Real-Time PCR Testing for mecA Reduces Vancomycin Usage and Length of Hospitalization for Patients Infected with Meticillin-Sensitive Staphylococci

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Nucleic acid amplification tests (NAATs) have revolutionized infectious disease diagnosis, allowing for the rapid and sensitive identification of pathogens in clinical specimens. Real-time PCR testing for the mecA gene (mecA PCR), which confers methicillin resistance in staphylococci, has the added potential to reduce antibiotic usage, improve clinical outcomes, lower health care costs, and avoid emergence of drug resistance. A retrospective study was performed to identify patients infected with methicillin-sensitive staphylococcal isolates who were receiving vancomycin treatment when susceptibility results became available. Vancomycin treatment and length of hospitalization were compared in these patients for a 6-month period before and after implementation of mecA PCR. Among 65 and 94 patients identified before and after mecA PCR, respectively, vancomycin usage (measured in days on therapy) declined from a median of 3 days (range, 1 to 44 days) in the pre-PCR period to 1 day (range, 0 to 18 days) in the post-PCR period (P < 0.0001). In total, 38.5% (25/65) of patients were switched to β-lactam therapy in the pre-PCR period, compared to 61.7% (58/94) in the post-PCR period (P = 0.004). Patient hospitalization days also declined from a median of 8 days (range, 1 to 47 days) in the pre-PCR period to 5 days (range, 0 to 42 days) in the post-PCR period (P = 0.03). Real-time PCR testing for mecA is an effective tool for reducing vancomycin usage and length of stay of hospitalized patients infected with methicillin-sensitive staphylococci. In the face of ever-rising health care expenditures in the United States, these findings have important implications for improving outcomes and decreasing costs.

The staphylococci are some of the most common agents of community-acquired and nosocomial infections (4, 6). Staphylococcus aureus is the most virulent staphylococcal species and causes disease in virtually any tissue site, most notably, skin and wound infections, as well as pneumonia, osteomyelitis, and sepsis (6). The other members of this genus, the coagulase-negative staphylococci (CoNS), although considered less virulent and commonly isolated as contaminants, also cause severe infections, particularly in patients with prosthetic devices or intravascular catheters and in immunocompromised hosts (12, 34).

Antimicrobial therapy against staphylococcus has historically been complicated by the ability of these organisms to acquire resistance to available drugs (6). The antistaphylococcal penicillins (methicillin, nafcillin, and oxacillin) were developed to counteract penicillin-resistant S. aureus, which emerged shortly after penicillin was introduced in the 1940s (2). However, in recent decades, methicillin-resistant S. aureus (MRSA) and methicillin-resistant coagulase-negative staphylococcus (MRCoNS) have emerged as leading agents of infectious disease both in health care settings and in the community (6, 22). Resistance to methicillin and the other antistaphylococcal penicillins is mediated by the acquisition of the mecA gene, which encodes an alternative penicillin-binding protein (PBP2a) that does not bind β-lactam antibiotics (19).

Vancomycin is a glycopeptide antibiotic used as first-line treatment of methicillin-resistant staphylococcus (8). Given the prevalence of MRSA and MRCoNS, vancomycin is frequently prescribed as empirical treatment for serious staphylococcal infections before susceptibility results are available. Thus, it is one of the most widely prescribed antibiotics in the United States (24). However, in recent years, its efficacy in the treatment of MRSA isolates with elevated vancomycin MICs of 1.0 to 2.0 μg/ml has been questioned (16, 18, 25, 29). Furthermore, a number of studies have shown that vancomycin is inferior to β-lactam antibiotics for the treatment of methicillin-sensitive staphylococcal organisms, resulting in poorer clinical outcomes and increased mortality (7, 14, 17, 32). Therefore, de-escalation of therapy from vancomycin to β-lactams is strongly recommended for methicillin-susceptible isolates when susceptibility results become available (9).

