Peptide Nucleic Acid Fluorescent In Situ Hybridization for Hospital-Acquired Enterococcal Bacteremia: Delivering Earlier Effective Antimicrobial Therapy

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Hospital-acquired vancomycin-resistant enterococcal bacteremia has been associated with increased hospital costs, length of stay, and mortality. The peptide nucleic acid fluorescent in situ hybridization (PNA FISH) test for Enterococcus faecalis and other enterococci (EFOE) is a multicolor probe that differentiates E. faecalis from other enterococcal species within 3 h directly from blood cultures demonstrating gram-positive cocci in pairs and chains (GPCPC). A quasiexperimental study was performed over two consecutive years beginning in 2005 that identified GPCPC by conventional microbiological methods, and in 2006 PNA FISH was added with a treatment algorithm developed by the antimicrobial team (AMT). The primary outcome assessed was the time from blood culture draw to the implementation of effective antimicrobial therapy before and after PNA FISH. The severity of illness, patient location, and empirical antimicrobial therapy were measured. A total of 224 patients with hospital-acquired enterococcal bacteremia were evaluated, with 129 in the preintervention period and 95 in the PNA FISH period. PNA FISH identified E. faecalis 3 days earlier than conventional cultures (1.1 versus 4.1 days; $P < 0.001$). PNA FISH identified Enterococcus faecium a median 2.3 days earlier (1.1 versus 3.4 days; $P < 0.001$) and was associated with statistically significant reductions in the time to initiating effective therapy (1.3 versus 3.1 days; $P < 0.001$) and decreased 30-day mortality (26% versus 45%; $P = 0.04$). The EFOE PNA FISH test in conjunction with an AMT treatment algorithm resulted in earlier initiation of appropriate empirical antimicrobial therapy for patients with hospital-acquired E. faecalis bacteremia.

VRE bacteremia has been associated with increased mortality, a longer hospital stay, and higher health care costs (5, 11, 20, 28, 33, 34). In a large meta-analysis of the outcomes of VRE infections, Salgado and Farr postulated that one of the reasons for the increased mortality in these patients was delayed effective antimicrobial therapy against VRE (28). Patients at the greatest risk for VRE are most likely to be critically ill and solid-organ transplant recipients and to have prolonged neutropenia and/or to have spent prolonged periods in the hospital (5, 6, 13, 15, 28, 39). Early appropriate antimicrobial therapy has been demonstrated to improve patient outcomes in the intensive-care unit (ICU) setting, which is clearly a high-risk setting for VRE infection (16, 30).

Peptide nucleic acid fluorescent in situ hybridization (PNA FISH) probes are DNA mimics in which a sugar-phosphate backbone of DNA is replaced with a noncharged peptide backbone. This allows the probes to target individual species-specific targets on the 16S rRNA within living bacteria. There are presently three commercially available FDA-approved probes, for Staphylococcus aureus, Candida albicans, and Enterococcus species (8, 24, 25). The E. faecalis and other enterococci (EFOE) PNA FISH probe (AdvanDx, Inc., Woburn, MA) is unique in that it is a multicolor probe that is applied to drop of blood from a positive blood culture that has gram-positive cocci in pairs and chains (GPCPC), and results can be obtained in about 2.5 hours. Typical results include green fluorescence.
for *E. faecalis*; red for other *Enterococcus* species, including *E. faecium*; and no color if the bacterium is not an *Enterococcus* species (e.g., a *Streptococcus* species) (8). PNA FISH is easier to perform than a Gram stain and, importantly, retains cellular morphology to assist in the identification of the organism under the fluorescence microscope (8).

Given this information, we can prescribe more appropriate directed therapy for patients with hospital-acquired enterococcal bacteremia and potentially decrease the delay in providing appropriate therapy to patients. The University of Maryland Medical Center microbiology laboratory has performed PNA FISH testing since 2004 and introduced the EFOE PNA FISH test in 2006. The reporting of results was incorporated into the routine of the hospital’s Antimicrobial Management Team (AMT), which consists of a full-time infectious-disease pharmacist and an infectious-disease physician who devotes 25% of his or her time to the AMT. This successful synergy between the AMT and PNA FISH has previously been reported by our institution, showing cost savings and reduction in length of stay (9, 10). Here, we present the impact of implementing the EFOE PNA FISH test on the clinical care of patients with hospital-acquired enterococcal bacteremia.

