Bloodstream Infections
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ABSTRACT Bacteremia and sepsis are conditions associated with high mortality and are of great impact to health care operations. Among the top causes of mortality in the United States, these conditions cause over 600 fatalities each day. Empiric, broad-spectrum treatment is a common but often a costly approach that may fail to effectively target the correct microbe, may inadvertently harm patients via antimicrobial toxicity or downstream antimicrobial resistance. To meet the diagnostic challenges of bacteremia and sepsis, laboratories must understand the complexity of diagnosing and treating septic patients, in order to focus on creating algorithms that can help direct a more targeted approach to antimicrobial therapy and synergize with existing clinical practices defined in new Surviving Sepsis Guidelines. Significant advances have been made in improving blood culture media; as yet no molecular or antigen-based method has proven superior for the detection of bacteremia in terms of limit of detection. Several methods for rapid molecular identification of pathogens from blood cultures bottles are available and many more are on the diagnostic horizon. Ultimately, early intervention by molecular detection of bacteria and fungi directly from whole blood could provide the most patient benefit and contribute to tailored antibiotic coverage of the patient early on in the course of the disease. Although blood cultures remain as the best means of diagnosing bacteremia and candidemia, complementary testing with antigen tests, microbiologic investigations from other body sites, and histopathology can often aid in the diagnosis of disseminated disease, and application of emerging nucleic acid test methods and other new technology may greatly impact our ability to bacterial and septic patients, particularly those who are immunocompromised.

INTRODUCTION
Bloodstream infections (BSIs) and sepsis rank among the top reasons for human mortality for hospitalized patients. The full breadth of sepsis syndromes are particularly dangerous to any immunocompromised patient. Sepsis is one of the oldest of medical syndromes and dates back to the time in which Hippocrates characterized it as a clinical syndrome (1). Worldwide estimates of sepsis prevalence exceed 19 million cases per year, with over 750,000 in the United States (2). While only 2% of patients are admitted to the hospital with severe sepsis, they represent at least 10% of all ICU admissions in the U.S. (3).

In this article, we will review the human impact of BSIs in the immunocompromised host, as well as standard definitions of clinical criteria, symptoms, predisposing factors linked to the development of bacteremia and fungemia, and common modes of bacterial pathogenesis. We will review current literature to describe the presence of nonculturable BSIs, such as those caused by some viruses, parasites, and other nonculturable microbes. We will also review key aspects of the Surviving Sepsis Campaign guidelines (4) and approaches for diagnosis, therapy, and resuscitation from the septic event. Finally, we will highlight key laboratory roles and responsibilities to support the diagnosis of sepsis as part of today’s interdisciplinary health care teams, detail the new Laboratory Medicine Best Practice guidelines for blood culture collection (5), and summarize the meta-analysis describing the impact of rapid diagnostics that identify bloodstream pathogens (6). We will show that expansion of the laboratory’s role, rapid diagnostics, and understanding of the complexities related to diagnosing and treating sepsis can not only improve patient survival, but also can limit the financial impact of sepsis on health care systems worldwide.
PATHOPHYSIOLOGY OF INFECTIONS
Pathophysiology of Sepsis

Due to the widespread immune response (2) when the human immune system is faced with infectious agents in the bloodstream, symptoms are widely variable and include fever, chills, hypotension, hypothermia (especially in the elderly), diaphoresis, apprehension, change in mental status, tachypnea, tachycardia, hyperventilation, reduced vascular tone, and the possibility of organ dysfunction. Hematologic findings include neutrophilic leukocytosis, thrombocytopenia, toxic granulations of neutrophils, or disseminated intravascular coagulation. Other metabolic findings include respiratory alkalosis; renal signs, such as acute tubular necrosis, oliguria or anuria; gastrointestinal signs such as upper gastrointestinal bleeding; cholestatic jaundice, increased transaminase levels, or hypoglycemia.

Despite modern advances, sepsis is one of the leading causes of death worldwide (8). If left untreated, sepsis can transform to septic shock (sepsis complicated by either hypotension that is refractory to fluid resuscitation or by hyperlactatemia), and eventually to organ dysfunction and death (3). There are several consensus definitions that define sepsis. For all conditions, the diagnosis primarily relies upon clinical recognition of symptoms, later confirmed by laboratory testing (4).

