CLINICAL OUTCOMES WITH RAPID DETECTION OF METHICILLIN-RESISTANT AND METHICILLIN-SUSCEPTIBLE STAPHYLOCOCCUS AUREUS ISOLATES FROM ROUTINE BLOOD CULTURES

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**Abstract**

*Staphylococcus aureus* is a common cause of bacteremia with substantial impact on morbidity and mortality. Because of increasing rates of methicillin-resistant *Staphylococcus aureus*, vancomycin has become the standard empiric therapy. However, beta-lactam antibiotics remain the best treatment choice for methicillin-susceptible strains. Placing patients quickly on optimal therapy is one goal of antimicrobial stewardship.

This retrospective, observational, single-center study compared 33 control patients utilizing only traditional full susceptibility methodology to 22 case patients utilizing rapid methodology with CHROMagar medium for detection and differentiation of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains hours before reporting full susceptibilities. Time to targeted therapy was statistically significantly different between control patients (mean 56.5 ± 13.6 hours) and case patients (44.3 ± 17.9 hours) (p=0.006). Intensive care unit status, time of day results emerged, and patient age did not make a difference in time to targeted therapy, either singularly or in combination. Neither length of stay (p=0.61) nor survival (p=1.0) was statistically significantly different.

Rapid testing yielded a significant result with a difference of 12.2 hours in time to targeted therapy. However, there is still room for improvement as the difference in time to susceptibility test result between the full traditional methodology and CHROMagar was even larger (26.5 hours). This study supports the hypothesis that rapid testing plays a role in antimicrobial stewardship by getting patients on targeted therapy faster.
**Introduction**

*Staphylococcus aureus* is frequently associated with nosocomial and community-acquired bacteremia worldwide (1,2). Furthermore, *S. aureus* bacteremia is associated with a high incidence of morbidity and mortality (3), with methicillin-resistant *S. aureus* (MRSA) bacteremia having a substantially higher rate of mortality than methicillin-susceptible *S. aureus* (MSSA) bacteremia (4). Because of increasing rates of MRSA and the severity of infection it produces, vancomycin has become standard empiric therapy for Gram-positive bacteremia.

Despite receiving the same empiric therapy until methicillin resistance is excluded, targeted treatment for MRSA and MSSA bacteremia differs. The treatment of choice for MSSA bacteremia is an intravenous (IV) penicillinase-resistant penicillin (e.g., oxacillin or nafcillin) or a first-generation cephalosporin (e.g., cefazolin), which have demonstrated superiority over vancomycin (5). Vancomycin also has the burden and expense of monitoring serum drug concentrations in addition to the concerns of minimum inhibitory concentration (MIC) creep with overuse (6). Placing patients on targeted therapy as quickly as possible is a goal of antimicrobial stewardship that has traditionally been limited by time to identification of the organism and determination of susceptibilities. Therefore, rapid detection of MRSA and MSSA may prove to be an effective tool for antimicrobial stewardship.

Traditional methodology for *S. aureus* identification and full susceptibilities at our institution, UNC Health Care, was done by VITEK2 (bioMérieux, Durham, NC) or disk diffusion, taking up to 48 hours to obtain results. In September 2011, we implemented a culture-based rapid susceptibility screen using BBL CHROMagar MRSA II (Becton
Dickinson, Sparks, MD). We used CHROMagar MRSA II for the detection and differentiation of MSSA and MRSA directly from positive blood culture bottles (BacT Alert, bioMérieux, Durham, NC). The manufacturer of CHROMagar MRSA II recommends that results be interpreted after 24 hours of incubation, with 92% positive agreement and 99.9% negative agreement for the determination of MRSA. However, it was later independently demonstrated that results could be obtained after only 12 to 16 hours of incubation, with the same high degree of sensitivity and specificity as 24 hours of incubation (7). Therefore, in January 2012, our institution began interpreting results after 12 to 16 hours of incubation.

Because of the added expense associated with rapid testing, it is important to assess the impact rapid testing has on the overall care to the patient. The objective of this study was to determine if a difference in clinical outcomes exists when comparing traditional and rapid testing for MRSA and MSSA in routine blood cultures. Clinical endpoints including time to targeted therapy, length of stay, and survival were examined.

**Materials and Methods**

**Study Design**

This was a retrospective, observational, single-center, institutional review board-approved study completed from October 2010-July 2012 in an 803-bed academic medical center. Patients were potentially included if they were retrospectively identified as having a routine blood culture positive for MSSA. The patients were designated as either a potential control or a potential case patient. Control patients were those where only traditional blood culture methodology was utilized for identification and
susceptibility testing. Case patients were those where CHROMagar medium was utilized in addition to the traditional culture work-up.