(These data were presented in part at the 44th Infectious Diseases Society of America meeting [slide presentation 131], Toronto, Canada, 12 to 15 October 2006.)

**MATERIALS AND METHODS**

**Study design.** This was a quasiexperimental study (one group, with pretest and posttest design) performed at the University of Maryland Medical Center over two years (2005 and 2006). The University of Maryland Institutional Review Board approved the study.

**Setting and patients.** The University of Maryland Medical Center is an ~600-bed inner-city tertiary-care teaching hospital that includes 130 ICU beds, the Marlene and Stewart Greenebaum Cancer Center, and a large solid-organ transplant program. All patients with hospital-acquired bacteremia due to *E. faecium* or *E. faecalis* during the study period were included as study subjects. Hospital-acquired bacteremia was defined as either type of bacteremia occurring more than 72 h after admission. This included the length of stay after transfer from another hospital setting (18). The AMT did not make interventions for patients with streptococcal infections, as these are often community acquired, present with admitting cultures, occur more often, and are frequently covered by many classes of antibiotics and would consume too much of the AMT time with minimal changes to antibiotic therapy. Only the first episode of enterococcal bacteremia per patient per admission was included. The following patient groups were excluded from the study analysis: pediatric patients and R. Adams Cowley Shock Trauma Center patients (because these patient groups are not evaluated by the hospital’s AMT), patients either group who died before the culture was positive (because the AMT was unable to intervene), and patients with documented endocarditis based on modified Duke criteria (because the developed treatment algorithm did not apply) (17). Surveillance for VRE with perirectal cultures was standard practice for all patients on the day of admission to an ICU or the cancer center and was then performed weekly during both the pre- and postintervention periods. VRE alerts were present in the chart for current and prior VRE colonization, and patients were appropriately isolated. A prior VRE culture in both time periods warranted early linezolid use.

**Laboratory.** Blood cultures were drawn according to standard hospital policy from two separate sites and collected in blood culture bottles (BacTAlert; bioMerieux, Marcy l’Etoile, France). The cultures were placed in a continuous automated detection incubator (BACTEC; Becton Dickinson, MD). When GPCPC were identified by Gram staining signal-positive blood cultures, they were plated onto standard growth media according to the standard laboratory protocol. These results were called in to the treating physician according to the AMT protocol. These results were called in to the physician with follow-up by the AMT. The final cultures were compared to the PNA FISH results. The AMT intervened at the time of the PNA FISH results to direct antibiotic therapy.

Based on our antibiograms from 2004, 100% of our *E. faecalis* organisms were ampicillin susceptible and 90% were vancomycin susceptible. Of our other *Enterococcus* species, 99% were *E. faecium*. None of the *E. faecium* isolates were ampicillin susceptible based on CLSI standards, and only 15% were vancomycin susceptible. With these data, the AMT developed the treatment algorithm shown in Fig. 1. The AMT discussed the PNA FISH results daily, including weekends, with the treating physician, who then determined whether a change in therapy was necessary. Prior VRE colonization and VRE surveillance cultures were also taken into consideration during the entire study period. The treatment algorithm had preferred therapies with ampicillin for *E. faecalis* (vancomycin when a patient was allergic to penicillin) and linezolid for *E. faecium*, and if the organism was not an *Enterococcus* species, then penicillin or ceftriaxone (vancomycin could be added if meningitis or febrile neutropenia was present) was used. High-dose daptomycin was considered as an alternative to linezolid when necessary with vancomycin-resistant *E. faecium* (29). Because enterococcal bacteremia is frequently associated with other organisms, especially gram-negative bacteria (28), piperacillin-tazobactam or imipenem/cilastatin could be utilized in place of ampicillin, as both these antibiotics have adequate *E. faecalis* activity (19, 23, 36). Ampicillin susceptibility was used as a marker for imipenem/cilastatin (36) and piperacillin-tazobactam (19) susceptibility. All central lines were removed after documentation of enterococcal bacteremia.