1. Bacteremia is defined as the presence of viable bacteria in the bloodstream.
2. Systemic inflammatory response syndrome is a systemic inflammatory response to bodily harm (e.g., from infection, burns, or trauma) that requires some of the following conditions:
   a. Fever or hypothermia
   b. Tachycardia
   c. Tachypnea
   d. Leukocytosis or leukopenia
   e. Elevated C-reactive protein
   f. Elevated plasma procalcitonin
   g. Altered mental status
   h. Significant edema or positive fluid balance
   i. Hyperglycemia
   j. Arterial hypotension
   k. Organ dysfunction
   l. Hyperlactatemia
   m. Decreased capillary filling or mottling
Non-specific causes of inflammation can cause systemic inflammatory response syndrome and must be considered in assessment of patients who present with inflammatory symptoms.

3. Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of infection. Sepsis includes organ dysfunction (i.e., low blood pressure, reduced urine output, intestinal paralysis, reduced circulation, increased creatinine, reduced oxygen in the blood, elevated lactate, elevated bilirubin, abnormal coagulation, or metabolic acidosis).
4. Septic shock is a condition in which the patient exhibits refractory arterial hypotension or hypoperfusion despite adequate intravascular fluid resuscitation. Hypoperfusion may be manifested as lactic acidosis, oliguria, or mental status changes.

Management of sepsis and septic shock is generally driven by clinical care bundles, detailed in the International Surviving Sepsis Campaign (4). Early recognition of the disease is essential, and treatment includes stabilization of blood pressure (early fluid management and vasopressors) and treatment of infection (immediate administration of antibiotics), driven by evidence that for every hour a septic patient is not appropriately treated, mortality increases by approximately 7% (9, 10). Ideally, blood cultures are obtained from the patient prior to the administration of antimicrobials, and empiric therapy with broad-spectrum antimicrobial agents is initiated within the first hour of diagnosis.

Along with administration of antimicrobial therapy, attention is keenly focused on identifying and treating any ongoing foci of infections and/or reactivation of latent infections, a process known as source control. Identifying the source of the infection (urinary tract, respiratory, wound, etc.) is crucial to proper management. Control measures are meant to reduce or eliminate the source of infection, such as an infected device (e.g., hardware, catheters, lines, etc.), removal or drainage of an abscess, or debridement of infected tissue. Once the source of the infection is identified, microbial cultures can be obtained and targeted therapy can be administered based on the culture results. Other key definitions related to BSIs include the following:

- **Endocarditis** is an infection of a natural or prosthetic heart valve.
- **Nosocomial BSI** is defined as a BSI occurring >48 h following admission to hospital or <48 h if the patient has been hospitalized within the previous 2 weeks (11).
- **Community-acquired BSIs** are present on admission (POA).
• Endogenous asymptomatic bacteremia (candidemia, fungemia) represents bacteremia that originates inside the host but that is not yet causing classic symptoms of BSI.
• Acute bacteremia (candidemia, fungemia) indicates the presence of microbes in the bloodstream with concomitant symptoms of bacteremia.
• Transient bacteremia represents sporadic and commonly asymptomatic bacteremia that is thought to occur under rare but normal circumstances. It occurs in a wide variety of procedures and manipulations, particularly those associated with mucous membrane trauma. It may also occur with such daily functions as tooth brushing and bowel movements. These intermittent and brief forms of bacteremia are especially common in tooth extraction and other dental procedures and can occasionally give rise to infective endocarditis in the susceptible patient.
• Persistent bacteremia is defined as bacteremia sustained for 3 days or longer, contributing to many Staphylococcus aureus bacteremia episodes and increasingly recognized among hospitalized patients.

Several scoring systems are commonly used to assess the severity and prognosis of a septic event. The Sequential Organ Failure Assessment (SOFA) score predicts intensive care unit (ICU) mortality based on laboratory results and clinical data. The Acute Physiological Assessment and Chronic Health Evaluation Score (Apache II Score) estimates ICU mortality based on laboratory values and patient signs, taking both acute and chronic disease into account. The use of the new “quickSOFA” (qSOFA) scores is described in the “Risk Factors and Outcomes” section of this article.

