Controlled Multicenter Evaluation of a Bacteriophage-Based Method for Rapid Detection of *Staphylococcus aureus* in Positive Blood Cultures

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Staphylococci are a frequent cause of bloodstream infections (BSIs). Appropriate antibiotic treatment for BSIs may be delayed because conventional laboratory testing methods take 48 to 72 h to identify and characterize isolates from positive blood cultures. We evaluated a novel assay based on bacteriophage amplification that identifies *Staphylococcus aureus* and differentiates between methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA, respectively) in samples taken directly from signal-positive Bactec blood culture bottles within 24 h of positive signal, with results available within 5 h. The performance of the MicroPhage KeyPath MRSA/MSSA blood culture test was compared to conventional identification and susceptibility testing methods. At four sites, we collectively tested a total of 1,165 specimens, of which 1,116 were included in our analysis. Compared to standard methods, the KeyPath MRSA/MSSA blood culture test demonstrated a sensitivity, specificity, positive predictive value, and negative predictive value of 91.8%, 98.3%, 96.3%, and 96.1%, respectively, for correctly identifying *S. aureus*. Of those correctly identified as *S. aureus* ($n = 334$), 99.1% were correctly categorized as either MSSA or MRSA. Analysis of a subset of the data revealed that the KeyPath MRSA/MSSA blood culture test delivered results a median of 30 h sooner than conventional methods (a median of 46.9 h versus a median of 16.9 h). Although the sensitivity of the test in detecting *S. aureus*-positive samples is not high, its accuracy in determining methicillin resistance and susceptibility among positives is very high. These characteristics may enable earlier implementation of appropriate antibiotic treatment for many *S. aureus* BSI patients.

Staphylococci are a frequent cause of bloodstream infections (BSIs). The incidence of *Staphylococcus aureus* bacteremia (SAB) has increased over the past decade, largely due to the increasing use of intravascular catheters and invasive devices (1). In a recent United States retrospective multicenter study, 31% of bloodstream isolates representing true infection were *Staphylococcus*, of which 74% were *S. aureus* and 26% were coagulase-negative staphylococci (CoNS) (1). In a prospective cohort of patients with nosocomial BSIs in the United States, *S. aureus* was the most commonly isolated organism representing true infection, accounting for 19% of cases (2).

Complications of SAB are common, and their incidence has also become more frequent, ranging from 10 to 50% (3). The mortality rate of SAB ranges from 11 to 20%, and relapse rates are high, especially if appropriate therapy is not administered or is delayed (1, 4, 5). It has been estimated that 33 to 60% of SAB bloodstream isolates in the United States are methicillin resistant (MRSA) (1, 6).

Patients with suspected BSI are often started empirically on broad-spectrum antimicrobial agents, which may entail added health care costs and adverse events. The most common broad-spectrum antibiotic prescribed for presumptive MRSA BSIs in the United States is vancomycin. Several studies have reported increased rates of treatment failure for patients with methicillin-susceptible *S. aureus* (MSSA) BSI treated with vancomycin (7–10). The selection of the most effective antibiotic treatment for SAB is often delayed because conventional microbiologic testing methods take 48 to 72 h to identify *S. aureus* and provide definitive susceptibility results. In this study, we evaluated a novel, phenotypic phage-based assay that requires no instrumentation to iden-