We excluded patients less than two years old, since antibiotic selection and dosing in this population is different. Patients were also excluded if they had a beta-lactam allergy that inhibited the ability to change therapy to a beta-lactam antibiotic after MSSA was reported. Growth of any other organism in a routine blood culture during treatment for the MSSA bacteremia was also an exclusion criterion. Lastly, patients were excluded if their first Gram stain from a positive blood culture was done at an outside hospital or if it was unclear when targeted therapy was initiated.

The primary endpoint of the study was the time to targeted therapy reported in hours. We defined time to targeted therapy as the time from report of Gram-positive cocci on Gram stain from the first positive blood culture to the time of pharmacy verification of a beta-lactam antibiotic. Pharmacy verification was necessary for dispensation of the antibiotic from the pharmacy to the patient and was a point in time we could easily capture unlike medication administration time, which was not documented electronically. Secondary endpoints that were examined in this study included length of stay, survival, and time to susceptibility test result.

Culture Methods

Blood cultures were collected from patients using standard procedures and incubated on the BacT/Alert3D system (bioMérieux, Durham, NC). Cultures that flagged positive by the instrument were Gram stained and the results reported to the responsible physician. Per routine laboratory protocol, positive bottles were sub-
cultured to chocolate, colistin-naladixic acid, and MacConkey agars (Becton Dickson, Sparks, MD). Organisms were identified using standard techniques, and full susceptibility testing was performed by Kirby Bauer disk diffusion or VITEK2 (bioMérieux, Durham, NC) as specified by the Clinical and Laboratory Standards Institute. During the study period, CHROMagar MRSA II media (Becton Dickson, Sparks MD) was added to the sub-culture set up for the first bottle demonstrating Gram-positive cocci in clusters on Gram stain for each new episode of bacteremia. In-house studies were performed to verify that determination of MRSA and MSSA could be accurately made as early as 12 hours after inoculation from a positive blood culture.

Statistics

The study was powered to detect a minimum difference of 8 hours in time to targeted therapy, a difference we considered meaningful since this would save at least one dose of vancomycin. We computed the necessary sample size assuming that the number of controls would be approximately twice the number of cases. Historical data suggested that time to targeted therapy was approximately normally distributed with a mean of 53.8 hours and a standard deviation (SD) of 9.8. Thus, 80% power would be achieved with 38 control patients and 19 case patients.

Power calculations and final analyses were conducted with R 3.0.0 for Windows (R Foundation for Statistical Computing, Vienna, Austria; 2013). The primary analysis was assessed with the t-test after the normality assumption had been checked with the Shapiro-Wilk test. Secondary analyses were assessed with t-tests with the Satterthwaite-Welch adjustment for non-constant variance, Mann-Whitney U-tests for
un-paired non-normal data, and the Wilcoxon signed rank test for paired non-normal data. The chi-squared test and Fisher’s exact test were used for some analyses where appropriate. We determined that the significant primary result would hold after adjusting for possible confounding factors with a multiple regression. We consider p-values <0.05 as significant.

**Results**

A review of 68 controls and 40 cases (CHROMagar) yielded 33 and 22 patients meeting inclusion criteria, respectively. Breakdown of the exclusion criteria met in each group is presented in Table 1. Patient characteristics at time of infection are presented in Table 2. The only characteristic analyzed that reached a statistically significant difference between groups was patient age (p<0.001), a characteristic that is not expected to have a major effect on the primary endpoint but possibly secondary endpoints. While there was not a statistically significant difference in characteristics including gender, dialysis, and total parenteral nutrition, there was a noticeable difference in percentage of patients in each group. Theoretically gender should not make a difference in any endpoint. Need for dialysis or total parenteral nutrition may require the patient to have a central venous catheter in place, potentially affecting treatment response and secondary endpoints. Also of note, 100% of control and case patients were initially started on empiric therapy with vancomycin. Change to a beta-lactam antibiotic, which was a criterion for inclusion, occurred in 100% of the control group and 95.5% of the case group. One patient in the case group was switched to trimethoprim-sulfamethoxazole instead of a beta-lactam antibiotic, but was included since this was still considered targeted therapy.
Another patient characteristic examined included the presence of a central venous catheter at the time of infection. For patients who had a central venous catheter at the time of infection, we aimed to determine if this catheter had no intervention, was replaced over a guide wire, was removed with a new catheter inserted, or was removed with no new catheter inserted. In the control group, three (33.3%) patients had no intervention to the catheter and six (66.7%) patients had the catheter removed and a new catheter inserted. In the case group, three (30%) patients had no intervention to the catheter, two (20%) patients had the catheter replaced over a guide wire, four (40%) patients had the catheter removed and a new catheter inserted, and one (10%) patient had the catheter removed with no new catheter inserted. While this characteristic may not affect the primary endpoint, it is important to mention since having a central venous catheter that is not removed or replaced when it is a likely source of bacteremia can affect the success of bacteremia treatment and patient outcome. About 30% of both groups had no intervention to a catheter that was present at the time of infection.

