Research Article

The Utility of Rapid Microbiological and Molecular Techniques in Optimizing Antimicrobial Therapy

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Early treatment of bloodstream infections with appropriate, definitive antimicrobial therapy has proven to reduce mortality, length of hospital stay, and healthcare costs. Culture-based testing methods require up to five days for final pathogen identification and susceptibility reporting, forcing use of broad spectrum empiric therapy. Recently, multiple rapid microbiological and molecular testing methods have been developed that reduce the time to identify the pathogen and susceptibility, allowing optimal antimicrobial therapy to be prescribed earlier. Real-time polymerase chain reaction and gene microarray have been described in literature, yet only peptide nucleic acid fluorescent in-situ hybridization has published data justifying its use based on clinical outcomes and cost savings. Target enriched multiplex polymerase chain reaction was developed to identify both the pathogen and multiple genes associated with resistance from blood within 6 hours and this methodology was studied in our hospital to assess effectiveness at optimizing antimicrobials in staphylococcal bloodstream infections.

1. Introduction

Culture positive healthcare-associated bloodstream infections (BSIs) are the tenth leading cause of death in the United States, with over 350,000 cases reported every year resulting in an estimated 90,000 attributable deaths [1, 2]. Studies have estimated the cost of treating BSIs to be approximately $27,000 per patient for community-associated bloodstream infections (CABSIs) and between $58,000 to $101,000 per patient for healthcare-associated bloodstream infections (HABSIs) [3, 4]. BSIs can quickly develop into life threatening situations such as sepsis and septic shock, requiring rapid identification of the causative pathogen to direct effective treatment [5]. The treatment of BSIs with early appropriate antibiotic therapy has proven to reduce morbidity, mortality, and healthcare costs [1, 6]. Current culture-based methods of phenotypically identifying BSI pathogens in BSIs have multiple inherent limitations that restrict their ability to rapidly identify the species and susceptibility. Because of this, clinicians are forced to prescribe empiric antimicrobial therapy with multiple antibiotics to cover all suspected pathogens while awaiting definitive reports from the laboratory. Therefore, the introduction of rapid microbial testing for BSIs into the clinical setting is absolutely necessary from both the patient and payer perspective. A recently proposed change by the Center for Medicare and Medicaid Services (CMS) to decrease reimbursement rates for healthcare-associated *Staphylococcus aureus* bloodstream infections is also a driving force behind the need for more rapid microbial diagnostic testing [5, 6].

2. Overview of Standard Microbiological Techniques and Their Drawbacks

The current standard of identifying BSIs in the hospital microbiology laboratory involves using culture-based techniques that have not evolved appreciably since their inception. This phenotypic method of culturing organisms has multiple inherent limitations that prevent it from providing rapid results, including time required for bacterial growth and limited sensitivity for slow growing, noncultivable, or intracellular organisms [7]. Under normal circumstances, a positive blood culture is typically detected by an automated microbial detection system within 8 to 36 hours from the time of collection. From that point, it can take anywhere from 12 to 48 hours to determine the species and another 24 to 48 hours to report the final pathogen identification and susceptibility report. Overall, the total time required by culture-based techniques to report the information needed to prescribe targeted, appropriate antimicrobial therapy is between 3 to 5 days. Previous studies have shown that treatment of BSIs with early, appropriate antimicrobial therapy reduces morbidity, mortality, and subsequent healthcare costs. Recommendations from the Infectious Diseases Society of America, the American Thoracic Society, and the Society for Healthcare Epidemiology of America suggest the use of broad-spectrum antimicrobials before blood samples are taken to cover for both gram negative and gram-positive organisms [8]. Although prescribing broad-spectrum antimicrobials before final culture and susceptibility testing is reported has proven to reduce mortality and healthcare costs, the opportunity to deescalate to targeted antimicrobial therapy sooner using rapid microbiological and molecular techniques can decrease the time patients are exposed to unnecessary treatment, potentially decreasing their length of stay and preventing antimicrobial resistance.

