Published in final edited form as:

Diagn Microbiol Infect Dis. 2015 March ; 81(3): 158–162. doi:10.1016/j.diagmicrobio.2014.11.014.

Evaluation of Real-time PCR and Pyrosequencing for Screening Incubating Blood Culture Bottles from Adults with Suspected Bloodstream Infection

Chase D. McCann¹, Miranda S. Moore^{1,*}, Larissa S. May², Matthew McCarroll¹, and Jeanne A. Jordan¹

¹Department of Epidemiology and Biostatistics, Milken Institute School of Public Health, The George Washington University, Washington, DC, USA

²Department of Emergency Medicine, The George Washington University Medical Faculty Associates, Washington, DC, USA

Abstract

Several molecular platforms can identify bacteria associated with bloodstream infections, but require positive culture bottles as starting material. Here we describe results of screening 1140 blood cultures at 8 hours post-inoculation, from 918 eligible adults being evaluated for bloodstream infection. DNA was extracted and analyzed by 16S and/or 23S rRNA real-time PCR/Pyrosequencing. Compared to culture, PCR/Pyrosequencing displayed 90.9% sensitivity, 99.6% specificity, 95.7% PPV, and 99.1% NPV. Overall concordance rate was 98.9% (1127/1140). In four cases with molecular-positive/culture-negative results, medical chart reviews provided evidence of identical bacteria from subsequent blood or concomitant urine/sputum cultures. Nine culture-positive/molecular-negative cases were associated with either polymicrobial growth, grew only in the anaerobic bottle of the clinical pair, and/or were detected by PCR/Pyrosequencing after 8 hours. In summary, this approach accurately detected and identified bacteria in ~91% of culture-confirmed cases significantly sooner than the phenotypic identification was available, having the potential to improve antibiotic stewardship.

Keywords

Molecular diagnosis; Screening incubating blood culture bottles for bacteria using a molecular approach; Bloodstream infections; ED and ICU patients; PCR/pyrosequencing methods used to more rapidly detect and identify bacteria compared to phenotypic identification; Incubating cultures

© 2014 Elsevier Inc. All rights reserved.

Corresponding Author: Jeanne A. Jordan, PhD, Department of Epidemiology and Biostatistics, Milken Institute School of Public Health, The George Washington University, 2300 Eye Street NW, Ross Hall Room 231, Washington, DC 20037, +01-202-994-7062, jajordan@gwu.edu.

*Current Affiliation: Epidemiology Fellow, New York City Department of Health and Mental Hygiene, NY, USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Bloodstream infections (BSI) are significant contributors to morbidity and mortality throughout the general population. Hospital-acquired BSI is the third most common cause of hospital mortality in the US [1, 2] while community-onset BSI is comparable to acute myocardial infarction, stroke and major trauma in burden of illness [3, 4]. Anderson et al. looked at BSI in community level hospitals in a multicenter cohort study and concluded that 1 in 3 patients with a culture confirmed BSI receives inappropriate empiric therapy [5] and in a prospective cohort study, Retamar et al. found that inappropriate empiric therapy is associated with worse outcomes [6]. Therefore, providing more rapid test results for ruling in or ruling out BSI could reduce the amount of delayed treatment while also promoting antimicrobial stewardship by decreasing the use of inappropriate, ineffective or unnecessary empiric antimicrobial therapy.

Automated blood culture systems are the current gold standard for diagnosis of BSI in hospitals [7, 8] but require significant time for the small number of organisms commonly present in the blood of patients with BSI to grow to detectable levels. Even after the instrument detects growth in an incubating blood culture bottle, fluid must be Gram stained and then sub-cultured onto solid agar for isolation and further phenotypic identification and antimicrobial susceptibility testing. One study found that growth of Gram-positive bacteria, which represent most BSI, require 24 to 48 hours (h) of incubation to be detected by the automated blood culture system, while Gram-negative bacteria require 12 to 24 h [9]. A second study found an automated system needs an average of 18 h (range 11–28 h) to detect growth of organisms, and 49 h (range 23–73 h) to complete the final identification of the organism [10].