Nucleic acid amplification tests (NAATs) are increasingly used in the clinical microbiology laboratory for rapid identification of pathogenic organisms and detection of resistance genes (10). Though NAATs can be more costly to perform than conventional microbiological testing, their sensitivity and rapid turnaround times may provide clinical benefits that offset...
their cost. In the case of methicillin susceptibility testing for staphylococci, conventional phenotypic methods use surrogate drugs (oxacillin or cefoxitin) and take about 48 h to become available from the time that positive cultures are detected. In contrast, detection of the mecA gene by real-time PCR takes less than 2 h and yields an unequivocal answer, and results are reported on the same day. In principle, the rapid availability of methicillin susceptibility results should allow clinicians to switch to more effective antibiotic therapy at an earlier time and thus improve clinical outcome, avoid drug toxicity, and minimize the risk for emergence of drug resistance (3, 28).

In this study, we measured the clinical impact of real-time PCR testing for mecA, which was instituted at a large academic hospital in November 2008. Isolates from various sources initially identified as staphylococci were submitted for PCR testing such that results for the presence of mecA, indicative of methicillin resistance, were reported on the same day as organism identification. The pharmacologic and hospitalization outcomes were measured for the same time period before and after PCR testing became available.

MATERIALS AND METHODS

Study cohort. The target study population included hospitalized adult patients at Stanford Hospital who were receiving empirical vancomycin therapy at the time positive microbiological cultures growing methicillin-susceptible staphylococcus species were reported. Data were retrospectively collected from December 2007 to May 2008 and December 2008 to May 2009 to compare vancomycin usage and length of hospitalization before and after implementation of real-time mecA PCR testing. This study was approved by an institutional review board, and the requirement for informed consent was waived.

Data collection. An electronic laboratory report was created to capture all patients with positive staphylococcal culture results during the study period. This report included ordering location, the specialty of ordering physician, and date and time of culture identification results and susceptibility results. An electronic pharmacy report was generated to capture antibiotic orders for the study patients during the study period. The two databases were merged in order to identify patients on empirical vancomycin therapy at the time positive culture results were reported. The following patient categories were excluded from the study: those patients placed on vancomycin after the reporting of microbiological culture results, patients on vancomycin therapy with concurrent methicillin-susceptible and methicillin-resistant staphylococcus culture results, and patients on vancomycin therapy in whom treatment was temporarily halted. For patients with multiple positive culture results during a single hospitalization episode, only the first positive culture result was included in the study. Vancomycin patient days were calculated, and replacement antibiotics were identified. To determine patient days of hospitalization from the time positive cultures were reported, electronic medical records were reviewed, and the date of discharge was extracted.

Laboratory procedures. Culturing of blood and certain sterile fluids was performed using a BacT/Alert automated system (bioMerieux, Durham, NC). All other specimens (tissue, body fluid, respiratory secretion, swab, and catheter tip) were cultured using standardized agar-based methods (26). Organisms with colony or microscopic morphology resembling staphylococci were tested for coagulase. For blood cultures, all tests, including susceptibility testing, were performed after enrichment of bacteria in cation-adjusted Mueller-Hinton broth for 3 h. S. aureus in any amount was considered significant. Coagulase-negative staphylococci present in more than one blood culture set, in sterile specimens, or in pure or predominant form in nonsterile specimens were considered significant. Clinically significant isolates were submitted for antibiotic susceptibility testing. Phenotypic testing was performed on a MicroScan Walkaway instrument (Siemens, Deerfield, IL). Prior to November 2008, methicillin resistance was also determined based on overnight growth on oxacillin-containing agar (Mueller-Hinton broth with 6% NaCl and 6 μg/ml oxacillin). Starting in November 2008, oxacillin screening agar was replaced with multiplexed, real-time PCR for mecA, S. aureus, and staphylococcus genus targets. PCR was performed in a 10-μl reaction volume on a Rotor-Gene 6000 instrument (Qiagen) as previously described (27). Electronic reporting of mecA PCR results occurred at the same time or on the same day as culture identification results.