tify *S. aureus* and differentiates between MSSA and MRSA isolates directly from positive blood cultures within 5 h. The KeyPath MSSA/MRSA blood culture test (Microphage, Longmont, CO) uses mixed lytic bacteriophage to differentiate *S. aureus* from other bacteria and predicts susceptibility to penicillinase-resistant β -lactams. The test consists of two simultaneous reactions: one (the ID reaction) contains bacteriophage specific for *S. aureus*, the other (the RS reaction) contains the bacteriophage plus cefoxitin. The bacteriophage amplify in the ID reaction if *S. aureus* is present in the sample. The bacteriophage amplify in the RS reaction if the *S. aureus* strains present are able to grow in the presence of cefoxitin. Because bacteriophage growth is dependent on host growth, resistant strains (MRSA) are positive and susceptible strains (MSSA) are negative for phage amplification in the RS reaction. Bacteriophage amplification is detected by phage-specific monoclonal antibodies in a dual-strip self-contained immunoassay cartridge. This assay represents one possible approach to reducing the analytical time required to identify MSSA and MRSA from positive blood culture bottles. Rapid, reliable identification and susceptibility can enable clinicians to initiate optimal antimicro-

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bial therapy sooner and thereby may lead to reductions in health care costs and adverse events and better patient outcomes.

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MATERIALS AND METHODS

Patient samples. This clinical trial was performed at the following four sites: University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School (RWJMS), Northwestern Memorial Hospital, Duke University Health System, and Denver Health and Hospital. All sites received approval to participate from their respective Institutional Review Boards. Each site was instructed to identify positive Bactec culture system (BD Diagnostics, Sparks, MD) blood culture bottles, 0 to 24 h after detection (i.e., signal positive). There were no limits placed on the number of samples tested from each patient enrolled in the study. Gram stains were performed on an aliquot of culture broth from each bottle. Subsequently, samples underwent both conventional testing (see below) and the KeyPath MRSA/MSSA blood culture test. Clinical information about each patient, such as recent administration of antibiotics and antiviral medications, was collected by physician chart review.

Inclusion and exclusion criteria. Enrollment in the trial was limited to signal-positive Bactec blood cultures drawn from adult patients (at least 18 years of age). Samples had to be enrolled in the trial within 24 h of the Bactec alarm signaling a positive blood culture. Samples were excluded if they were mislabeled or misidentified.

Standard method tests. Conventional methods for the identification of organisms in positive blood cultures, including tube coagulase, catalase, and Staphaurex (Remel, Lenexa, KS) tests, were performed on all specimens. The comparative standard for *S. aureus* identification was defined as concordant results between the catalase, coagulase, and Staphaurex tests (all positive). For blood cultures that were judged to be positive for *S. aureus*, additional testing was performed to distinguish between MRSA and MSSA using both cefoxitin and oxacillin Sensi-Discs (BD, Franklin Lakes, NJ). These assays were interpreted using the following criteria: cefoxitin Sensi-Disc test with a zone of inhibition (ZOI) of ≥ 21 mm was considered susceptible (MSSA), and a ZOI of < 21 mm was considered resistant (MRSA) (11). Oxacillin Sensi-Disc with a ZOI of ≥ 13 mm was considered susceptible (MSSA), a ZOI of 11 to 12 was considered intermediate susceptibility, and a ZOI of < 10 was considered resistant (MRSA). The Oxoid PBP2a latex agglutination test (Thermo Scientific, Basingstoke, United Kingdom) was also performed. The gold standard for distinguishing MRSA from MSSA was based on the results of the cefoxitin Sensi-Disc test.

MicroPhage MRSA/MSSA blood culture test. An aliquot of broth from the positive blood culture bottle was transferred to a tube. KeyPath reaction media (ID and RS media) containing phage cocktail and broth were added to respective reaction tubes (ID and RS) containing dry reagents. The device contains both an identification and susceptibility component which are read in tandem. A total of 10 μ l of the blood culture sample was then added to each of the two separate reaction tubes. The tubes were incubated for $5 \text{ h} \pm 20 \text{ min}$ at $35 \pm 2^\circ\text{C}$ to allow phage amplification from phage-susceptible bacteria (*S. aureus*), if present. One of the two tubes (RS) contained cefoxitin (a surrogate for methicillin). At the end of the incubation period, aliquots were taken from the reaction tubes and applied to a lateral flow device that detects phage antigens as a surrogate for the presence of bacteria. Cultures that yielded positive results (i.e., phage amplification) in both tubes were interpreted as positive for MRSA, those in which only the tube which did not contain cefoxitin gave a positive result were interpreted as positive for MSSA, and those that gave negative results for the ID reaction were interpreted as negative for *S. aureus*. When performed in batches of 5 to 10, the total time from specimen acquisition to result was approximately 7 h, with a hands-on time for the laboratory technician of approximately 60 to 90 min.

