EDUCATIONAL COMMENTARY – RECENT DEVELOPMENTS FOR RAPID IDENTIFICATION OF BLOODSTREAM PATHOGENS

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**Florida licensees, please note: This exercise is NOT intended to fulfill your state education requirement for Molecular Pathology. It will fulfill requirements for Microbiology.**

Learning Objectives

On completion of this exercise, the participant should be able to

- define sepsis;
- explain the key aims of the Surviving Sepsis Campaign;
- discuss methods for the detection of bloodstream infections;
- list the most common organisms isolated from blood cultures; and
- compare the advantages and disadvantages of rapid detection methods vs traditional culture for identification of bloodstream pathogens.

Introduction

Sepsis is a systemic inflammatory response to a bloodstream infection. It is a major health care burden, affecting more than one million Americans annually. The mortality rate associated with bloodstream infections ranges from 20% to 50%; in addition, sepsis is responsible for health care-associated costs of $400 billion annually.¹

The Surviving Sepsis Campaign promotes evidence-based guidelines for early, goal-directed management of sepsis.² These guidelines stress the early diagnosis of the infection at the root of the patient’s sepsis and the timely administration of appropriate antimicrobial therapy as key to patient survival.

Antimicrobial therapy is a critical determinant of clinical outcome for patients with bloodstream infections. The correct initial choice of antibiotic saves more lives than any other intervention. For each hour after the onset of hypotension until initiation of effective antibiotics, there is a mean decrease of 7.6% in survival rate.³ Use of inappropriate antibiotics within the first 6 hours after recognition of septic shock is associated with a five-fold increase in mortality risk.⁴ Current practice in the critical care of a patient with suspected sepsis favors the early use of antibiotics, guided by local pathogen surveillance; usually a broad-spectrum, high-potency antibiotic is initially used to ensure all potential pathogens are addressed.² The widespread use of broad-spectrum antibiotics, however, is a major cause of the emergence of
drug-resistant organisms and the rising rates of opportunistic infections, including *Clostridium difficile* and yeast. Therefore, the Surviving Sepsis Campaign guidelines advocate early de-escalation of antimicrobial therapy to reduce this problem.²

Rapid and accurate identification of the microorganism responsible for the sepsis is crucial to initiate pathogen-specific antimicrobial therapy and decrease mortality rates. Furthermore, rapid identification methods may provide antimicrobial stewardship programs with the information needed to de-escalate antibiotic therapy and slow the emergence of antibiotic-resistant strains of pathogens. Timely and accurate reporting of microbiologic data from positive blood cultures improves clinical outcomes and reduces health care costs.⁵⁻⁹

New diagnostic technologies significantly reduce the time required to identify the pathogens that cause bloodstream infections. This commentary will review the current diagnostic technologies available for rapid identification of bloodstream pathogens.

**Use of Blood Cultures in Sepsis Diagnosis**

Blood cultures are central to the diagnosis of sepsis.² Positive results on culture guide antimicrobial therapy once the pathogen has been identified and antimicrobial susceptibility testing has been performed. Blood cultures are considered the criterion standard in the diagnosis of sepsis. In addition, cumulative surveillance data from positive blood cultures at an institution provides an antibiogram on which clinicians can base empiric antimicrobial treatment.²

Many different microorganisms can cause bloodstream infections, including bacteria (bacteremia) and fungi (fungemia). A large multicenter study found that of hospital-acquired bloodstream infections in the United States, 65% of cases resulted from gram-positive bacteria, 25% from gram-negative bacteria, and 10% from fungi.¹⁰ The most commonly isolated pathogens in this study were coagulase-negative *Staphylococcus* species (31%), *Staphylococcus aureus* (20%), and *Enterococcus* species (9%). Gram-negative bacteria frequently detected in blood cultures are *Escherichia coli* (27%), *Pseudomonas aeruginosa* (15%), and *Klebsiella pneumoniae* (7%).¹¹ Nosocomial fungal infections are mostly caused by *Candida* species.¹²

A number of clinical and technical factors play a role in isolating the pathogen from a positive blood culture. Of those, a preanalytic factor, the volume of blood drawn for the sample, is the most critical for detecting a bloodstream infection. The number of organisms present in adult bacteremia is low, often 1 to 100 colony-forming units per mL of blood.¹³ A direct relationship has been demonstrated between the
volume of blood collected and the detection rate of bloodstream infections. The standard collection practice for adult patients is to collect at least two culture bottles (aerobic and anaerobic), with 8 to 10 mL of blood each, from a single venipuncture. Two to three sets of blood cultures collected via separate venipunctures increases sample volume and pathogen identification, in addition to helping differentiate a bloodstream infection from contamination.