The primary endpoint, time to targeted therapy in hours, was significantly different between controls with a mean ± SD of 56.5 ± 13.6 hours and cases with a mean ± SD of 44.3 ± 17.9 hours (p=0.006). Intensive care unit status (p=0.21), time of day results emerged (p=0.54), and patient age (p=0.17) did not make a difference in time to targeted therapy in isolation or in combination. Time to targeted therapy remained significant (p=0.01) even after adjusting for these potential covariates. While the number of patients in the intensive care unit and whose test results emerged after first shift were very small, we adjusted for these two covariates because they had the
potential to affect the primary endpoint by altering the time it took for the physician to see that the test had resulted.

Secondary endpoints evaluated included length of stay and survival. Mean length of stay in days was not significantly different, with a mean ± SD of 13.4 ± 11.5 days in the control group and 10.0 ± 5.0 days in the case group (p=0.61). Survival was also not significantly different between groups with 97% survival in the control group and 100% in the case group (p=1.0).

An additional secondary endpoint that was assessed was time to susceptibility test result. The time to full susceptibility results in the control group (46.1 ± 10.9 hours) compared to the case group (48.0 ± 12.1 hours) was not significantly different (p=0.62). When we compared the time to full susceptibility determination in the control group (46.1 ± 10.9 hours) to the CHROMagar result in the case group (19.6 ± 5.9 hours) we found a significant difference (p<0.001). When we compared the case group full susceptibility results (48.0 ± 12.1 hours) to the case group CHROMagar result (19.6 ± 5.9 hours), we similarly found a significant difference (p<0.001). See Figure 1 for a graph comparing time to targeted therapy with time to susceptibility test result.

**Discussion**

Results from the CHROMagar were reported in our electronic medical record as a comment that reads “Oxacillin susceptible *S. aureus* predicted by rapid resistance testing. Approximately 2% of *S. aureus* may be resistant to oxacillin due to mechanisms not detected by this method.” This comment was based on historical data of MRSA isolates from our laboratory; however all of the isolates reported as MSSA by
CHROMagar in this study were confirmed to be MSSA upon full susceptibility results. Full susceptibility results were reported in our electronic medical record as a list of susceptibility testing for oxacillin, gentamicin, vancomycin, erythromycin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole. The medical team was not alerted that the CHROMagar or full susceptibility results had been posted in the medical record. Therefore, the change from empiric to targeted therapy was reliant on the medical team noticing that the results were available.

We found that there was a significant decrease in time to targeted therapy in the CHROMagar group, while there was not a significant difference in the time it took for full susceptibilities to be reported. This suggests that the decrease in time to targeted therapy is attributed to the utilization of the CHROMagar medium. Despite the fact that we found a significant difference in time to targeted therapy in the CHROMagar group, Figure 1 demonstrates that there is still room for improvement, as the difference in time to targeted therapy was 12.2 hours but the difference in time to susceptibility test result was even larger at 26.5 hours. Theoretically, the time to targeted therapy could have been up to 26.5 hours, which would provide another 14.3 hours of targeted therapy; a difference that could be clinically relevant. Change in treatment occurred within 24 hours of full susceptibility results in 84.8% of the control group. Change in treatment occurred within 24 hours of the CHROMagar result in only 31.8% of the case group. However, 54.5% in the CHROMagar group were changed within 24 hours of the full susceptibility results instead. The failure to change antibiotics within 24 hours of CHROMagar result suggests that the medical staff may not have trusted the rapid results enough to change therapy or they did not rapidly notice the results in the medical
record. Both of these theories provide an opportunity for intervention that could further improve time to targeted therapy.