STATISTICAL ANALYSIS. Wilcoxon’s test of medians or Student t tests were used to compare differences in medians and means, respectively. All statistical tests were computed for a two-sided type I error rate of 5%. The Tukey-Kramer method was used to adjust for multiple comparisons. SAS, version 9.3 (Cary, NC), was used to perform statistical analyses. Vancomycin days were defined as the number of days from the time a positive culture identification was reported in the hospital information system to the time of vancomycin treatment termination. The Wilcoxon’s test was used to test the difference in median number of days on vancomycin in the two time periods, and an analysis of variance (ANOVA) model, using the logarithmic transformation of days, was used to test the difference in mean vancomycin days before and after mecA PCR, with adjustment for patient characteristics (age and sex), species of susceptible organism (S. aureus or CoNS), and the medical specialty of the ordering physician (classified as medical, surgical, or other). The proportion of patients switched to a new antibiotic regimen following susceptible culture result was compared in the two time periods using a χ2 test. A Kaplan-Meier curve was used to characterize time to antibiotic replacement in the pre- and post-PCR time periods. For this analysis, patients infected with methicillin-susceptible isolates who were not switched to a different regimen were censored. Patient hospital days were defined as the number of days from the time positive culture identification was reported in the hospital information system to the time of discharge from the hospital. An ANOVA model, utilizing a logarithmic transformation of hospital days, was used to test differences in mean inpatient stays before and after mecA PCR, with adjustment for patient characteristics (age and sex) and species (S. aureus or CoNS).

RESULTS

Study sample. A total of 933 and 1,012 staphylococcal isolates were cultured from patient specimens between December 2007 to May 2008 (before mecA PCR implementation) and December 2008 to May 2009 (after mecA PCR implementation), respectively. Of these, 175 and 185 cultures, respectively, met study inclusion criteria as first-time isolates from hospitalized patients receiving empirical vancomycin treatment at the time of their positive culture result. The final sample selected included 65 methicillin-susceptible isolates (53 methicillin-susceptible S. aureus [MSSA] and 12 methicillin-susceptible CoNS [MSCoNS]) in the pre-PCR group and 94 methicillin-susceptible isolates (74 MSSA and 20 MSCOoNS) in the post-PCR group. The characteristics of the patients, specimens, and organisms are shown in Table 1. Pre- and post-PCR groups differed with respect to age (P = 0.02) but not with respect to sex, specimen source, or species (P > 0.05).

Effect on vancomycin usage. In the pre-PCR period, patients with methicillin-susceptible culture results were on empirical vancomycin for a median of 3 days (range, 1 to 44 days), compared to a median of 1 day (range, 0 to 18 days) for those patients in the post-PCR period (P < 0.0001) (Fig. 1A). Vancomycin days also declined when usage was analyzed by species from a median of 3 days to 1 day for patients infected with MSSA (P < 0.0001) (Fig. 1B) and from a median of 5 days to 2 days for patients infected with MSCOoNS (P = 0.003) (Fig. 1C). Time period was a significant independent predictor of reduction in vancomycin days in a multivariable model after patient age, sex, species of sensitive isolate, and the specialty of the ordering physician were accounted for. As a control for confounding variables, vancomycin usage was also assessed in 201 patients infected with methicillin-resistant isolates. In the pre-PCR period, 58 patients with MRSA culture results remained on vancomycin for a median of 5 days (range, 1 to 32 days), compared to a median of 3.5 days (range, 0 to 39 days) for 56 patients with MRSA culture results in the post-PCR time period (P = 0.15) (Fig. 1D). In the pre-PCR period, 52 patients with MRCoNS culture results were on vancomycin.
therapy for a median of 5.5 days (range, 1 to 29 days), compared to a median of 3 days (range, 0 to 18 days) for 35 patients with MRCoNS culture results in the post-PCR time period ($P = 0.05$) (data not shown).