In recent years several molecular-based approaches have been developed, validated and implemented that can rapidly identify microorganisms from positive blood culture bottles including MALDI-TOF MS analysis, PNA FISH and PCR [11,12]. We describe combining real-time PCR and Pyrosequencing to provide an accurate identification of the bacteria present within positive blood culture bottles [13].

MALDI-TOF MS analysis has been used to improve antibiotic stewardship programs by more rapidly identifying bacteria within positive blood culture bottles. Huang et al. describes using MALDI-TOF to detect microorganisms within positive blood culture bottles to improve time to effective antibiotic therapy and optimal antibiotic therapy [14]. In a separate study, Perez et al. describes how MALDI-TOF MS analysis of positive blood culture fluids from bottles containing gram-negative bacteria coupled with antimicrobial stewardship significantly improves time to optimal therapy and decreased hospital length of stay and total costs [15].

Recently we reported on using real-time PCR and Pyrosequencing to accurately identify bacteria from incubating blood culture bottles, after limited enrichment time, before the automated instrument detected growth. On average, this approach identifies the bacterial pathogen in culture-confirmed BSI 16 hours sooner than growth is detected by the instrument and the Gram stain results is called to the unit, and a full 3 days sooner than the

phenotypic identification is available [16]. In fact we found that 73% of all culture-confirmed cases of BSI are detected and accurately identified from incubating blood culture bottles after just 5 hours of enrichment using this molecular approach [16].

In this prospective study we describe the overall analytical performance characteristics of this molecular approach to screen 1140 blood culture fluids after 8 h of pre-enrichment compared to culture from 918 eligible patients being evaluated clinically for suspected BSI.

Materials and Methods

Participant Enrollment and Sample Collection

This prospective study enrolled adult participants from both the Emergency Department (ED) and three intensive care units (ICU) at The George Washington University Hospital between May 2009 and June 2012. Participants needed only to have a physician-ordered blood culture being drawn due to suspicion of BSI to be eligible and were provided information about the study and consented by clinical staff. Both the physician-ordered blood cultures and the research blood samples were collected from the same needle stick, with the former collected first, by either a venous or arterial draw. Eight to ten ml of blood were collected directly into each of the following: one BD Bactec Plus Aerobic/F Medium (Cat# 442192; Becton Dickinson (BD), Sparks, MD) and one Standard Anaerobic/F Medium blood culture bottles (Cat# 442191; BD) according to standard of care for the clinical sample. Eight to ten ml of blood were collected also into one yellow top vacutainer tube containing sodium polyanetholesulfonate, sodium chloride (Cat# 364960, BD) for the research sample. The Institutional Review Board of The George Washington University (GWU), Office of Human Research approved this study.

Clinician-ordered Automated Blood Culturing and Phenotypic Identification

Standard of care dictates that physician-ordered blood cultures must be received by the clinical microbiology laboratory within 1 hour of draw time where they are placed in a Bactec 9240 (BD) automated blood culture system according to manufacturer recommendations. Bottles flagged by the instrument had fluid removed for Gram staining and sub-culturing onto appropriate agar-based culture plates. Purified bacterial colonies were analyzed either by an automated identification system (Vitek 2[®], bioMérieux, Durham, NC) or with the appropriate biochemical reagents for manual phenotypic identification.

Research Blood Culture Protocol

Research blood samples collected from enrolled participants into yellow-top Vacutainer tubes were subsequently inoculated into Bactec Plus Aerobic/F blood culture bottles (BD# 442192) upon receipt in the research lab and immediately incubated at 37°C with continuous motion (150 rpm, Excella E24 Incubator Shaker Series, New Brunswick Scientific, Enfield, CT). Samples were rejected for this study if the blood in the vacutainer tube was grossly hemolyzed, clotted, or contained half or less of the fill blood volume. Time of inoculation was noted for each culture bottle, with 1.5 ml aliquots of culture fluid removed aseptically from each aerobic blood culture bottle after 8 h for this analysis. Research specimens were

inoculated only into aerobic blood culture bottles, as this study was not intended to evaluate strict anaerobic organisms.