**Data collection.** We collected the following information on study subjects via chart review: age, sex, and length of stay; central-line status, APACHE II score at the time of blood culture (21), serum chemistry, complete blood count, and hospital location (at all of the onset of bacteremia); and time from report of Gram stain to final enterococcal susceptibilities, other organisms in the blood culture, initial antimicrobial therapy, time to change antimicrobial therapy to appropriate therapy after report of GPCPC, and survival status at 30 days. Neutropenia was defined as having an absolute neutrophil count of less than 500 cells at the onset of bacteremia. The prospective intervention group also included the time from the Gram stain to the PNA FISH report and the accuracy of the PNA FISH result compared to the final culture.
Appropriate antimicrobial therapy was defined as the receipt of an antibiotic with activity against the cultured enterococci. The time to appropriate therapy was the period from the time the blood culture was drawn to the time of receipt of appropriate therapy (21). We assessed whether the selected empirical therapy had coverage of all organisms from the blood culture based on their final susceptibilities. Mortality was all-cause mortality and was determined 30 days after the first positive blood culture.

**Statistical analysis.** The primary objective of this study was to determine if the EFOE PNA FISH test would lead to earlier initiation of appropriate antimicrobial therapy for patients with *E. faecium* and *E. faecalis* bacteremia. The secondary objective was to determine the effects of PNA FISH on survival and hospital length of stay. We initially described the study population using proportions for categorical variables and medians for continuous variables. Because the intervention was likely to have a greater effect in patients with *E. faecium* bacteremia than in those with *E. faecalis* bacteremia, we stratified our results by enterococcal species. We compared categorical variables using chi-square analysis or the Fisher exact test (if appropriate) and continuous variables using a t test in the pre- and postintervention periods, stratified by enterococcal species. The survival curves of the time to effective antibiotic therapy were compared by a log rank test. Data analysis was performed using SPSS version 15 (SPSS, Chicago, IL). All tests were two tailed, and the level of statistical significance was set at a P value of <0.05.

**RESULTS**

**Description of the study population.** Overall, there were 650 patients with blood cultures positive for GPCPC. All blood cultures positive for gram-positive cocci in pairs during 2006 had the PNA FISH test in the laboratory. Of these, 271 patients had hospital-acquired *E. faecium* or *E. faecalis* bacteremia during the study period, with 50 patients excluded (23 patients had a repeat episode of enterococcal bacteremia, 10 died before the blood culture turned positive, 3 were from pediatric and 7 were from shock trauma units, and 7 had endocarditis), and 379 were infected with a Streptococcal species and were excluded from analysis. Thus, 224 patients were included, with 129 patients in the intervention period and 95 patients in the postintervention period. The EFOE PNA FISH test identified all 48 *E. faecalis* organisms and 44 other enterococcal organisms, all of which were *E. faecium* by probe and confirmed by culture in the postintervention group, and identified three patients with both *E. faecalis* and *E. faecium* in the same blood culture.

The study population had a median age of 56 years (range, 19 to 90 years), with 118 (53%) males. One hundred of 224 patients (45%) were in an ICU at the onset of their enterococcal bacteremia, and 40 (18%) were located in the cancer center. We compared categorical variables using chi-square analysis or the Fisher exact test (if appropriate) and continuous variables using a t test in the pre- and postintervention periods, stratified by enterococcal species. The survival curves of the time to effective antibiotic therapy were compared by a log rank test. Data analysis was performed using SPSS version 15 (SPSS, Chicago, IL). All tests were two tailed, and the level of statistical significance was set at a P value of <0.05.

**Comparison of pre- and post PNA FISH interventions.** Obtaining species level identification was 2.6 days quicker with PNA FISH than with conventional culture reporting (P < 0.001; t test). The effects of PNA FISH on antibiotic selection and clinical outcomes with *E. faecalis* and other enterococci separately offer more information.