There are many tests available for the rapid determination of MRSA from MSSA in positive blood cultures, including molecular methodologies. With the many rapid tests that are available, choosing which test to use at an institution will depend on variety of factors including cost, reported specificity and sensitivity, time to test result, U.S Food and Drug Administration approved indications, and the data available to support their use in regards to improvement of patient outcomes (8). Their role in antimicrobial stewardship ultimately relates to how they promote the goals of stewardship, namely, improving patient care and health care outcomes (8). For this study we utilized CHROMagar media which, although not as rapid as molecular tests, can cost five to 20 times less than a molecular assay. Importantly, the utilization of a culture-based approach requires no extra equipment or specialized expertise, making it accessible to any institution.

We acknowledge that our study has several limitations. We performed a retrospective, observational, single-center study. We had to rely on the accuracy of documentation in medial records to derive some of our data. We also chose to base time to targeted therapy on pharmacy verification time instead of drug administration time, because our medication administration records were not available in an electronic format and were more difficult to access. Moreover, we were unable to fully evaluate cost differences. Unlike oxacillin treatment, which costs $78 per day for an adult patient with MSSA bacteremia at our institution (receiving 2 grams IV every four hours), the cost of vancomycin treatment is variable. Since vancomycin dosage is based on age,
weight, renal function, and patient specific serum trough concentrations, it was not possible to calculate an expected daily cost of vancomycin. Further analysis would be needed to determine whether total drug costs would be reduced with the use of rapid testing.

In addition to the above limitations, there were two secondary endpoints that we were hoping to evaluate in this study but were unable to do so. Time to culture clearance was a patient outcome where we expected we might see a difference if patients were placed on targeted therapy faster. However, repeat cultures were drawn at different times in every patient based on physician preferences. The other endpoint was time to defervescence, but this was also too difficult to accurately examine in a retrospective study. We found that most patients had their first fever at home, which was not documented in our medical record. We also found that some patients were started on antibiotics before they ever had a documented fever in the hospital. This variability made these endpoints too unreliable to analyze and report.

This is one of a limited number of studies that has looked at the impact rapid testing may have on clinical outcomes and on antimicrobial stewardship. It is also one of the first studies that specifically evaluated these outcomes with CHROMagar detection of MRSA and MSSA. In this study, patients were placed on targeted therapy 12.2 hours faster with CHROMagar utilization. However, as noted, there is further room for improvement given the larger difference of 26.5 hours between time to traditional and rapid test results. To try to further improve the time to targeted therapy, we will implement an antimicrobial stewardship intervention, in which CHROMagar results will be paged to an on-call pharmacist who will then collaborate with the medical team to
switch to targeted therapy. This approach has previously been successful at our institution (9). Regardless of our future endeavors, this study supports the idea that rapid culture-based detection of MRSA and MSSA plays a role in antimicrobial stewardship by decreasing the time that it takes to place patients on targeted antimicrobial therapy.

Acknowledgments: This research is partly supported by a grant from the National Institutes of Health (UL1TR000083) for biostatistical support through the North Carolina Translational and Clinical Sciences Institute.

References


Table 1. Exclusion Criteria Breakdown.

<table>
<thead>
<tr>
<th>Exclusion Criteria</th>
<th>Control Group</th>
<th>Case Group (CHROMagar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age less than two years old</td>
<td>5 (7.4%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Beta-lactam allergy</td>
<td>4 (5.9%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Another organism in blood</td>
<td>5 (7.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Outside hospital Gram stain</td>
<td>6 (8.8%)</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>Targeted therapy time unclear</td>
<td>15 (22.1%)</td>
<td>7 (17.5%)</td>
</tr>
</tbody>
</table>
Table 2. Patient Characteristics at Time of Infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Group</th>
<th>Case Group (CHROMagar)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (average)</td>
<td>56 years</td>
<td>31 years</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male</td>
<td>19 (57.6%)</td>
<td>18 (81.8%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Dialysis</td>
<td>5 (15.2%)</td>
<td>2 (9.1%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
<td>1 (3.0%)</td>
<td>2 (9.1%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Catheter in place</td>
<td>9 (27.3%)</td>
<td>10 (45.5%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>5 (15.2%)</td>
<td>4 (18.2%)</td>
<td>1</td>
</tr>
<tr>
<td>Empiric vancomycin</td>
<td>33 (100%)</td>
<td>22 (100%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Change to a beta-lactam antibiotic</td>
<td>33 (100%)</td>
<td>21 (95.5%)</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Figure 1. Time to Susceptibility Test Result Compared to Time to Targeted Therapy Reported in Hours (Error bars are ± 1 standard error).