A blood culture can become contaminated through the collection process itself, producing a false-positive result. Documented rates of contamination vary between institutions and range from 0.6% to 6%. Duplicate blood culture sets are primarily utilized to recognize contamination vs. a bloodstream infection.

False-negative blood cultures can result from the use of prophylactic antibiotics or antifungal agents, or administration of these therapies before blood for culture has been drawn from the patient. Despite techniques aimed at neutralizing the antimicrobial substances present in a blood sample, the sensitivity of blood cultures decreases greatly when drawn after the initiation of antimicrobial therapy. In addition, certain fastidious and slow-growing organisms, including fungi, that can cause bloodstream infections may not be recovered.

Test parameters that guide management of sepsis in patients include the detection and identification of the pathogen and the antimicrobial susceptibility results. The gold standard method for pathogen identification in bloodstream infections has traditionally been culturing patient blood in an automated continuous monitoring system, followed by Gram staining, subculturing on agar plates, biochemical testing, and antimicrobial susceptibility testing once the blood culture has flagged positive. General principles and procedures are described in the Clinical and Laboratory Standards (CLSI) guidelines.

Traditional culture-based methods to detect and identify microorganisms can take 2 to 3 days from initial sampling of the blood culture, or much longer for a fastidious organism. Subculturing from a positive blood culture takes 18 to 24 hours. Pathogen identification requires biochemical testing, which, depending on the microorganism, can add 24 hours or more. Gram staining and rapid biochemical tests can provide an initial diagnosis of the etiologic agent, but detailed pathogen identification and susceptibility testing can take up to several days and cause a significant delay in time to results.

**Rapid Identification of Bloodstream Pathogens**

Newer technologies increase the speed of diagnosis and improve sensitivity in identification of bloodstream pathogens. When combined with antimicrobial stewardship programs, these new methods improve clinical outcomes and reduce health care expenditures. Technologies for rapid identification of
bloodstream pathogens can be separated into two types of rapid testing: from positive cultures or direct blood samples. This commentary focuses on the methods that can be used after a positive blood culture, because those are the methods available in the U.S.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a molecular diagnostic application that is becoming more common in the clinical microbiology laboratory. This method detects the nucleic acid of a microorganism to identify the isolate. Diagnostic PCR assays can be fully or partially automated.

A low multiplex, fully automated PCR test for identification of \textit{S aureus} and methicillin resistance in positive blood cultures containing gram-positive cocci in clusters was developed because \textit{S aureus} is the cause of 20% of nosocomial bloodstream infections in the United States.\textsuperscript{10} Empiric treatment with vancomycin is typically prescribed for patients with suspected \textit{S aureus} bacteremia because of the increasing prevalence of methicillin-resistant \textit{S aureus} (MRSA) infections. However, for treating methicillin-susceptible \textit{S aureus} (MSSA) bacteremia, \(\beta\)-lactam antibiotics are more effective than vancomycin.\textsuperscript{15-17} Therefore, a rapid test that detects \(\beta\)-lactam susceptibility in \textit{S aureus} isolates in a positive blood culture would lead to directed therapy faster and improve patient outcomes. This rapid multiplex real-time assay detects two genes: the staphylococcal protein A (\textit{spa}) gene, which is specific for \textit{S aureus}; and the \textit{mecA} gene, which encodes the penicillin-binding protein 2a that is resistant to \(\beta\)-lactam antibiotics (except those with anti-MRSA activity). The turnaround time for this assay is less than one hour from the time a blood culture turns positive with gram-positive cocci in clusters. The MRSA/SA test has 98.3% to 100% sensitivity and 98.6% to 99.4% specificity for MSSA and MRSA identification.\textsuperscript{18}

Recently developed, highly multiplexed PCR tests identify large panels of pathogens in addition to specific antibiotic-resistance genes for bloodstream infections. Because 90% of bloodstream infections are caused by approximately 25 pathogenic organisms, rapid identification tests capable of identifying 20 to 30 targets can identify the majority of bloodstream infections.\textsuperscript{19}