The Systemic Inflammatory Response Syndromes and Sepsis
BSIs can lead to sepsis, in the following manner. At onset, a bacterial cell triggers the host immune response. For Gram-negative pathogens, the bacterial endotoxin, a lipopolysaccharide from the cell wall, initiates the human inflammatory response. For Gram-positive pathogens, lipoteichoic acid, peptidoglycan, and extracellular products (toxins) trigger the response. An inflammatory response follows, mounting vascular, cellular, and chemical responses designed to stop injury with edema, which dilutes toxins, and with phagocytosis, which removes bacteria and cell debris.

The Role of the Human Immune Response in Sepsis
Classically immunocompromised patients have high mortality and can present with a wide variety of offending pathogens and common or unusual infections. While immunocompromised patients may sometimes die of infection due to their lack of ability to mount an effective immune response, in an immunocompetent host, the immune response is actually implicated in the disease symptoms; therefore, patient survival is dependent on whether the patient immune response to microbial pathogens is sufficient and can be ultimately balanced to regain immune homeostasis (12).

The pathogenesis of sepsis involves proinflammatory mediators, anti-inflammatory mediators, and vaso-inflammatory mediators. There are several important inflammatory responses, which are linked to several different human cells that respond to bacterial invasion: 1) phagocytes (monocytes/macrophages, neutrophils, eosinophils); 2) mast cells, which are induced by lipopolysaccharide and complement (C3a and C5a) to release immune mediators; and 3) natural killer cells, which cause lysis of target cells and production of cytokines, like gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), in a process called cell-mediated cytotoxicity. Other physiological changes include reduced protein C activity, microplugging of vessels, cellular necrosis (ischemic injury), the inhibition of fibrinolysis, apoptosis, leukocyte-mediated tissue injury, endothelial dysfunction, and cytopathic hypoxia (12).

Sepsis is a complicated and variable condition and has a prolonged host immune response. During the immune response, human proinflammatory responses, aimed at eliminating pathogens, combine with the anti-inflammatory responses, aimed at limiting tissue injury. Unfortunately, the same immune responses contribute to concurrent organ injury and susceptibility to secondary infections, respectively (12), and can vary widely during the clinical course of the disease. The immune response depends on various factors, including the causative pathogen (microbial density and relative virulence), the host (genetic characteristics, immunologic condition, comorbidities, and other concurrent infection), and the therapeutic regimen(s) (Fig. 1).

The Host’s Innate Immune System
As part of the host’s innate immunity, pathogens activate immune cells by interacting with pattern-recognition receptors, which recognize pathogen-associated molecular patterns that are conserved among microbial species. Binding of pathogen-associated molecular patterns
to the human pattern-recognition receptors results in the upregulation of inflammatory gene transcription and initiation of the innate immune system. Human pattern-recognition receptors are categorized into four main classes: toll-like receptors, C-type lectin receptors, retinoic acid-inducible gene 1-like receptors, and nucleotide-binding oligomerization domain-like receptors, which partially act in protein complexes called inflammasomes (Fig. 1) (3, 13). The receptors are also capable of sensing molecules called damage-associated molecular patterns or alarmins, which are released from injured cells. Examples of alarmins include the high-mobility-group
protein B1, S100 proteins, as well as extracellular RNA, DNA, and histones (3). Since alarmins are also released during trauma and other sterile tissue injury, the prevailing concepts that describe the pathogenesis of multiple organ failure in sepsis are not intrinsically different from those found in some noninfectious critical illnesses (3).

Organ Dysfunction
Although reduced oxygen supply to human organs is clearly an important contributor to tissue damage, inflammation can also cause dysfunction of the vascular endothelium and can contribute to cell death and loss of cell integrity, giving rise to subcutaneous and body-cavity edema (14). In parallel, injured mitochondria release alarmins, which include mitochondrial DNA and formyl peptides that can activate neutrophils and cause further tissue injury (15). Oxidative stress also damages mitochondria, and other mechanisms impair cellular oxygen use. Mechanisms that contribute to oxygen reduction include hypotension, reduced red-cell deformability, and microvascular thrombosis (16).