DNA Extraction from Culture Fluid 8 Hours Post-inoculation

Five hundred microliters of the drawn culture fluid was extracted using a combination of Zirconium silica bead-based lysis and organic nucleic acid extraction as described previously [16]. Culture fluids were stored at 4°C for a maximum of 1 week before DNA extraction was completed. DNA extracts were stored at -20°C until analyzed by PCR and pyrosequencing.

Real-time PCR Assays Used to Screen DNA Extracts for 16S rRNA and/or 23S rRNA Gene Targets

DNA extracts were amplified in 25 µl reactions by PCR using SmartCycler instruments (Cepheid, Sunnyvale, CA) as previously described [16]. The customized 2.5X PCR master mix used in amplifying the 16S rRNA universal gene target (base pair location 1155–1525) [13] contained a final MgCl₂ concentration of 2 mM (catalog no. S-026–0250, Molzyme GmbH & Co. KG). If a Universal 16S rRNA gene target was detected by real-time PCR and identified as either a *Staphylococcus* sp., *Streptococcus* sp. or enteric Gram negative rod by Pyrosequencing, then the corresponding real-time PCR assay was set up using a 2X SYBR Premix Ex Taq polymerase PCR master mix (catalog no. RR420A; TaKaRa Biotechnology, Inc., Dalian Corp, Ltd., Japan) to amplify either a *Staphylococcus*-specific 16S rRNA gene target (base pair location 16–258), a *Streptococcus*-specific 23S rRNA gene target (base pair location 602–834) or an enteric Gram-negative rod-specific 23S rRNA gene target (base pair location 1346–1625) [13], respectively for subsequent Pyrosequencing analysis. Positive controls included 1 µl DNA extracts from purified isolates of either *S. aureus* or coagulase-negative *Staphylococcus* spp. (CoNS) for the 16S rRNA *Staphylococcus* targets, or *S. pneumoniae* or *Enterococcus* spp. for the 23S rRNA *Streptococcus* targets, or *Escherichia coli* or *Klebsiella pneumoniae* for the 23S rRNA enteric Gram-negative rod targets. Any of these bacterial DNA extracts served as a positive control for the Universal 16S rRNA target. Negative controls consisting of the appropriate master mix and molecular grade water were included in each PCR run, one at the beginning of each run and one at the end of each run.

Pyrosequencing of PCR Amplicons for Bacterial Identification

The entire 25 µl volume of biotin-labeled PCR product was analyzed by Pyrosequencing (PyroMark ID Pyrosequencer, Qiagen, Germantown, MD) using PyroMark Gold Q96 reagents (Cat# 972804; Qiagen) as previously described [16]. The dispensation programs varied with the target and included: 11(ACTG) dNTP for the Universal, *Staphylococcus*, and *Streptococcus* rDNA targets, or AGC, 12(CTGA) dNTP for the enteric rDNA target. PyroMark Identifire v1.0.5.0 software (Qiagen) was used to analyze the resulting sequences, and then compared to our reference library that included validated sequences from previously identified clinical isolates, ATCC reference sequences, and validated sequences found in the NCBI GenBank using the BLAST algorithm [10, 13, 17]; greater than 95% sequence concordance was required when identifying bacteria.

Statistical Calculations

Sample size estimates for paired two-sample comparison were made to achieve 90% power at a 5% significance level using a two-sided equivalence test of correlated proportions [18]. Analytical performance characteristics; sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) with accompanying 95% confidence intervals (95% CI) were calculated using Stata version 12.1 (StataCorp, College Station, TX).

Results

Demographic Characteristics of Study Participants

A total of 918 patients being evaluated clinically for BSI were enrolled in this prospective study at The George Washington University (GWU) hospital; 49% (446/918) of them had blood cultures drawn in the Emergency Department (ED), while the remaining 51% (472/918) had their blood cultures drawn while hospitalized in one of three intensive care units (ICU). The average age of those enrolled was 57 years (y) (range 19–103 y), with those enrolled in the ED being slightly younger in age (55 y) (range 20–103) compared to those enrolled in an ICU (60 y) (range 19–99). Slightly more than half (516/918; 56.2%) of the participants were male and 58.0% (532/918) self-reported their race/ethnicity as Black (Table 1). A total of 1142 research blood samples were collected from these 918 eligible participants. Two of the 1142 samples were excluded from analysis due to mislabeling (1) or missing specimen (1), leaving 1140 samples for the final analysis. Those individuals with more than one pair of research and clinical samples analyzed had these pairs of specimens collected during different hospital stays.