3. Overview of Currently Available Rapid Microbiological and Molecular Techniques

Several microbial diagnostic methods such as real-time polymerase chain reaction (RT-PCR), target enriched multiplex polymerase chain reaction (Tem-PCR), and gene microarray have been described in literature, but only peptide nucleic acid fluorescent in-situ hybridization (PNA FISH) has published data justifying its use based on clinical outcomes and associated cost savings [9–11]. In general, all of these methods identify and differentiate pathogens on a genotypic, rather than phenotypic, basis by detecting species specific expressed gene products directly from blood samples. This eliminates the time needed to obtain adequate bacterial growth that is required by current culture-based techniques.

Polymerase chain reaction (PCR) based methods have been used in the research laboratory for more than two decades but have not been user friendly enough to be efficiently utilized in the clinical laboratory until 5–7 years ago [12]. PCR is considered to be a type of nucleic acid amplification technique that works by amplifying and measuring small specific sequences of DNA directly from a blood sample using fluorescence resonance emission transfer probes, molecular beacons, or Taqman probes [12]. Each probe is designed to bind to a specific gene associated with the species of an organism, antimicrobial resistance (e.g., the *mecA* gene in methicillin-resistant *S. aureus*), or a subtype of these genes for epidemiological purposes (e.g., the *mecA* cassette IV associated with community-acquired *S. aureus* infection) [12]. There are also probes available for detection of virulence factors (e.g., Panton-Valentine leukocidin cytotoxin commonly found in community-acquired methicillin-resistant *S. aureus*), providing more detailed information about the pathogen [12]. When the probe binds to the amplified gene product, it creates a measurable amount of fluorescent signal that can be quantified exponentially by an RT-PCR machine [13]. Although RT-PCR can provide rapid and accurate results, it is only able to detect one gene at a time, requiring the lab to wait for bacterial growth to identify the organism and resistance genes. This problem has been solved by a more advanced nucleic acid amplification technique called target enriched multiplex polymerase chain reaction (Tem-PCR), which uses similar techniques as RT-PCR, but is able to identify multiple species and resistance genes at the same time. The whole process can be completed in 6 hours [14]. The cost effectiveness and clinical utility of this broad detection method in identifying BSIs have yet to be published.

PNA FISH is a peptide nucleic acid test that can identify organisms directly in positive blood cultures and is considered to be the simplest of the rapid microbiological tests. It is similar to nucleic acid amplification techniques in that it uses fluorescein labeled probes, but it differs by binding to the 16 s rRNA of the live bacteria rather than binding to amplified gene products [12]. Once bound to the specific 16 s rRNA sequence, a fluorescent signal is created and the sample is read under a fluorescent microscope by a laboratory technician [15]. The species of the organism is then determined by examining the color of the fluorescein probes, along with morphologic characteristics of the live bacteria [12]. The drawback of this test compared to PCR methods is that it does not divulge any resistance or epidemiological information, requiring prescribers to rely on local antibiograms to guide appropriate therapy. With the PNA FISH several species of various organisms can be identified once the genus is determined. PNA Fish currently offers FDA approved tests which provide individual fluorescent labels for the identification of the following microorganisms: *S. aureus*, coagulase-negative staphylococcus, *E. faecalis*, other enterococcus, *C. albicans*, *C. glabrata*, *C. tropicalis*, *E. coli*, and *P. aeruginosa. C. parapsilosis, C. krusei*, and *K. pneumonia* can also be identified by a fluorescent label that is shared with *C. albicans*, *C. glabrata*, and *E. coli*, respectively. Although, PNA FISH is only cost effective to use once a positive blood culture is obtained and a gram stain is performed to pinpoint which organisms are in question as to allow for the specific species to be determined.

4. Impact of Rapid Microbiological and Molecular Techniques

Rapid microbiological and molecular techniques have become part of routine practice for many commercial

industries, but until recently have not been adequately sensitive and reliable to be used in the clinical setting. Although many major advancements in technology have made rapid microbiological and molecular testing available for use in the hospital setting, PNA FISH is the only technique with published data describing its clinical impact in rapidly identifying BSIs [16–19].