Time to result. Three of the study sites (Duke, RWJMS, and Denver Health) recorded the date and time of reporting the initial Gram stain, routine bacterial identification, and final susceptibility results in the hospital laboratory information system. In addition, these 3 sites recorded the date and time of completing the KeyPath MRSA/MSSA blood culture test for each positive blood culture specimen included in the study ($n = 708$ for conventional methods, 706 for the KeyPath MRSA/MSSA blood culture test). Time data were not recorded for 2 of the KeyPath assays.

Data analysis. We compared the performance of the KeyPath MRSA/MSSA blood culture test to the conventional methods as a consensus standard. Analysis was also stratified based on exposure of patients to antimicrobials and antiviral medications. For analysis of turnaround times, start time was defined as the point at which the blood culture bottle signaled positive. The gold standard methods were conducted per standard protocol in each laboratory, while the KeyPath MRSA/MSSA blood culture test was routinely batched for clinical trial purposes. Finish time for the KeyPath MRSA/MSSA blood culture test assay was defined as the time when the research technologist recorded the results. Standard method finish time was defined as the time when the results were entered into the computer system by clinical laboratory staff. The medians of the times to results for each method were compared.

Discrepant analysis. Sample repeat testing was not performed on any discrepant samples at the sites. Three samples were initially invalid on the MRSA/MSSA blood culture test detector and were rerun immediately with the remaining volumes from reaction tubes. However, these data were excluded from analysis.

This study was registered with ClinicalTrials.gov, identifier number NCT01184339.

RESULTS

A total of 1,165 samples were collected at four sites for KeyPath MRSA/MSSA blood culture test and predicate testing. The overall prevalence of *S. aureus* was 32% and ranged from 21% to 43% between sites. Among *S. aureus* samples, 53% were MRSA, with a range of 39% to 69% between sites.

Forty-nine positive blood culture bottles were excluded for the following reasons: 5 missing catalase, coagulase, or Staphaurex results; 8 disagreements between catalase, coagulase, or Staphaurex results; 3 invalid KeyPath MRSA/MSSA blood culture test detector results; and 33 invalid KeyPath MRSA/MSSA blood culture test results (performed > 24 h after positive signal). Thus, 1,116 samples were included in the data set used to characterize the ability of the KeyPath MRSA/MSSA blood culture test to accurately identify *S. aureus*. All samples included in the analyzed data set had valid coagulase (4 or 24 h), catalase, Staphaurex, and KeyPath MRSA/MSSA blood culture tests performed.

Samples that had Gram stain results other than Gram-positive cocci were considered to be negative for *S. aureus* even if no further testing was performed. At the start of the study, all specimens (regardless of Gram stain result) underwent subsequent testing; however, as the trial progressed, to enrich the population for *S. aureus*-positive samples, only those specimens identified as Gram-positive cocci underwent subsequent testing as described above. All of the non-Gram-positive cocci that underwent KeyPath MRSA/MSSA blood culture testing were correctly categorized as not *S. aureus*.