Coagulation Abnormalities Related to Tissue Injury
Four subtypes of protease-activated receptors (PARs) form a link between inflammation and coagulation. PAR1 is particularly important; it exerts cytoprotective effects when stimulated by activated protein C or low-dose thrombin, but it exerts disruptive effects on endothelial-cell barrier function when activated by high-dose thrombin (17, 18). Because of these interactions, human coagulation is commonly altered during sepsis (3) (Fig. 2), and the disruption can lead to disseminated intravascular coagulation. In disseminated intravascular coagulation, the coagulation cascade drives excess fibrin deposition through depression of the fibrinolytic system, which triggers fibrin removal, and via activation of a transmembrane glycoprotein, which is expressed by various cell types because of impaired anticoagulant mechanisms (including the protein C system and antithrombin) (18).

Immunosuppression and Its Association with Anti-inflammatory Processes
The immune system is composed of humoral, cellular, and neuroinflammatory mechanisms that mitigate the harmful effects of the human proinflammatory cytokines and the proinflammatory response (Fig. 1). Phagocytes can switch to an anti-inflammatory phenotype, combining with regulatory T cells, myeloid-derived suppressor cells, and neural mechanisms to promote tissue repair and reduction of inflammation (3, 12, 19). By nature of the septic event itself, patients who survive early sepsis but remain under intensive care have evidence of immunosuppression, partly from reduced expression of HLA-DR on myeloid cells (20). Reduced responsiveness of leukocytes, functional impairment of splenocytes, and immunosuppression in the spleen and lungs are all associated with sepsis (12, 20). In addition, epigenetic regulation of gene expression may also contribute to sepsis-associated immunosuppression (21) and apoptosis, especially of B cells, CD4+ T cells, and follicular dendritic cells. Sepsis-associated immunosuppression has been implicated in sepsis-associated immunosuppression and death (22, 23).

Agents of Bloodstream Infections
In an immunocompromised host, the spectrum of microbes that cause BSIs has changed over the last decade, shifting from Gram-negative to Gram-positive organisms (24). Reasons for the change can be found in recent changes in clinical practice that include antimicrobial therapy, changes to immunosuppressive or chemotherapeutic regimens, and significant increases in the use of long-term indwelling devices. In addition, emerging antimicrobial resistance impacts the epidemiology of BSIs found in immunocompromised hosts (24).

Historically, patients with hematologic malignancies and febrile granulocytopenia constituted the majority of immunocompromised hosts (24, 25); however, there are currently many other types of immunocompromised hosts, including those with hematopoietic stem cell and solid organ transplants, as well as a wide variety of other immunosuppressive states, detailed later in this article. Today, many of these patients have concurrent intravascular catheters and are besieged with Gram-positive microorganisms, especially staphylococci, which have become the most frequent cause of BSIs. Additionally, BSIs caused by nontuberculous mycobacteria (NTM) and non-Candida yeasts are on the rise. Unfortunately, blood cultures are not a fully reliable diagnostic test—in some populations, only one-third of sepsis cases result in positive blood cultures (3).