There were 112 individual episodes of *E. faecalis* bacteremia evaluated, with 64 in the preintervention period and 48 in the intervention period (Table 1). The clinical characteristics of the two groups were similar, with a slightly greater severity of illness within the PNA FISH group (APACHE II score, 14 versus 12; P = 0.08). The reporting of PNA FISH for *E. faecalis* was 3 days earlier than for conventional cultures (1.1 versus 4 days; P < 0.001); however, most patients received effective empirical antimicrobial therapy (99% versus 96%; P = 0.4). There was no difference in mortality between the groups (13% versus 10%; P = 0.73).

There were a total of 112 episodes of non-*E. faecalis* bacteremia. All were *E. faecium* except one episode of *Enterococcus avium* bacteremia, which was in the preintervention period. There were 65 patients in the preintervention period and 47 patients in the intervention period. The demographics were similar, with a trend toward more patients with neutropenia from the cancer center in the intervention group (20% versus 36%; P = 0.06). Over 75% of the patients with *E. faecium* bacteremia were either from the ICU or neutropenic. The median lengths of stay until onset of bacteremia in the groups were similar (12 versus 14 days; P = 0.7). Reporting of *E. faecium* with the PNA FISH test was a median 2.3 days earlier than with conventional reporting (P < 0.001; t test). The initial empirical antimicrobial therapy was inadequate in 53 of 65 patients (82%) in the preintervention group and 41 of 47 (87%) in the intervention group (P = 0.4; chi-square test). However, as shown in Fig. 2, there was a significant difference between the lengths of the time it took for the intervention group and the preintervention group to receive appropriate therapy (P < 0.001; log rank test). There was also lower mortality in the intervention group (26% versus 45%; P = 0.04), though there was no difference in the length of stay.

**DISCUSSION**

This study used the EFOE PNA FISH test in a clinical setting and showed its ability to identify enterococcal species more rapidly than conventional culture. In addition, for *E. faecium* bacteremia, its use reduced the time to effective antimicrobial therapy. PNA FISH was accurate (the sensitivity, specificity, and positive and negative predictive values were 100%) and was significantly faster, by almost 3 days, in identifying the *Enterococcus* species than standard microbiological methods. Identification of enterococci by standard methods is often prolonged because they are often part of mixed bacteremia with other organisms. By implementing the test with a treatment algorithm and incorporating it into the AMT responsibilities, we reduced the time to effective antibiotic therapy for those with *E. faecium* bacteremia. We did not see the same benefits of PNA FISH with *E. faecalis* bacteremia, which reflects the fact that, in our analysis, all of these infections received effective empirical antibiotic therapy prior to receipt of culture information. We also observed improved survival...
during the PNA FISH intervention period for those with *E. faecium* bacteremia; however, we did not detect a difference in the length of stay, which may reflect the severity of illness of the study population. Vergis et al. and Lodise et al., in separate studies, showed that up to 80% of patients with VRE bacteremia had received ineffective therapy (20, 35). In addition, Vergis et al. demonstrated that among patients with monomicrobial enterococcal bacteremia, receipt of effective antimicrobial therapy within 48 h of the blood culture independently predicted survival (odds ratio for death, 0.21 [confidence interval, 0.06 to 0.80]; \( P = 0.02 \)). These authors have suggested that delays in appropriate therapy may be associated with increased mortality (20, 35). Early initiation of linezolid therapy could affect outcomes in patients with VRE bacteremia (1). Our results are consistent with these and other studies. Taken together, they show that earlier identification of *E. faecium* is important for initiating earlier effective therapy, and this may improve survival in patients with hospital-acquired *E. faecium* bacteremia (14, 22, 40).