To determine whether the use of PNA FISH to rapidly differentiate *E. faecium* from *E. faecalis* would lead to earlier initiation of appropriate antimicrobial therapy and improve mortality and length of stay in patients with hospital acquired enterococcal BSIs, Forrest et al. conducted a quasi-experimental study that included 223 patients over a 2-year period. An antimicrobial management team (AMT), consisting of an infectious disease physician and pharmacist, made treatment decisions in the preintervention group using standard microbiological methods then based treatment decisions of PNA FISH results and standard methods the next year in the intervention group. PNA FISH identified *E. faecalis* 2.9 days earlier than conventional culture methods (1.1 versus 4 days; $P < .001$) and *E. faecium* 2.3 days earlier (1.1 versus 3.4 days; $P < .01$). Most patients with *E. faecalis* BSIs in both the preintervention and intervention group received effective empiric therapy with ampicillin (99% versus 96%, resp.; $P = .4$), and there was no difference in mortality between the groups (13% versus 10%; $P = .73$). In contrast, over 80% of patients with *E. faecium* BSIs in both groups received initial inappropriate empirical therapy, and the use of PNA FISH in the intervention group was associated with statistically significant reductions in the time to initiating effective therapy with linezolid (1.3 versus 3.1 days; $P < .001$) and decreased 30 day mortality (26% versus 45%; $P = .04$). Cost savings associated with using PNA FISH in this population were not reported.

A retrospective, cost-effectiveness analysis done by Forrest et al. in 2006 evaluated the impact of rapid differentiation of *Staphylococcus aureus* from coagulase-negative staphylococci (CoNS) with PNA FISH in blood culture samples positive for gram-positive cocci in clusters (GPCC) [9]. Vancomycin usage, length of hospital stay, and hospital costs were evaluated. One hundred and thirty-nine positive blood cultures were tested with PNA FISH, with a majority of them being on nonintensive care unit (ICU) services. A control sample of 84 positive blood cultures containing GPCC underwent standard microbiology procedures and did receive PNA FISH. In the non-ICU setting, there was a trend towards a decrease in the amount of vancomycin usage in the PNA FISH group (4.9 defined daily doses (DDD) compared to the control group 6.78 DDD with no P value reported). There was a statistically significant difference in the number of patients who received one or less doses of vancomycin between the PNA FISH group and control group (43% versus 15%, resp.; $P \leq .005$), and a decrease in the median length of stay (LOS) (4 days versus 6 days, resp.; <0.05). Overall cost per patient was approximately $4000 less in the PNA FISH group compared to control group ($9616 versus $13621, resp.), due mostly to a decrease in LOS, along with a decrease in the amount of antibiotics given and laboratory tests ordered.

Another study done by Ly et al. evaluated further decreasing the time to reporting PNA FISH data, by adding a clinical laboratory liaison that rapidly relayed results to physicians 3 hours after results [10]. Use of the laboratory clinical liaison reduced overall mortality (16.8% control group versus 7.9% active group; $P = .05$) with the biggest impact seen in ICU patients (47.8% control group versus 9.5% active group; $P = .01$). Use of a laboratory clinical liaison also reduced further overall antibiotic use by 2 days ($P = .01$) and antimicrobial use in patients with blood cultures positive for CoNS by 2.5 days ($P = .01$). Early notification of PNA FISH results reduced the LOS in patient with CoNS by 2 days in the non-ICU setting and 7 days in the ICU, and subsequently a trend towards reduced median hospital charges of $19,441 per patient ($P = .09$).