Phenotypic Identification of Bacterial Isolates

The 1140 physician-ordered blood cultures resulted in 102 cultured bacterial isolates from 99 clinically confirmed BSI; three of the positive blood cultures were polymicrobial growing *Klebsiella pneumoniae*/*Stenotrophomonas maltophilia*, *Proteus mirabilis*/*Enterobacter cloacae*, or Group G Streptococcus/Viridans streptococci. Table 2 displays the types of bacteria isolated. The most common organisms cultured from blood were classified as Gram-positive cocci (79/102; 77%); either *Staphylococcus* spp. (51/102; 50%) or *Streptococcus* spp. (28/102; 27%). The most common *Staphylococcus* spp. isolated was coagulase-negative *Staphylococcus* spp. (32/51; 63%), while *Streptococcus pneumoniae*, Viridans streptococci and *Enterococcus faecalis* were each found at equal rates (5/28; 18%). Gram-negative rods (GNR) comprised 22% (22/102) of all BSI with *Escherichia coli* being by far the most common GNR identified (9/22; 41%). Strict anaerobic bacteria, yeast and fungi isolated during this study were not included in the analysis.

Analytical Performance Characteristics of Molecular Approach Using 8 h Culture Fluid Compared to Standard Culture

The overall concordance rate in this prospective study was 98.9% (1127/1140). Compared to clinician-ordered hospital blood culture results, the molecular approach using culture fluid removed from incubating blood culture bottles exhibited 90.9% (95% CI: 83.4–95.8%) sensitivity and 99.6% (99.0–99.9%) specificity with a PPV and NPV of 95.7% (89.5–

98.8%) and 99.1% (98.4–99.6%), respectively (Table 3). Nine organisms isolated in culture were undetected by PCR/Pyrosequencing from the 8 h culture fluid (3 *E. coli*, 2 *K. pneumoniae*, and 1 each of *Proteus mirabilis*, *E. cloacae*, *S. maltophilia* and *S. aureus*). Three of these, 2 *E. coli* and *S. aureus*, were detected and identified by PCR/Pyrosequencing, but only in culture fluids collected after 8 h of pre-enrichment so they were counted as being missed in this analysis. Three others, *K. pneumoniae*, *P. mirabilis* and *E. cloacae*, were undetected by our molecular approach, but grew only in the clinician-ordered anaerobic blood culture bottles, and not in the aerobic blood culture bottles; the research blood samples were only inoculated into aerobic blood culture bottles. The remaining 3 cases had multiple sets of culture-confirmed blood culture bottles drawn either before or after our pair of bloods were obtained (data not shown).

Four organisms detected by PCR/Pyro but not isolated by standard culture included 1 each of *S. aureus*, Group B *Streptococcus* spp., *K. pneumoniae* and *P. aeruginosa*. Medical records were examined for the 4 enrolled participants in whom an organism was detected and identified by PCR/pyrosequencing using both the Universal and target specific protocols but whose matching clinical culture showed no evidence of growth after 5 days. Table 4 illustrates the clinical and/or lab-based evidence found for these 4 discordant cases, along with the Ct values for the target specific PCR results. For each of these 8 h time point aliquots tested, the Ct values generated ranged from <12 to 27.6, with a median of 14.4. This data were consistent with high levels of target DNA in the extracted specimens. In one case, the same bacteria identified in the research sample by PCR/Pyrosequencing (*P. aeruginosa*) were also isolated from urine (>100,000 CFU/ml) and sputum cultures collected on the same day as the clinical and research blood cultures. In 2 other cases, research sample results matched the clinical blood culture results from the same patients collected on subsequent days.

Discussion

Several molecular-based platforms including real-time PCR [19, 20], PNA FISH [21, 22], Verigene (Nanosphere Inc., Northbrook, IL) [23], FilmArray (BioFire, Salt Lake City, UT) [24] and MALDI-TOF MS [25, 26] have been used to successfully detect and identify microorganisms associated with BSI, however these assays all require fluid from positive blood culture bottles as starting material. In contrast, this study illustrated our success in combining real-time PCR and Pyrosequencing to detect and identify bacteria in incubating blood culture fluid prior to growth being detected in culture. The results described here could have broad application to the general adult population being evaluated for BSI.