***S. aureus* detection.** A total of 366 samples were identified as *S. aureus* by the standard methods. Thirty *S. aureus* isolates were incorrectly characterized as “not *S. aureus*” (NSA) by the KeyPath MRSA/MSSA blood culture test (false negatives) and 13 NSA isolates were mischaracterized as *S. aureus* (false positive) by the KeyPath MRSA/MSSA blood culture test (Fig. 1). The overall accuracy

		Gold Standard		
		SA	NSA	
KeyPath™	SA	336	13	
	NSA	30	737	
		SN = 92% SP = 98%		PPV = 96% NPV = 96%

FIG 1 Performance characteristics of the KeyPath MRSA/MSSA blood culture test for identification of *S. aureus*. Of 366 isolates identified as *S. aureus* by the standard methods (catalase, tube coagulase, and Staphaurex), the KeyPath MRSA/MSSA blood culture test correctly identified 336 directly from the positive Bactec blood culture bottle. Thirteen non-*S. aureus* blood culture isolates were misidentified by the KeyPath MRSA/MSSA blood culture test as *S. aureus*, and 30 *S. aureus* isolates were misidentified as non-*S. aureus*. SA, *S. aureus*; NSA, not *S. aureus*; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

(concordance) of standard (predicate) methods and Microphage KeyPath MRSA/MSSA blood culture test for detection of *S. aureus* was 96.2%. Sensitivity and specificity of the MRSA/MSSA blood culture test versus standard (predicate) methods was 91.8% and 98.3%, respectively. For the MRSA/MSSA blood culture test, the positive predictive value (PPV) was 96.3% and the negative predictive value (NPV) was 96.1%. Further evaluation of discrepant results revealed that half the false negatives, when isolated as colonies and retested, gave results that matched those obtained by the standard method. The false negatives were not concentrated in any one pulsed-field gel electrophoresis (PFGE) type. All false-positive results were CoNS except for one enterococcus. No discrepant results were observed from blood cultures which grew multiple organisms. There was no difference of test performance characteristics among the different sites in the study (data not shown).

Differentiating MRSA from MSSA. Two of the 366 *S. aureus* isolates were not evaluated by disk diffusion and were excluded from resistance and susceptibility analyses. Of the remaining 364 isolates, 334 were correctly identified as *S. aureus* by the KeyPath MRSA/MSSA blood culture test; these samples were further analyzed for correct categorization as MRSA or MSSA. Of these 334 *S. aureus* isolates, 180 were categorized as MRSA and 154 as MSSA based on the standard method results. A total of 99.1% of these were correctly categorized as either MRSA or MSSA using the Microphage KeyPath MRSA/MSSA blood culture test (Fig. 2). Two *S. aureus* isolates judged to be cefoxitin resistant with the disk test (cefoxitin disk test zone diameters of 9 mm and 10 mm) and PBP2a positive were misclassified as susceptible by the KeyPath MRSA/MSSA blood culture test (1.3% very major error rate). One cefoxitin-susceptible isolate (cefoxitin disk test zone diameter of >30 mm) which was also PBP2a negative was misclassified as resistant by the KeyPath MRSA/MSSA blood culture test (0.8% major error rate). Repeat KeyPath MRSA/MSSA testing on two of the isolates (one incorrectly classified as susceptible and one incorrectly classified as resistant) yielded results concordant with the standard method. Sensitivity and specificity of the KeyPath MRSA/MSSA blood culture test compared to cefoxitin disk testing for determination of MSSA was 88.4% and 98.7%, respectively, with a PPV of 92.7% and an NPV of 97.9%.

For *S. aureus*, MRSA, and MSSA detection, there were no significant differences for accuracy, sensitivity, specificity, PPV, or NPV between antimicrobial or antiviral-treated patient populations when comparing the MRSA/MSSA blood culture test to standard methods (data not shown).

		Cefoxitin Disk		
		R	S	
KeyPath™	R	178	1	
	S	2	153	
Category Agreement		99.4%	98.7%	

FIG 2 Categorical agreement between the KeyPath MRSA/MSSA blood culture test and cefoxitin disk test for prediction of oxacillin susceptibility. Two *S. aureus* isolates judged to be cefoxitin resistant using the disk test were misclassified as susceptible by the KeyPath MRSA/MSSA blood culture test (very major error). One cefoxitin susceptible isolate was misclassified as susceptible by KeyPath MRSA/MSSA blood culture test (major error). Repeat testing on two of the isolates (one incorrectly classified as susceptible and one incorrectly classified as resistant) yielded results concordant with those of cefoxitin disk testing.