A study done by Forrest et al. evaluated the impact of rapid identification of *Candida albicans* using PNA FISH on the selection and expenditure of antifungal therapy [8]. Blood cultures positive for yeast by gram stain in 2004 underwent PNA FISH testing, and positive *C. albicans* cultures were reported to clinicians within 3 hours. PNA FISH results were also relayed to an antimicrobial management team whose approval was required to release antifungal therapy to the prescribing physician, ensuring the preferred use of fluconazole for tests positive for *C. albicans*. Effects of PNA FISH on antifungal usage and expenditure was compared to a control group whose data was collected in 2003 when only standard culture techniques were used for detecting *C. albicans*. PNA FISH significantly reduced the median time required for identification compared to standard culture techniques (9.5 hours versus 44 hours; $P < .001$). PNA FISH significantly reduced the DDD/patient usage of caspofungin versus the control group (3.2 versus 8.7; $P < .05$) which resulted in cost savings of $1,978 per patient. A trend towards a decrease in caspofungin usage was noted in patients who had bloodstream infections caused by *Candida* species other than *C. albicans*, but it was not significant (11.9 versus 8.7; P value not reported). The overall cost savings were $130,231, or $1,729 per patient after accounting for PNA FISH testing cost.

5. Potential Drawbacks of Rapid Microbiological and Molecular Techniques

While the previously mentioned studies have successfully described and confirmed the clinical and potential economic benefits of rapidly identifying BSIs, there are some serious technical and nontechnical drawbacks to rapid microbiological and molecular testing that prevent them from completely negating the need for performing standard culture-based techniques in the future.

A major concern from a clinical standpoint is basing treatment decisions on the presence or absence of resistance genes alone without knowing the organism's susceptibility profile. Because rapid microbiological and molecular tests can only detect known mechanisms of resistance, it is possible that they can miss unknown or mutated resistance

genes. This fundamental flaw requires culture-based techniques to be performed alongside rapid microbiological and molecular tests to report resistance and susceptibility on a phenotypic level and assure that appropriate therapy is being provided.

A common concern for laboratory and hospital administration is not only the cost of rapid microbiological tests, but also the ease of use, amount of space required for the equipment, and the ability for laboratory personnel to quickly adapt to using a new technology. Many rapid microbiological and molecular tests are expensive when compared to standard techniques, costing anywhere from $25 to $80 on average for each sample tested depending on contracting agreements [20]. Because there are currently very few studies evaluating the cost effectiveness of using rapid microbiological and molecular tests, it is difficult to justify the cost of buying the equipment and training laboratory personnel to run them on a routine basis to hospital administration.

Although rapid microbiological and molecular testing have proven to provide results much faster than standard culture-based methods, the logistics of relaying the information to prescribers in a timely manner is just as important as the tests themselves. As these tests become more widely available for use in the future, a clinical pharmacist can play an integral part in the process by ensuring timely reporting of results while concurrently making recommendations on the most appropriate antimicrobial therapy based on cost and local resistance patterns.

Based on current literature evaluating the time to targeted antimicrobial therapy in relation to mortality, length of hospital stay, and healthcare costs, the implementation of rapid microbiological testing into the clinical setting is necessary to improve patient care, minimize resistance, and decrease healthcare costs. At the present time, PNA FISH is the only rapid microbiological test that has published data evaluating its clinical impact on identifying BSIs. Studies evaluating the utility of PNA FISH have shown that it can reduce overall hospital costs by $4000 to $20, 000 depending on the type of infection. It has also been shown to reduce the amount of antibiotics utilized as well as decrease mortality. As these tests become less expensive to use and further studies are published supporting their use based on clinical and economical outcomes, clinical pharmacists will have the opportunity to become an integral part of the process by acting as a liaison between the microbiology laboratory and the prescriber, ensuring that patients are receiving the most appropriate and cost-effective antimicrobial treatment sooner.