Strengths of this study included the fact that participants enrolled in this study were not pre-selected for a specific disease condition or particular bacterial infection when evaluating this molecular approach. Additionally, participants were distributed evenly between the ICU and ED and therefore represented both hospital- and community-acquired BSI. This assumption was supported by the fact that 100% of the *S. pneumoniae* isolates, a common community-acquired pathogen, were from blood cultures of ED participants, while the majority of *Enterococcus* sp. (88%) and *Pseudomonas aeruginosa* (75%) were isolated from ICU patients. We also showed how our molecular approach was able to provide valuable

information for 4 culture-negative specimens; in 3 of these cases, the identical microorganism was isolated in subsequent blood cultures and/or urine/sputum cultures obtained on the same day.

Because the molecular approach was used in conjunction with incubating blood culture bottles, it does not eliminate the need for culture, but rather permitted significantly faster results to be generated in the majority of the culture-confirmed cases of BSI sooner than conventional culture. Having results sooner could enable clinicians to consider tailoring the antibiotics given from two broad-spectrum antibiotics to cover both Gram-positive organisms and Gram-negative organisms to a single, more narrow-spectrum antibiotic based on the hospital's antibiogram.

The major limitation of this study was its labor-intensive nature, requiring manual extraction of DNA from culture fluids followed by lengthy manual preparation of the biotinylated PCR amplicon for Pyrosequencing. From start to finish, DNA extraction, PCR and Pyrosequencing steps required 5.5 h to 6 h to complete, with roughly half of that time requiring technical effort. Automating the two sample preparation steps would greatly diminish the hands-on time of this approach.

Another limitation was the fact that this study did not screen for antibiotic resistance profiles of the microorganisms detected. This is an important consideration that needs to be included going forward in order for molecular-based approaches to be most useful to the clinicians treating patients with suspected BSI.

Lastly, detecting certain combinations of polymicrobial infection could be problematic using this approach because it is not possible to differentiate two different bacteria from the same broad classification (e.g. *Staphylococcus* spp., or *Streptococcus* spp. or enteric gram-negative rod). Additionally, detecting the organism present at much lower concentration in a polymicrobial infection, even if different can be difficult (data not shown).

In conclusion, this study demonstrated that over 90% of all cases of culture-confirmed BSI were accurately identified using this molecular approach, with results being available in ~14 hours. Ideally, each incubating blood culture bottle would be screened once using this approach, roughly after 8 hours of pre-enrichment. However, in many labs, one may only have molecular testing performed on first shift, or lack adequate staff to complete this additional screening in a timely manner. This would mean that a portion of the incubating blood culture fluids would in fact incubate longer than 8 hours before they are screened by PCR and Pyrosequencing, which in turn lengthens the average turn-around-time to obtain results to a time closer to when the automated instrument detects growth in incubating blood culture bottles. However, one must remember that even then the molecular approach would provide bacterial identification in the same amount of time that the culture-based approach would provide only the Gram stain result. In summary, before this type of rapid molecular approach for screening incubating blood culture fluids could be implemented within clinical laboratories, one needs to consider hours of operation, staffing levels, additional costs incurred when most specimens are culture negative, workflow logistics that would arise, and

lastly whether the results generated can be used in conjunction with antimicrobial stewardship efforts.

Acknowledgments

Funding

This work was supported by the National Institute for Allergy and Infectious Diseases, National Institutes of Health [R01 AI073342 to J.A.J.].