### Antibiotic replacement regimens
Vancomycin has been shown to be significantly inferior to β-lactams for the treatment of methicillin-susceptible staphylococcal infections (7, 14, 17, 32). We determined whether introduction of mecA PCR was associated with more appropriate antimicrobial regimens in patients with methicillin-susceptible culture results. Vancomycin therapy was replaced with another antibiotic regimen in 47.7% (31/65) of the patients in the pre-PCR group and in 73.4% (69/94) of the post-PCR patients ($P = 0.001$) (Table 2) with methicillin-susceptible culture results. Vancomycin was switched to a β-lactam, alone or in combination with other antibiotics, in 38.5% (25/65) of the patients in the pre-PCR period and in 61.7% (58/94) of patients in the post-PCR period ($P = 0.004$). Of those patients who received replacement therapy, 81% (25/31) in the pre-PCR period and 84% (58/69) in the post-PCR period ($P = 0.67$) received a replacement regimen consisting of β-lactams alone or of β-lactams with other antibiotic combinations (Table 2). The most common β-lactam substitution in both time periods was nafcillin with or without combination therapy (64% and 50% of vancomycin replacements, respectively). The median time to vancomycin replacement was 5 days in the pre-PCR group and 2 days in the post-PCR group ($P = 0.0001$) (Fig. 2).

### Effect on length of hospitalization
To investigate the impact of mecA PCR on hospitalization, we compared the hospitalization stay from the time of availability of methicillin susceptibility results in the pre- and post-PCR groups. In 159 patients

### Table 1. Characteristics of patients, specimens, and organisms isolated from study subjects before and after implementation of real-time mecA PCR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-PCR value</th>
<th>Post-PCR value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (no. [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>43 (66.2)</td>
<td>53 (56.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Female</td>
<td>22 (33.8)</td>
<td>41 (43.6)</td>
<td></td>
</tr>
<tr>
<td>Mean age (yr [SD])</td>
<td>58 (17)</td>
<td>52 (18)</td>
<td>0.02</td>
</tr>
<tr>
<td>Specimen type and source (no. [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>40 (61.5)</td>
<td>49 (52.1)</td>
<td>0.24*</td>
</tr>
<tr>
<td>Blood</td>
<td>20 (30.8)</td>
<td>26 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>16 (24.6)</td>
<td>17 (18.1)</td>
<td></td>
</tr>
<tr>
<td>Sterile fluid</td>
<td>4 (6.2)</td>
<td>5 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Catheter tip</td>
<td>0</td>
<td>1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Nonsterile</td>
<td>25 (38.5)</td>
<td>45 (47.9)</td>
<td></td>
</tr>
<tr>
<td>Wound</td>
<td>10 (15.4)</td>
<td>22 (23.4)</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>14 (21.5)</td>
<td>20 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>1 (1.5)</td>
<td>3 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Organism group (no. [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA</td>
<td>53 (81.5)</td>
<td>74 (78.7)</td>
<td>0.66*</td>
</tr>
<tr>
<td>MSCoNS</td>
<td>12 (18.5)</td>
<td>20 (21.3)</td>
<td></td>
</tr>
</tbody>
</table>

* $P$ value compares sterile and nonsterile sources.

b $P$ value compares MSSA and MSCoNS groups.

c $n$, number of patients.

![FIG. 1. Vancomycin patient days for study patients before and after implementation of real-time mecA PCR. (A) Methicillin-sensitive S. aureus and methicillin-susceptible coagulase-negative staphylococcus isolates. (B) Methicillin-susceptible S. aureus isolates. (C) Methicillin-susceptible coagulase-negative staphylococcus isolates. (D) Methicillin-resistant S. aureus isolates. Wilcoxon's test was used to compare differences in medians (indicated by horizontal lines).](image-url)
infected with methicillin-susceptible staphylococci, total hospital days declined from a median of 8 days (range, 1 to 47 days) in the pre-PCR period to a median of 5 days (range, 0 to 42 days) in the post-PCR period ($P = 0.03$) (Fig. 3). The decline in inpatient stays remained significant after patient age, sex, and staphylococcus species were accounted for.