Time to results. Time to result (TTR) data were recorded for a subset of isolates from three sites as specified in Materials and Methods. For 706 isolates for which TTR data were available for conventional testing, the median TTR was 46.9 h (range, 21.8 h to 76.3 h). For 708 isolates for which KeyPath MRSA/MSSA blood culture test TTR data were available, the median TTR was 16.9 h (range, 13.4 h to 20.9 h).

Bottle type. The KeyPath test was performed on samples from Bactec Plus Aerobic/F ($n = 812$), Plus Anaerobic/F ($n = 296$), and Standard 10 Aerobic/F ($n = 8$) bottle types. No significant differences in performance between bottle types were observed.

DISCUSSION

S. aureus is a virulent organism which causes significant morbidity and mortality (12–14). With the increasing incidence of *S. aureus* bacteremia (SAB) and the rising prevalence of MRSA, it is imperative to consider approaches that may improve the timeliness of appropriate therapeutic interventions. Using standard microbiologic methods, initiation of directed therapy requires definitive identification and susceptibility testing, which can take up to 72 h. Although Gram stain is used for presumptive diagnosis based on bacterial morphology, it is unable to discriminate between similar-appearing organisms and does not provide susceptibility results. Because approximately 30% of *S. aureus* isolates in U.S. hospitals are methicillin resistant, the most common antimicrobial used for presumptive *S. aureus* infection is vancomycin. Multiple studies have suggested that patients who have MRSA bacteremia have a longer length of stay in the hospital than those with MSSA bacteremia, resulting in higher hospital costs (3, 15–18) and higher mortality as a result of inappropriate initial antibiotics (3).

Clearly, nafcillin is superior to vancomycin for treating MSSA infection (5, 7, 8, 10) but is ineffective for treating MRSA infection (8). *In vitro* studies suggest β -lactam antibiotics achieve bactericidal activity significantly faster than glycopeptides (5, 19–21). Receipt of nafcillin or cefazolin is independently associated with decreased treatment failure (5) and mortality (9) compared to vancomycin in the treatment of MSSA bacteremia, a benefit that persists even if receipt is delayed until definitive culture results are available.

These facts, coupled with the potential to increase the emergence of staphylococci and enterococci with increased resistance to vancomycin, make initial therapy with vancomycin for patients with presumptive *S. aureus* infection suboptimal. More rapid definitive identification of *S. aureus*, along with β -lactam suscepti-

bility testing, should allow earlier initiation of β -lactams if appropriate, thereby reducing the overall usage of vancomycin in these patients. While it is unclear whether appropriate initial antibiotic therapy for the treatment of SAB affects mortality (19), earlier therapy appears to decrease the length of bacteremia and fever in the patient (22, 23), and delayed treatment is an independent predictor of infection-related mortality and associated with increased length of hospital stay (24).

There are several accelerated SAB identification assays that are commercially available. These methods employ a variety of technologies, including molecular amplification and/or detection, antigen detection, and culture-based methods (25–28). None of them are performed directly on blood specimens but rather require a signal-positive blood culture or the growth of a colony on media. The MicroPhage KeyPath assay is the first to use a bacteriophage as the surrogate marker for the presence of bacteria in a positive blood culture, providing a phenotypic result in as little as 5.5 h. In our study, it performed comparably to the aforementioned assays and does not require additional instrumentation. Moreover, it provides a phenotypic assessment of susceptibility to methicillin which may potentially avoid some of the limitations of molecular testing for the *mecA* gene. Such limitations have recently been illustrated in reports that describe the inability of such assays to accurately categorize some *S. aureus* isolates. A recent analysis revealed that of 217 patients who tested positive by rapid PCR for MRSA carriage, 12.7% had *S. aureus* isolates with a staphylococcal cassette chromosome (SCC) element that lacked the *mecA* gene; these patients were mislabeled as being MRSA carriers because the rapid test was targeted to detect the SCC rather than the *mecA* gene (25). The KeyPath assay determines susceptibility through phenotypic testing using ceftiofur rather than genotypic assessment. Although the PCR test evaluated in the above-referenced study is not designed to test directly from signal-positive blood cultures, it serves to illustrate the potential benefit of phenotypic susceptibility analysis compared to molecular approaches.