An alternative to rapid microbiological testing would be to use a risk-based approach to empirically treat patients with linezolid at “high risk” for VRE bacteremia before or after identification of GPCPC by the laboratory. The high-risk pa-

### TABLE 1. Characteristics of unique patients with *E. faecalis* and *E. faecium* bacteremia pre- and postimplementation of the PNA FISH test

<table>
<thead>
<tr>
<th>Characteristica</th>
<th>Value</th>
<th>E. faecalis</th>
<th>E. faecium</th>
<th>Pre-PNA FISH</th>
<th>PNA FISH</th>
<th>Pre-PNA FISH</th>
<th>PNA FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [median yr (range)]</td>
<td></td>
<td>53 (18–90)</td>
<td>60 (19–78)</td>
<td>0.2</td>
<td>54 (22–83)</td>
<td>53 (21–84)</td>
<td>0.5</td>
</tr>
<tr>
<td>Male sex [no. (%)]</td>
<td></td>
<td>29 (45)</td>
<td>29 (60)</td>
<td>0.11</td>
<td>37 (57)</td>
<td>23 (49)</td>
<td>0.4</td>
</tr>
<tr>
<td>ICU at BC draw [no. (%)]</td>
<td></td>
<td>28 (44)</td>
<td>19 (40)</td>
<td>0.66</td>
<td>33 (51)</td>
<td>20 (41)</td>
<td>0.39</td>
</tr>
<tr>
<td>Neutropenic at BC draw [no. (%)]</td>
<td></td>
<td>6 (9)</td>
<td>2 (4)</td>
<td>0.29</td>
<td>13 (20)</td>
<td>17 (36)</td>
<td>0.06</td>
</tr>
<tr>
<td>Central line at BC draw [no. (%)]</td>
<td></td>
<td>57 (89)</td>
<td>44 (92)</td>
<td>0.65</td>
<td>59 (91)</td>
<td>43 (91)</td>
<td>0.9</td>
</tr>
<tr>
<td>Median LOS until BC draw (days) [median (range)]</td>
<td></td>
<td>5 (0–64)</td>
<td>2 (0–101)</td>
<td>0.68</td>
<td>12 (0–99)</td>
<td>14 (0–123)</td>
<td>0.72</td>
</tr>
<tr>
<td>LOS from BC to discharge (days) [median (range)]</td>
<td></td>
<td>9 (2–50)</td>
<td>10 (1–60)</td>
<td>0.304</td>
<td>13 (1–85)</td>
<td>14 (1–175)</td>
<td>0.44</td>
</tr>
<tr>
<td>Median WCC at BC draw ((\text{cells/dl})) [median (range)]</td>
<td></td>
<td>10.8 (0.1–38.3)</td>
<td>8.7 (1–47.3)</td>
<td>0.9</td>
<td>9.2 (0.1–40.5)</td>
<td>5.7 (0.1–31.7)</td>
<td>0.04b</td>
</tr>
<tr>
<td>Median APACHE II score at BC draw [median (range)]</td>
<td></td>
<td>12 (3–28)</td>
<td>14 (5–28)</td>
<td>0.08</td>
<td>16 (5–25)</td>
<td>16 (5–23)</td>
<td>0.9</td>
</tr>
<tr>
<td>Ampicillin susceptible [no. (%)]</td>
<td></td>
<td>64 (100)</td>
<td>48 (100)</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Vancomycin susceptible [no. (%)]</td>
<td></td>
<td>57 (89)</td>
<td>43 (90)</td>
<td>0.93</td>
<td>11 (17)</td>
<td>2 (4)</td>
<td>0.04b</td>
</tr>
<tr>
<td>Other bacteria in same BC draw [no. (%)]</td>
<td></td>
<td>41 (64)</td>
<td>30 (63)</td>
<td>0.87</td>
<td>22 (34)</td>
<td>15 (34)</td>
<td>0.9</td>
</tr>
<tr>
<td>Total time in days from BC drawn to final microbiological report [median (range)]</td>
<td></td>
<td>4 (2.4–9.8)</td>
<td>4.1 (2.3–8.5)</td>
<td>0.33</td>
<td>3.3 (2.0–8.6)</td>
<td>3.4 (2.5–7.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>Total time in days from BC drawn to PNA FISH report [median (range)]</td>
<td></td>
<td>1.1 (0.5–3.3)</td>
<td>&lt;0.001b</td>
<td>1.1 (0.5–3.5)</td>
<td>&lt;0.001b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time in days from BC drawn to appropriate therapy [median (range)]</td>
<td></td>
<td>0 (0–5.3)</td>
<td>0.3 (0–6.5)</td>
<td>1</td>
<td>3.1 (0–9)</td>
<td>1.3 (0–4.3)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Initial appropriate empirical therapy [no. (%)]</td>
<td></td>
<td>63 (98)</td>
<td>46 (96)</td>
<td>0.4</td>
<td>12 (18)</td>
<td>6 (13)</td>
<td>0.42</td>
</tr>
<tr>
<td>Received appropriate therapy after final microbiological report [no. (%)]</td>
<td></td>
<td>64 (100)</td>
<td>48 (100)</td>
<td>1</td>
<td>56 (86)</td>
<td>46 (98)</td>
<td>0.04b</td>
</tr>
<tr>
<td>30 day mortality [no. (%)]</td>
<td></td>
<td>8 (13)</td>
<td>5 (10)</td>
<td>0.73</td>
<td>29 (45)</td>
<td>12 (26)</td>
<td>0.039b</td>
</tr>
</tbody>
</table>