**DISCUSSION**

Numerous measures have been undertaken to curtail the use of vancomycin in the hospital, including the requirement for approval from an infectious diseases clinician prior to the initiation of therapy, education of clinical staff, automatic stop orders, computer-generated messages, and the use of pharmacology specialists to report susceptibility results (1, 5, 20, 30, 31). These interventions have met with various degrees of success. At our adult academic hospital, the implementation of a rapid mecA PCR test to detect methicillin resistance in staphylococci resulted in a significant decrease in inappropriate antibiotic usage. In contrast to prior studies, delivery of our results was by the standard hospital information system interface with no need for a specialized pharmacist or antimicrobial team member to deliver results directly to the clinician (5, 11). A similar trend toward decreased vancomycin usage was seen among pediatric patients at the affiliated children’s hospital, but the data were excluded from this study due to the small population size. We conclude that mecA PCR is not only a rapid and sensitive diagnostic tool but also an effective intervention to limit vancomycin usage even in the absence of an antibiotic stewardship program.

Notably, although the actual turnaround time for methicillin susceptibility results decreased by 1 day, the median decrease in vancomycin usage was 2 days of antibiotic therapy. This discrepancy likely reflects the 52% of patients in the pre-PCR period who were never switched to alternative therapy despite the identification of their isolate as being methicillin susceptible. In contrast, only 27% of patients infected with methicillin-susceptible isolates remained on vancomycin therapy in the post-PCR period. Empirical antibiotic treatment is often started to broadly cover for potential pathogens when there is suspicion of infection. The identity of the organism and its susceptibility pattern are important for subsequently narrowing down the therapeutic coverage (9). As previously suggested (3), we hypothesize that the availability of both of these results simultaneously allows clinicians to make appropriate antibiotic decisions at a single time point without the need to follow up on pending results. Thus, even a single day’s improvement in susceptibility reporting time resulted in a disproportionate improvement in the initiation of appropriate therapy.

The reduction in vancomycin usage is likely to have significant clinical and epidemiologic consequences although the comprehensive measurement of these effects was beyond the scope of the current study. A significant decrease in hospitalization days was observed among patients in the post-PCR compared to the pre-PCR period and may reflect quicker recovery due to more effective treatment or reduced toxicity. A similar decrease in hospitalization days was also seen in a study using fluorescence in situ hybridization for early differentiation of *S. aureus* from CoNS in blood cultures (11). In our study, rapid species identification likely did not contribute to study results because same-day identification of *S. aureus* and CoNS by coagulase testing was already being performed prior to the introduction of mecA PCR. Our study focused on methicillin-susceptible infections and the switch from vancomycin to more

**FIG. 2.** Time to vancomycin replacement for study patients infected with methicillin-sensitive isolates before and after implementation of real-time mecA PCR. Kaplan-Meier plot shows the cumulative percentage of patients on vancomycin therapy. Patients who were not switched to a different regimen are shown as censored. The $P$ value computed with the Wilcoxon statistic is shown.

**FIG. 3.** Hospitalization days for study patients infected with methicillin-sensitive staphylococci before and after implementation of real-time mecA PCR. Wilcoxon’s test was used to compare differences in medians (indicated by horizontal lines).