Earlier identification of organisms should allow institution of appropriate antimicrobial therapies. A pharmacoeconomic analysis of several publications determined the impact of PCR to identify MRSA and concluded that mortality rate when appropriate empirical antimicrobials were administered was significantly lower than the mortality rate in patients who required a change in therapy. Cost analysis indicated PCR testing for MRSA would be less costly than empirical therapy as long as the cost of the test was less than \$2,400 (29). However, given that this was a composite of multiple other studies and a theoretical model which proposes a 60-min turnaround time from positive blood culture result to administration of optimal antibiotic therapy, it is difficult to apply the results in an actual laboratory/hospital setting.

Furthermore, studies that analyzed rapid diagnostics used in combination with direct communication of results to the prescribing physician by the laboratory or an antimicrobial stewardship team have shown that communication is essential for affecting outcomes (30–35). The results of this combination were shorter length of stay (30, 32, 35, 36), shorter time to switch to a β -lactam antibiotic (30–32, 35, 36), and decreased mortality (35). All of these studies were carried out in large academic centers, and it is unclear if similar benefits would be realized in a mixed practice or community practice-based center. Even with the faster turnaround time than conventional methods of the assay we evaluated,

timely communication of the results so that clinicians can implement changes, if needed, is essential to potentially improving patient care.

Since the KeyPath MRSA/MSSA blood culture test has a sensitivity of 92%, labs may wish to delay reporting of negative results until they are confirmed by standard methods, during which time patients would continue on empirical therapy. Labs could continue to use standard-of-care methods with the KeyPath as a supplementary laboratory test. Accuracy of susceptibility determination is high once an organism is identified as *S. aureus*. On repeat testing of isolates that were not correctly identified initially as *S. aureus* or NSA, 50% of isolates produced results concordant with the gold standard result. This was likely a result of the fact that the repeat tests were performed on bacterial isolates as opposed to the initial method of testing directly from signal-positive blood culture bottles which may contain lower concentrations of organisms or interfering substances. Other possibilities for incorrect identification include technical errors related to reading strips, transfer of reagents, and inadequate mixing of reagents with the blood culture specimen. An isolate identified as *S. aureus* by both the KeyPath MRSA/MSSA blood culture test in conjunction with a positive catalase and tube coagulase or Staphaurex test (to confirm *S. aureus*) would provide reliable and actionable data. In this study, all testing with the KeyPath assay was performed in batch mode. In practice, it would be practical to do the same to streamline the workflow of the laboratory technician. The test can be easily performed without any specific technical training and expertise, unlike fluorescence *in situ* hybridization testing. Another option would be to perform the test on demand when a blood culture bottle signaled positive and Gram stain (routinely performed) revealed Gram-positive cocci. While not impossible, this may be more difficult to integrate into laboratory workflow and lead to errors if a laboratory technician needs to track multiple incubation periods for the assay in addition to their routine assignments.

Study limitations. Only one type of blood culture system, Bactec, was evaluated. In addition, this rapid test was not compared directly to other rapid methods.

In summary, the KeyPath MRSA/MSSA blood culture test provides moderate sensitivity (91.8%) and excellent specificity (98.3%) for the identification of MRSA and MSSA directly from positive blood cultures with a significantly shorter turnaround time than that of conventional testing methods.

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