a BC, blood culture; LOS, length of stay; WCC, white cell count.

b Significant value.
tients could be patients with prolonged ICU admission, neutropenia, or liver transplantation with VRE colonization based on surveillance cultures or prior colonization. The presence of VRE colonization has been strongly associated with subsequent VRE bacteremia (22, 37, 39). However, VRE screening is limited in its ability to detect VRE colonization prior to bacteremia and has been an asset in controlling spread. Garbutt et al. showed that less than 20% of patients were positive for VRE before an index blood culture, and Zaas et al. showed that 10 out 24 (42%) patients had developed VRE bacteremia up to 24 h before the identification of colonization (12, 39). Our center tracked a prior history of VRE colonization or infection and performed admission and weekly perirectal surveillance cultures for VRE in the ICUs and cancer center. These practices were in place and available to clinicians and the AMT during the entire study period. Thus, the decrease in the time to effective therapy, and perhaps the mortality difference, was due to the use of the PNA FISH test and the AMT algorithm. We believe that risk factors can be helpful in making empirical antibiotic decisions; however, they are a supplement to rather than a substitute for more rapid microbiological testing. We believe that the PNA FISH test is a relatively easy-to-implement, positive step toward more rapid microbiological testing.

The conclusions of this study are limited by its nonrandomized design. Because of its uncontrolled before-after quasiexperimental design, there may have been other, unmeasured factors that changed during the intervention period that could account for our results. However, there were no differences between the pre- and postintervention groups in baseline characteristics, including the severity of illness, and the AMT was active during both periods.

The conclusions of this study are also limited by the generalizability of the test and the AMT intervention to other medical centers. We have experienced laboratory staff accustomed to performing the test; however, the test is technically easy to perform (10). Our intervention required an active AMT that could implement a simple treatment algorithm, and the treatment algorithm was based on high rates of VRE among our *E. faecium* isolates. The majority of the decrease in the time to effective therapy was driven by more rapid treatment of VRE with linezolid. The development and use of a test that could rapidly identify vancomycin resistance would remove the need for the treatment algorithm.

There are other rapid tests available for the identification of enterococci. At present, there are several PCR tests that can screen patients as carriers of VRE infection, which could mark these patients to begin early VRE therapy at the start of their infections (7, 26, 31). These PCR tests are performed off swabs, but with the technology evolving, they may become available for use with blood cultures in the near future. In fact, Peters et al. have performed real-time PCR for *E. faecalis* directly from blood from ICU patients, with a sensitivity of 73% and a specificity of 96% (27). We believe that rapid testing with the ability to identify antibiotic susceptibility would be superior to the PNA FISH test with the AMT algorithm.

In conclusion, use of the EFOE PNA FISH test in conjunction with a treatment algorithm led to earlier identification of the *Enterococcus* species for patients with hospital-acquired enterococcal bacteremia and the earlier initiation of effective antimicrobial therapy for patients with hospital-acquired *E. faecium* bacteremia. Earlier appropriate therapy may decrease the hospital mortality for such patients. Further evaluation in a prospective interventional trial is needed to determine the benefit of rapid identification of antibiotic-resistant pathogens, such as VRE, in hospitalized patients.

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