely to provide any benefit.

The laboratory pitfalls of PCR for detection of antimicrobial resistance have been described previously (43). However, in addition to the usual technical and quality control issues related to PCR in the clinical laboratory, direct detection from patient samples (e.g., perirectal, stool, and nares swab specimens) with complex mixed flora may be compromised by sample inhibition (32). It is therefore important to include an internal inhibition control for each reaction (43) so that potential false-negative results can be more readily detected and standard culture-based assays can be performed, if needed.

Another important limitation of the rapid detection of resistance genes by methods that do not require culture is that the organism itself may not be available. Isolation of the organism in culture is important for evaluation for resistance to other antimicrobials and for molecular typing to obtain evidence of patient-to-patient spread in the hospital setting (36). It is therefore important to use some of the patient sample to inoculate a culture, which can be discarded if the PCR is negative and there is no evidence of sample inhibition.

SUMMARY

The rates of antimicrobial resistance in the hospital continue to increase and contribute substantially to morbidity, mortality, and health care costs in the United States and worldwide. The two most important resistant organisms in U.S. hospitals are MRSA and VRE, both of which are commonly transmitted from patient to patient. Early and accurate detection of MRSA and VRE carriers is necessary to focus isolation and prevention strategies. While the potential for rapid (real-time) detection of MRSA and VRE now exists, it largely remains an unmet clinical need. Future development of rapid methods for detection of MRSA, VRE, and other epidemiologically important pathogens (e.g., *Clostridium difficile* and extended-spectrum β-lactamase-producing gram-negative organisms) should greatly improve our ability to focus prevention and control measures.

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