6. Findings from an Analysis Involving Tem-PCR in a Large Community Hospital

We conducted a prospective, randomized study at Huntsville Hospital, an 881-bed regional referral center, and to evaluate how Tem-PCR testing improves the pharmacists' ability to intervene and improve clinical outcomes in patients with staphylococcal BSIs. Our study was completed over a three-month period from 2/1/09 to 5/18/09 and included a total of 57 patients. Patients were randomized on a 1 : 1 basis to an intervention or usual care group based on the time the blood sample was identified as positive for GPCC by the microbiology laboratory. Patients enrolled in the study received both routine culture-based microbiological testing and Tem-PCR testing. Patients in the intervention group had Tem-PCR results reported to the physician by a clinical pharmacist, while those in the usual care group did not. All Tem-PCR results were faxed and emailed to the clinical pharmacist. The intervention consisted of notifying the prescribing physician of the results and providing recommendations on antimicrobial therapy optimization. Moreover, our study design allowed us to measure the impact of the Tem-PCR as opposed to traditional care, and since we had Tem-PCR results on all patients, we measured some variables using the entire population. The analysis concerning turnaround time was conducted with T-tests. Descriptive statistics were used to present information on the other items of interest. Additional endpoints of interest were the number of opportunities to optimize therapy, deescalate therapy, or stop contamination therapy before standard microbiology reports were available. The primary goal of the analysis was to evaluate the timeliness and utility of phenotypic versus genotypic testing methodologies.

Our study found that Tem-PCR provided culture results more quickly with an average turnaround time of 11.78 hours versus 50.64 ($P < .001$) hours by standard culture based microbiological techniques. The two testing methods agreed on the species in 93% of cases and on the sensitivities in 80% of the cases. Tem-PCR provided culture data used to optimize and deescalate therapy in 27% and 23% of the patients, respectively. Antibiotics were stopped in 19% of the patients due to an identified blood contaminate. Investigation of all 57 patients found that 40% of cases could have been optimized, 28% de-escalated, and 21% stopped due to contamination if all patients had Tem-PCR results reported to their physicians. Patients in the intervention group on average had antimicrobial costs of $394 while those in the control group had average antimicrobial costs of $493. We observed a difference in intensive care unit (ICU) length of stay (LOS) with the average ICU LOS being 9.3 days for the intervention group versus 12.2 days for the control. Age, mortality, and Apache II scores were approximately the same in both groups on average. Organism, resistance, and susceptibility varied between groups.

7. Discussion

The finding from our investigation that Tem-PCR turnaround was 11.78 hours and traditional testing turnaround was 50.64 is quite significant based on prior research reports regarding delayed therapy. These two average time points representing when results were made available fall on either side of the 44.75 hours reported in the Lodise et al. study as delayed therapy [17]. In addition, Lodise et al. found that patients that had a delay in treatment longer than 44.75 hours were 3.8 times more likely to die due to an

infection and on average stayed in the hospital 6 days longer. Therefore, the difference of approximately 39 hours in report receipt between genotypic and phenotypic testing methodologies found in our study could have had significant impact on patient outcomes. Moreover, Khatib et al. [6] found that patients with *S. aureus* bacteremia who received inappropriate initial therapy had a 35 percent chance of death while those with proper initial medication had only a 21 percent chance of death.

In conclusion, our analysis adequately evaluated the utility of Tem-PCR by a clinical pharmacist verifying this technology's ability to aid in the accomplishment of early, appropriate, and less costly treatment, minimized antimicrobial exposure, and improved antimicrobial stewardship practices. However, a limitation of this analysis was that small sample size which limited the opportunities for analysis. A larger sample size is needed to see what the impact on overall patient outcomes would be. In addition future models should control for variables that were mentioned. The different organisms and their resistance and sensitivities likely play a large role in the therapy that is required. Despite these limitations, the difference in turnaround time was substantial and should not be impacted by the covariates. However, the long-term clinical and financial outcomes will need more complex modeling and a larger data sample to analyze most appropriately. Therefore, based on this evidence, Tem-PCR looks very promising and warrants future investigations with a larger sample size. Since the number of potential participants receiving Tem-PCR testing is relatively small and will likely be based on risk factor stratification (i.e., patients with sepsis, neutropenia, serious wound infection, etc.), the investigators will consider only having one study arm and no control group in future investigations as data from prior time periods could be used as the control or comparator group.

Acknowledgments

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