**TABLE 2.** Vancomycin replacement therapy for study patients infected with methicillin-susceptible staphylococci before and after implementation of real-time mecA PCR

<table>
<thead>
<tr>
<th>Patient therapy group and/or agent</th>
<th>No. (%) of patients</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-PCR ($n = 65^a$)</td>
<td>Post-PCR ($n = 94^a$)</td>
</tr>
<tr>
<td>Replacement$^a$</td>
<td>31 (47.7)</td>
<td>69 (73.4)</td>
</tr>
<tr>
<td>$\beta$-Lactam</td>
<td>25 (80.6)</td>
<td>49 (71)</td>
</tr>
<tr>
<td>$\beta$-Lactam plus other antibiotic</td>
<td>0</td>
<td>9 (13)</td>
</tr>
<tr>
<td>Other antibiotic</td>
<td>6 (19.4)</td>
<td>11 (15.9)</td>
</tr>
<tr>
<td>No replacement</td>
<td>34 (52.3)</td>
<td>25 (26.6)</td>
</tr>
</tbody>
</table>

$^a$ $\beta$-Lactam includes amoxicillin, ampicillin-sulbactam, piperacillin-tazobactam, nafcillin, cefazolin, cephalaxin, ceftriaxone, cefepime, ceftepim, and imipenem; other antibiotics include trimethoprim-sulfamethoxazole, daptomycin, linezolid, dindamycin, ciprofloxacin, moxifloxacin.

$^b$ $P$ value compares replacement to no-replacement group.

$^c$ $P$ value compares $\beta$-lactam to other antibiotics within the replacement group.

$^d$ $n$, number of patients.
effective β-lactam therapy in these patients; however, even patients with MRSA infections are likely to benefit from rapid identification. As the efficacy of vancomycin has become more limited, identification of resistant organisms may prompt a switch to alternative MRSA agents in patients with life-threatening infection or poor response to empirical treatment (8, 23).

The decrease in vancomycin usage also has implications for the spread of antibiotic resistance (33). For example, increased rates of vancomycin-resistant enterococcus (VRE) have been associated with high vancomycin and third-generation cephalosporin usage in intensive care units (13). Among S. aureus strains, the frequency of vancomycin-resistant or -intermediate strains is low (2). However, high vancomycin usage may select for strains with higher MICs in the susceptible range or strains harboring heteroresistance (8, 21, 35). Although still considered susceptible, isolates with vancomycin MICs of 1.0 to 2.0 μg/ml are more frequently associated with treatment failure (15). Limiting vancomycin usage may help prevent or counteract these phenomena.

The findings in this study are promising and warrant further validation. However, the main limitation of the study is the before/after design such that trends (e.g., changes in susceptibility result reporting, physician prescribing behavior, or hospital directives) occurring over the study period may influence the results, in addition to the specific mecA PCR intervention being investigated. The same 6-month interval from December to May was compared before and after PCR implementation to minimize effects due to events occurring at particular times during the calendar year, such as house staff changes. The similarity before and after PCR in the distribution of specialties ordering empirical vancomycin and in the replacement regimens for isolates later identified as susceptible suggests that differences in physician behavior or knowledge base was not a significant factor. Furthermore, patients with the same eligibility criteria as the study group but who were infected with methillin-resistant isolates were used as a control group to evaluate for confounding variables that may have affected antibiotic usage. The methillin-resistant infection group did not demonstrate a significant change in days on vancomycin therapy, indicating that the decreased vancomycin usage seen in the methillin-susceptible infection group was not due to general trends in vancomycin usage (other than the recommended change to β-lactams for methillin-susceptible isolates) that would have affected the resistant infection group as well. The current study could also be enhanced by more stringent selection of patients with clinically significant infections from blood or other sterile sites and exclusion of those with comorbid diagnoses that could influence the clinical outcome. A follow-up study incorporating systematic matching of patient characteristics, resistance rate, and randomization of the intervention should allow us to perform a more accurate comparison of clinical outcome and cost savings associated with rapid mecA PCR.

In summary, the role of rapid diagnosis in the clinical microbiology laboratory in improving antibiotic usage, clinical outcomes, and resistance should be emphasized. In the face of ever-rising health care expenditures in the United States, it is assumed that diagnostic tests only contribute to high health care costs. Our findings suggest that further opportunities to employ diagnostic advances to decrease costs and improve outcomes should be explored and exploited.

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REFERENCES


mecA PCR REDUCES VANCOMYCIN USAGE 789