References

1. Klouche M. Rapid diagnostic tests to detect pathogenic microorganisms. *Clin Chem Lab Med*. 2008; 46:885–887. [PubMed: 18624613]
2. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis*. 2004; 39(3):309–317. [PubMed: 15306996]
3. Laupland KB, Gregson DB, Flemons WW, Hawkins D, Ross T, Church DL. Burden of community-onset bloodstream infection: a population-based assessment. *Epidemiol Infect*. 2007; 135:1037–1042. [PubMed: 17156500]
4. Dalager-Pedersen M, Sogaard M, Schonheyder HC, Nielsen H, Thomsen RW. Risk for myocardial infarction and stroke after community-acquired bacteremia: a 20-year population-based cohort study. *Circulation*. 2014; 129(13):1387–96. [PubMed: 24523433]
5. Anderson DJ, Moehring RW, Sloane R, Schmader KE, Weber DJ, Fowler VG Jr, Smathers E, Sexton DJ. Bloodstream infections in community hospitals in the 21st century: a multicenter cohort study. *PLoS One*. 2014; 9(3):e91713. [PubMed: 24643200]
6. Retamar P, López-Prieto MD, Nátera C, et al. Reappraisal of the outcome of healthcare-associated and community-acquired bacteremia: a prospective cohort study. *BMC Infect Dis*. 2013; 13:344. [PubMed: 23883281]
7. O'Grady NP, Barie PS, Bartlett JG, Bleck T, Carroll T, Kalil AC, Linden P, Maki DG, Nierman D, Pasculli W, Masur H. Guidelines for evaluation of new fever in critically ill adult patients: 2008 update from the American College of Critical Care Medicine and the Infectious Disease Society of America. *Crit Care Med*. 2008; 36:1330–1349. [PubMed: 18379262]
8. Ntusi N, Aubin L, Oliver S, Whitelaw A, Mendelson M. Guideline for the optimal use of blood cultures. *S Afr Med J*. 2010; 100:839–843. [PubMed: 21414279]
9. Jordan JA, Durso MB. Real-time polymerase chain reaction for detecting bacterial DNA directly from blood of neonates being evaluated for sepsis. *J Mol Diagn*. 2005; 7:575–581. [PubMed: 16258155]
10. Jordan JA, Butchko AR, Durso MB. Use of pyrosequencing of 16S rRNA fragments to differentiate between bacteria responsible for neonatal sepsis. *J Mol Diagn*. 2005; 7:105–110. [PubMed: 15681481]
11. Pence MA, TeKippe EM, Burnham CD. Diagnostic assays for identification of microorganisms and antimicrobial resistance determinants directly from positive blood culture broth. *Clin Lab Med*. 2013; 33:651–684. [PubMed: 23931843]
12. Bauer KA, Perez KK, Forrest GN, Goff DA. Review of rapid diagnostic tests used by antimicrobial stewardship programs. *CID*. 2014; 59(Suppl 3):S134–S145.
13. Jordan JA, Jones-Laughner J, Durso MB. Utility of pyrosequencing in identifying bacteria directly from positive blood culture bottles. *J Clin Microbiol*. 2009; 47(2):368–372. [PubMed: 19091813]
14. Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J, Collins CD, Nagel JL. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. *CID*. 2013; 57:1237–1245.

15. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA, Peterson LE, Musser JM. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Arch Pathol Lab Med*. 2013; 137:1247–1254. [PubMed: 23216247]
16. Moore MS, McCann CD, Jordan JA. Molecular detection of culture-confirmed bacterial bloodstream infections with limited enrichment time. *J Clin Microbiol*. 2013; 51(11):3720–3725. [PubMed: 23985915]
17. Jordan JA, Durso MB, Butchko AR, Jones JG, Brozanski BS. Evaluating the near term infant for early onset sepsis: progress and challenges to consider with 16S rDNA polymerase chain reaction testing. *J Mol Diagn*. 2006; 8:357–363. [PubMed: 16825509]
18. Lewis JA. Statistical principles for clinical trials (ICH E9): an introductory note on an international guideline. *Stat Med*. 1999; 18:1903–1904. [PubMed: 10440877]
19. Wang HY, Kim S, Kim J, Park SD, Uh Y, Lee H. Multiplex real-time PCR assay for rapid detection of methicillin-resistant staphylococci directly from positive blood cultures. *J Clin Microbiol*. 2014; 52(6):1911–20. [PubMed: 24648566]
20. Wang HY, Kim S, Kim H, et al. Real-time PCR TaqMan assay for rapid screening of bloodstream infection. *Ann Clin Microbiol Antimicrob*. 2014; 13(1):3. [PubMed: 24393579]
21. Rigby S, Procop GW, Haase G, Wilson D, Hall G, Kurtzman C, Oliveira K, Von Oy S, Hyldig-Nielsen JJ, Coull J, Stender H. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. *J Clin Microbiol*. 2002; 40(6):2182–2186. [PubMed: 12037084]
22. Deck MK, Anderson ES, Buckner RJ, Colasante G, Davis TE, Coull JM, Crystal B, Della-Latta PD, Fuchs M, Fuller D, Harris W, Hazen K, Klimas LL, Lindao D, Meltzer MC, Morgan M, Shepard J, Stevens S, Wu F, Fiandaca MJ. Rapid detection of *Enterococcus* spp. direct from blood culture bottles using *Enterococcus QuickFISH* method: a multicenter investigation. *Diagn Microbiol Infect Dis*. 2014; 78(4):338–342. [PubMed: 24439447]
23. Beal SG, Ciurca J, Smith G, John J, Lee F, Doern CD, Gander RM. Evaluation of the Nanosphere Verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. *J Clin Microbiol*. 2013; 51(12):3988–3992. [PubMed: 24048531]
24. Altun O, Almuhayawi M, Ullberg M, Ozenci V. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. *J Clin Microbiol*. 2013; 51(12):4130–4136. [PubMed: 24088863]
25. Wang MC, Lin WH, Yan JJ, Fang HY, Kuo TH, Tseng CC, Wu JJ. 2013 Dec 30. Early identification of microorganisms in blood culture prior to the detection of a positive signal in the BACTEC FX system using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *J Microbiol Immunol Infect*. S1684–1182(13)002000–4
26. Machen A, Drake T, Wang YF. Same Day Identification and Full Panel Antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. *PLoS One*. 2014; 9(2):e87870. [PubMed: 24551067]

Highlights

Culture fluids screened by PCR after 8 h had >90% sensitivity compared to culture.

MDx testing provided results more rapidly than automated blood culturing.

Lab logistics must be considered before implementing MDx testing for BSI.

Table 1

Demographic Characteristics of Study Participants

	Emergency Department (<i>n</i> = 446)	Intensive Care Unit (<i>n</i> = 472)	Combined Total (<i>N</i> = 918)
Characteristic	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Age; mean [range]	55 [20–103]	60 [19–99]	57 [19–103]
Sex			
Male	249 (55.8)	267 (56.6)	516 (56.2)
Female	194 (43.5)	205 (43.4)	399 (43.5)
Not Reported	3 (0.7)	0 (0.0)	3 (0.3)
Race/Ethnicity			
Black	278 (62.3)	254 (53.8)	532 (58.0)
White	113 (25.3)	147 (31.1)	260 (28.3)
Other	23 (5.2)	28 (5.9)	51 (5.6)
White Hispanic	18 (4.0)	11 (2.3)	29 (3.2)
Unknown	2 (0.5)	19 (4.0)	21 (2.3)
Not Reported	5 (1.1)	7 (1.5)	12 (1.3)
Asian	3 (0.7)	5 (1.1)	8 (0.9)
Black Hispanic	4 (0.9)	0 (0.0)	4 (0.4)
American Indian	0 (0.0)	1 (0.2)	1 (0.1)