A multiplex PCR-based assay that uses nested multiplex PCR to identify 19 bacterial pathogens, five yeast, and three antimicrobial resistance markers directly from positive blood cultures has been developed. This rapid test panel includes 27 targets including staphylococci, streptococci, Enterobacteriaceae, \textit{Enterococcus}, \textit{Acinetobacter}, \textit{Neisseria meningitidis}, \textit{P aeruginosa}, \textit{Listeria}, and five \textit{Candida} species. In addition to organism identification, the test detects the presence or absence of \textit{mecA}, \textit{vanA/B}, and \textit{K pneumoniae} carbapenemases genes. Only one test can be performed at a time, and turnaround time for the assay is one hour, with less than five minutes of hands-on time required for
set-up. Sensitivities for organism identification are greater than 90% and for resistance markers, 100%.20,21

Microarray-based assays have recently been developed for clinical laboratory detection of bloodstream pathogens. A microarray-based assay for detecting a panel of gram-positive bacteria and antibiotic-resistance gene targets in blood cultures has been approved by the US Food and Drug Administration (FDA), and another test for identifying gram-negative bacteria in blood cultures is currently pending approval.22 These two tests are fully automated, and the turnaround time is 2.5 hours.

The gram-positive microarray test panel can identify 12 gram-positive bacterial pathogens and three genetic markers of antibiotic resistance directly from positive blood cultures.22 The test can identify *S aureus*, *Staphylococcus lugdunensis*, *Staphylococcus epidermidis*, other *Staphylococcus* species, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Listeria* species. It also identifies the *mecA* and *VanA/B* resistance genes. For identification of the microorganisms, sensitivity ranges between 92.6% and 100% and specificity from 95.4% to 100%. For the resistance markers, sensitivity ranges from 98.6% to 100% and specificity from 94.3% to 100%.22

The gram-negative panel has nine bacterial targets, which include *P aeruginosa*, *Acinetobacter* species, and select *Enterobacteriaceae* organisms. It also detects six resistance markers, including *K pneumoniae* carbapenemase and New Delhi metallo-β-lactamase genes.

**Peptide Nucleic Acid Fluorescence In Situ Hybridization Molecular Stains**

Peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) stains have been used in rapid diagnosis of bloodstream infections and direct identification of selected pathogens from positive blood cultures for more than ten years. Several of these fluorescently-labeled oligonucleotide probes specific for ribosomal RNA have been approved by the FDA and can identify more than 95% of pathogens commonly found in bloodstream infections.23,24 Depending on the assay, the PNA-FISH tests have turnaround times of 30 to 145 minutes and hands-on times of 5 to 15 minutes.23,24 One drawback of FISH assays is that each assay tests for a small number of pathogens, so multiple FISH reactions are required to cover all possible bloodstream pathogens. PNA-FISH kits are currently available to differentiate between *S aureus* and coagulase-negative staphylococcus; *E faecalis* and other *Enterococcus* species; *E coli*, *K pneumoniae*, and *P aeruginosa*; and *Candida* species. Depending on the assay, sensitivity and specificity range from 96% to 100%.23-24
Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a new approach to microbial identification in the clinical microbiology laboratory. This analyzer ionizes biomolecules through an electric field and separates them according to their mass to charge (m/z) ratio. The m/z ratio of each biomolecule is then compared with a reference database to identify the microorganism. It has been used in laboratories to detect gram-negative, gram-positive, and fungal pathogens. MALDI-TOF is currently utilized to identify colonies on an agar plate, but several studies have shown promising data for MALDI-TOF analysis on direct positive blood cultures. MALDI-TOF identified the pathogen in approximately 75% of positive blood cultures but it does have limitations. This method exhibits reduced sensitivity for gram-positive bacteria, and for bloodstream infections with more than one pathogen.\textsuperscript{25,26} Identification with this method takes approximately one hour, depending on the number of samples analyzed. MALDI-TOF is also being evaluated for identifying antibiotic resistance markers, but this application is still in the developmental stage.

Conclusion

Bloodstream infections are associated with high mortality and increased health care costs. Early diagnosis of a patient’s sepsis is important and depends on several factors, including recognition of the disease and laboratory tests. Rapid and accurate identification of the specific microorganism is critical for initiating pathogen-specific antimicrobial therapy.

Culture-based methods are still the gold standard for detecting and identifying bloodstream pathogens, but this method is time consuming, sometimes taking several days for final identification. Newer technologies have been developed for rapid identification of bloodstream pathogens. These assays can detect a bloodstream pathogen and specific antimicrobial-resistance genes within a few minutes to hours of a positive blood culture. Rapid identification can lead to earlier initiation of appropriate antimicrobial therapy, shorter hospital stays, and improved outcomes.

References


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