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Clinical Studies

Evaluation of rapid phenotypic identification and antimicrobial susceptibility testing in a pediatric oncology center

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ABSTRACT

Identification (ID) and antimicrobial susceptibility testing (AST) remain rate limiting steps in producing actionable data for clinical care of bloodstream infections. Rapid, automated phenotypic ID and AST by fluorescent in situ hybridization and automated microscopy were used to characterize blood stream infections in a predominantly pediatric oncology patient population. Results were compared to standard of care (SOC) phenotypic methods. The Accelerate Pheno System (AXDX) had a sensitivity of 91.2% and an accuracy of 100% to the genus level for identification, and an overall categorical agreement 91.2–91.8% for susceptibility, depending on the breakpoints used. The AXDX required a mean time of 1.4 hours for identification and 6.6 hours for susceptibility testing compared to SOC, requiring 32.5 and 46.7 hours, respectively. Identification and susceptibility by rapid phenotypic methods shows a high degree of accuracy; the marked reduction in time to results may have significant implications for patient care.

1. Introduction

The detection and characterization of bloodstream infections is a fundamentally important task for the clinical microbiology laboratory. Over time, improved culture systems have reduced time to detection and improved culture yield (Endimiani et al. 2002; Vigano et al. 2002). However, identification and (particularly) antimicrobial susceptibility testing remain rate limiting steps in producing actionable data for clinical care. Earlier work showed that reducing the time for identification and susceptibility testing (ID-AST) can be associated with both lower mortality and lower cost of care (Doern et al. 1994); time to notification of bloodstream infection has been correlated with increased length of hospital stay (Beekmann et al. 2003), and appropriateness of initial antimicrobial therapy has been tied to reduced mortality rates (Kang et al. 2005). In fact, great strides have been made over the last decade in addressing these issues.

The advent of commercially available systems for rapid identification of blood stream pathogens has been seen with the availability of fluorescence in-situ hybridization (FISH), matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry, and a variety of PCR-based assays. Data has been published demonstrating impact on care, outcome, and resource utilization using such methodologies, particularly when paired with an active antimicrobial stewardship program (Box et al. 2015; Huang et al. 2013; Lockwood et al. 2016; MacVane and Nolte 2016; Messacar et al. 2016; Nagel et al. 2014; Verroken et al. 2016). MALDI-TOF is typically carried out only from culture isolates, with direct detection from positive blood culture vials remaining an off-label application in the United States (Chen et al. 2015; Huang et al. 2013; Lockwood et al. 2016; Verroken et al. 2016). Beyond this limitation remains the challenge of rapidly assessing antimicrobial susceptibility (Arena et al. 2015; Banerjee et al. 2015; Barenfanger et al. 1999; Idelevich et al. 2016; Lee and Chung 2015; Machen et al. 2014; March et al. 2015; Nimer et al. 2016; Verroken et al. 2016). MALDI-TOF has again been used for this purpose primarily in the research setting (Idelevich et al. 2016; Machen et al. 2014; March et al. 2015; Verroken et al. 2016). However, multiplexed or broad-panel PCR methods are available that include certain high-impact molecular markers of resistance (Banerjee et al. 2015; MacVane and Nolte 2016). These can be applied directly to positive blood cultures, with a rapid time to result and well-characterized performance characteristics.

Again, published data suggest that such methods can improve stewardship, cost and potentially clinical outcome (Box et al. 2015; Huang et al. 2013; Lockwood et al. 2016; MacVane and Nolte 2016; Messacar et al. 2016; Nagel et al. 2014; Verroken et al. 2016). Such methods, however, are limited to a relatively small number of molecular targets.

The phenotypic correlation of such targets is imperfect, and only a relatively small subset of microbial resistance can be detected in this

Abbreviations: AST, antimicrobial susceptibility testing; AXDX, Accelerate Pheno System; SOC, standard of care; ID-AST, identification and antimicrobial susceptibility testing; GEF, gel electrofiltration system.

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manner (Machen et al. 2014; Uno et al. 2015). Molecular mechanisms of resistance continue to evolve, and the number of drug-bug pairs with targetable markers accounting for most or all cases of resistance is limited. In turn, "conventional" AST must still be carried out in most cases of diagnostic importance to assure a high degree of sensitivity.

Such challenges have lead toward an increasing exploration of methods for rapid phenotypic characterization of resistance (Li et al. 2016; Price et al. 2014). These methods potentially obviate the need to constantly update molecular assay targets and have the potential for supplanting, rather than supplementing current AST methods, as reflex testing might not be needed to ensure sensitivity for detection of resistant organisms. Several such methodologies have been described, based on principles of phenotype microarrays (Li et al. 2016), automated microscopy tests (Metzger et al. 2014; Price et al. 2014), and pyrolysis mass spectrometry (Wilkes et al. 2005). The Accelerate Pheno™ system (Accelerate Diagnostics, Tucson, AZ) is based on the principles of FISH for identification and morphokinetic cellular analysis for antimicrobial susceptibility testing, and represents one of the first such technologies to reach commercial availability. Here we describe the performance of this system for the rapid identification and susceptibility testing of bloodstream infections in a predominantly pediatric oncology population.