Table 2**Bacteria Isolated from Standard Blood Culture**

Organism	Emergency Department (<i>n</i> = 52)	Intensive Care Unit (<i>n</i> = 50)	Combined Total (<i>N</i> = 102)
	N (%)	N (%)	N (%)
<i>Staphylococcus</i> spp. (n=51)			
CoNS	13 (41)	19 (59)	32 (31)
MSSA	4 (31)	9 (69)	13 (13)
MRSA	2 (33)	4 (67)	6 (6)
<i>Streptococcus</i> spp. (n=28)			
GAS	1 (50)	1 (50)	2 (2)
GBS	1 (50)	1 (50)	2 (2)
GCS	1 (50)	1 (50)	2 (2)
GGG	3 (75)	1 (25)	4 (4)
<i>Enterococcus faecalis</i>	0 (0)	5 (100)	5 (5)
<i>Enterococcus faecium</i>	1 (50)	1 (50)	2 (2)
<i>Enterococcus</i> spp.	0 (0)	1 (100)	1 (1)
Viridans streptococci	5 (100)	0 (0)	5 (5)
<i>Streptococcus pneumoniae</i>	5 (100)	0 (0)	5 (5)
Enteric gram negative rods (n=17)			
<i>Escherichia coli</i>	9 (100)	0 (0)	9 (9)
<i>Klebsiella pneumoniae</i>	2 (100)	0 (0)	2 (2)
<i>Enterobacter aerogenes</i>	0 (0)	1 (100)	1 (1)
<i>Serratia marcescens</i>	0 (0)	1 (100)	1 (1)
<i>Proteus mirabilis</i>	0 (0)	1 (100)	1 (1)
<i>Providencia rettgeri</i>	1 (100)	0 (0)	1 (1)
<i>Salmonella</i> sp.	1 (100)	0 (0)	1 (1)
<i>Citrobacter</i> sp.	1 (100)	0 (0)	1 (1)
Non-fermentative GNR rods (n=5)			
<i>Pseudomonas aeruginosa</i>	1 (25)	3 (75)	4 (4)
<i>Stenotrophomonas maltophilia</i>	1 (100)	0 (0)	1 (1)
Gram positive rod (n=1)			
<i>Bacillus</i> spp. (not anthracis)	0 (0)	1 (100)	1 (1)

spp.; species, GNR; Gram-negative rods, CoNS; Coagulase-negative Staphylococci, MSSA; Methicillin-sensitive *Staphylococcus aureus*, MRSA; Methicillin-resistant *Staphylococcus aureus*, GAS; Group A Streptococcus, GBS; Group B Streptococcus, GCS; Group C Streptococcus, GGS; Group G Streptococcus.

Table 3

Contingency table comparing results of PCR/Pyro from the 8 h culture fluid aliquots to standard blood culture method.

PCR/Pyro	Culture		Total
	Positive	Negative	
Positive	<u>90</u>	4	<u>94</u>
Negative	<u>2</u>	1037	<u>1046</u>
Total	99	1041	1140

Analytical performance: % (95% CI)

Sensitivity: 90.9% (83.4–95.8%)

Specificity: 99.6% (99.0–99.9%)

PPV: 95.7% (89.5–98.8%)

NPV: 99.1% (98.4–99.6%)

Table 4

Clinical and/or laboratory findings from participants with clinical blood culture-negative, PCR/pyrosequencing-positive 8 h time point aliquot test results.

Discordant Specimen	Target-specific PCR Ct values	Pyrosequencing Identification	Participant's Symptoms, Diagnosis and Other Lab Results
1	<12	<i>S. aureus</i>	ED: Fever, chills and hemorrhagic findings consistent with a diagnosis of either food poisoning or Dengue fever from international travel. Positive Dengue fever IgM (3.80). No recent Hx of antibiotic usage.
2	<12	GBS	ED: Fever of unknown origin, shaking chills, rigors, elevated WBC count ($17.63 \times 10^3/\mu\text{l}$), elevated absolute granulocyte count ($0.11 \times 10^3/\mu\text{l}$) and positive rapid Influenza A test. GBS was isolated from clinical blood cultures drawn on subsequent day. No recent Hx of antibiotic usage.
3	<12	<i>K. pneumoniae</i>	ED: History of vomiting and diarrhea for 3 days in an HIV-positive individual. Diagnosis of gastroenteritis and UTI based on presence of neutrophils, proteinuria, ketones, blood and leukocyte esterase findings in urine. Only blood was collected for culture. KPN was isolated from clinical blood cultures drawn on subsequent day.
4	20.6	<i>P. aeruginosa</i>	ICU: Primary diagnosis of VAP and UTI. Participant was already receiving ciprofloxacin and meropenem for previous culture-positive respiratory and urine cultures. On the same day this blood culture was drawn, <i>P. aeruginosa</i> was again cultured from both sputum and urine cultures (Urine; >100,000 CFU/ml).

WBC; white blood cell, GBS; Hx; history, Group B Streptococcus, VAP; ventilator-associated pneumonia, UTI; urinary tract infection, KPN; *K. pneumoniae*, CFU; colony forming units.