2. Materials and methods

2.1. Study design

A total of 104 blood cultures were collected using BACTEC™ liquid media bottles (BACTEC™ Peds Plus/F Medium, BACTEC™ Plus Aerobic/F Medium, BACTEC™ Plus Mycolytic/F Medium, BACTEC™ Plus Anaerobic/F Medium, Becton-Dickenson, Franklin Lakes, New Jersey) between February 2016 and November 2016 at St. Jude Children's Research Hospital. Within eight hours of flagging positive on the BACTEC™ FX (Becton-Dickenson) blood culture system, aliquots of positive blood cultures were loaded on the Accelerate Pheno™ system (AXDX). (Samples were removed from BACTEC™ FX immediately upon positivity, but processed within 8 hours as per manufacturer's instructions.) Samples from patients with positive blood cultures run on the AXDX within the previous four days were excluded from the study. As most probes for the AXDX were genus level probes, analysis of identification was done to the genus level only; identification to the species level is described. Exclusions included samples with control failures, nonviable by standard of care (SOC), or off-panel ID results (Fig. 1). Polymicrobial infections were not included in the analysis, but were also described. Results from the AXDX were compared to those of standard of care methodology, primarily VITEK® MS and VITEK® 2 (bioMérieux, Marcy l'Etoile, France). Latex agglutination testing (Staphaurex® Plus, Remel, Lenexa, KS), was used to identify some *Staphylococcus* species. A few other species (*Bacillus, Corynebacterium*, etc.) were identified using BBL™ Crystal Identification Systems (Becton, Dickinson and Company). Organisms with discrepant identifications underwent 16S sequencing (D2 rDNA gene sequencing for yeast) as a reference standard method. Sequencing was performed, blinded, at Mayo Medical Laboratories, Rochester, MN. VITEK®2 was primarily used for AST testing. Daptomycin was tested using E-test (bioMérieux). Additional E-tests were used for confirming vancomycin and penicillin resistance testing (bioMérieux). All isolates producing AST results on the Accelerate system were also tested using broth microdilution as a reference standard (performed, blinded, at Accelerate Diagnostics). AST results were compared based on MIC and interpretation, with errors in the latter classified as very major, major and minor (CLSI, 2016). For samples containing more than one morphotype of the same species, all were tested, but only the most resistant minimum inhibitory concentration (MIC) for each drug was used for the analysis, as the AXDX system cannot differentiate morphotypes of the same species and only results the most resistant MIC.

2.2. Standard of care methods

VITEK®*MS* – Positive blood cultures were sub-cultured on solid media for 16–24 hours prior to identification on the VITEK®MS. A 1 μL inoculation loop was used to add cellular material to the individual spot of the Fleximass-DS slide, then Matrix DHB (+ 0.5 μL 25% Formic acid for yeast isolates) was added to the spot. Spectral data was collected and analyzed on the VITEK® analysis software, Myla® (bioMérieux).

VITEK®*2 Compact* – Positive blood cultures were sub-cultured on solid media for 16–24 hours, then used to make a saline McFarland solution of 0.5–0.63 for bacterial isolates, and 1.8–2.2 for yeast isolates. Appropriate test cards (VITEK®2 GP, VITEK®2 GN, VITEK®2 AST-XN06, VITEK®2 AST-GN69, and VITEK®2 AST-GP67) were run as per VITEK®2 operator's manual, with VITEK®Observa® software (bioMérieux).

2.3. Accelerate pheno system

The Accelerate Pheno™ system (Accelerate Diagnostics) performs identification and antimicrobial susceptibility testing (AST) of bacteria and yeast using fluorescence in situ hybridization (FISH) and automated microscopy-based, single-cell analysis, directly from blood culture media. The original concept of simultaneous identification and quantitation using automated microscopy was described for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Metzger et al. 2014) and for

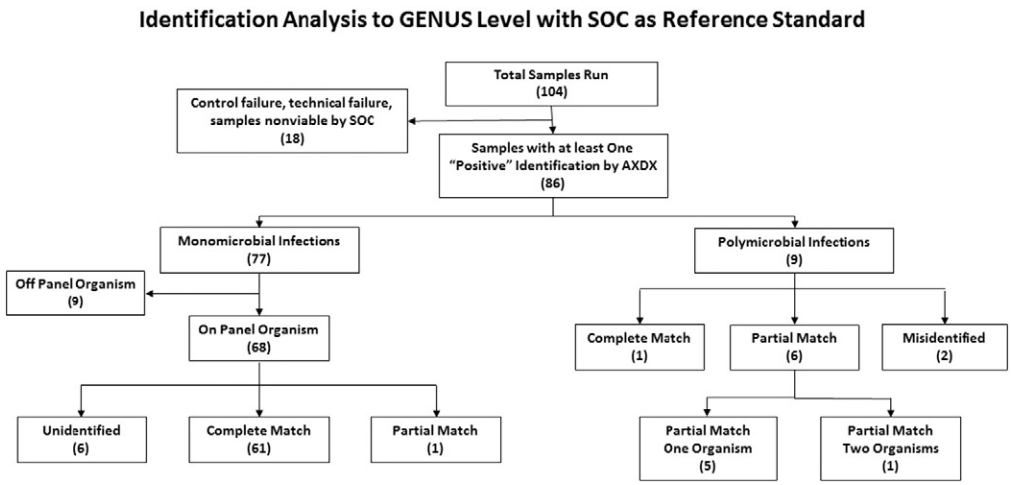

Fig. 1. Study population. A total of 104 samples including 77 monomicrobial infections and 9 polymicrobial infections after excluded samples. Excluded monomicrobial samples include those with control failures, technical failures, samples nonviable by SOC, and off panel ID results.