Gram-positive and Gram-negative bacteria
Severe sepsis can be the result of either community-acquired or health care-associated infections, but it can also follow dissemination of primary infections. Primary infections such as pneumonia, urinary tract, and intra-abdominal infections are often the source of BSIs and can lead to sepsis and septic shock (8). Common causative bacterial agents of BSIs include Staphylococcus
FIGURE 2  Organ failure in severe sepsis and dysfunction of the vascular endothelium and mitochondria. Sepsis is associated with microvascular thrombosis caused by concurrent activation of coagulation (mediated by tissue factor) and impairment of anticoagulant mechanisms as a consequence of reduced activity of endogenous anticoagulant pathways (mediated by activated protein C, antithrombin, and tissue factor pathway inhibitor), plus impaired fibrinolysis owing to enhanced release of plasminogen activator inhibitor type 1 (PAI-1). The capacity to generate activated protein C is impaired at least in part by reduced expression of two endothelial receptors: thrombomodulin (TM) and the endothelial protein C receptor. Thrombus formation is further facilitated by neutrophil extracellular traps (NETs) released from dying neutrophils. Thrombus formation results in tissue hypoperfusion, which is aggravated by vasodilatation, hypotension, and reduced red-cell deformability. Tissue oxygenation is further impaired by the loss of barrier function of the endothelium owing to a loss of function of vascular endothelial (VE) cadherin, alterations in endothelial cell-to-cell tight junctions, high levels of angiopoietin 2, and a disturbed balance between sphingosine-1 phosphate receptor 1 (S1P1) and S1P3 within the vascular wall, which is at least in part due to preferential induction of S1P3 through protease-activated receptor 1 (PAR1) as a result of a reduced ratio of activated protein C to thrombin. Oxygen use is impaired at the subcellular level because of damage to mitochondria from oxidative stress. Reprinted from reference (3), with permission.
aureus, Staphylococcus epidermidis, Enterococcus spp., Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp.

Not only does the range of disease and pathogens vary greatly, but the way in which an individual responds to sepsis also depends on the immune condition of the host and on the pathogenic characteristics (virulence factors and bacterial load). For example, the absence of specific host immune responses such as B-cell depletion from rituximab or complement deficiency in systemic lupus erythematosus may predispose a patient to a BSI. In many instances, the underlying reason for immunosuppression provides clues to the type of microorganism causing infection. For example, a complement-depleted patient with systemic lupus erythematosus who presents with sudden onset of septic shock is at increased risk for a bloodstream infection with Neisseria meningitidis (26). Similarly, unusual pathogens such as Vibrio vulnificus may cause BSIs in patients with severe liver disease or liver transplantation. When anatomic barriers such as skin or mucosa are compromised from graft-versus-host disease or cytotoxic chemotherapy, patients are more likely to become infected with microorganisms colonizing the skin or oropharyngeal and gastrointestinal tracts. The recovery of Leptotrichia spp. or Capnocytophaga spp. from blood culture may suggest disruption of the mucosal integrity from the oropharynx or gastrointestinal tract in a granulocytopenic patient with severe mucositis.

Anaerobic bacteria

Anaerobic bacteremia is an important consideration in any patient, particularly in the immunocompromised and vulnerable populations. Anaerobic infections in the general population are most frequently related to pelvic, abdominal, and skin/soft-tissue infections, in 50 to 70%, 5 to 20%, and 5 to 20% of cases, respectively. Publications in the immunocompromised population exist but are limited in number (27–29). The Bacteroides fragilis group and Clostridium spp. are the most frequent anaerobic blood culture isolates (28), and associated conditions include cancer and malignant tumors, diabetes mellitus, post-surgery status, and advancing age. Older persons are at higher risk of mortality with anaerobic bacteremia (25 to 44%) (30, 31). In general, the risk of mortality for anaerobic BSIs is high and the prevalence of anaerobic bacteremia is increasing (30, 31). As antimicrobial resistance is also increasing among anaerobes, there is a need for more rapid and easier anaerobic antimicrobial susceptibility testing (AST) methods to be deployed.

Mycobacteria

For the diagnosis of disseminated Mycobacterium tuberculosis infections or NTM infections, such as Mycobacterium chelonae and Mycobacterium haemophilum, it is necessary to consider collection of specimens, like sputum or skin lesions, to improve diagnostic yield over that of blood cultures alone (32, 33). There are case reports of septicemia caused by M. chelonae complex, in which appropriate therapy is likely delayed, resulting in significant morbidity and multiple hospital admissions (34). Gram staining of these organisms from blood culture should not be confused with debris or with Corynebacterium spp. For immunocompromised individuals, the identification of a Gram-positive bacillus that is not able to be identified by phenotypic methods should prompt an acid-fast bacilli stain to distinguish between Bacillus spp. and the rapidly growing mycobacteria.

Likewise, rapidly growing NTM, while considered rare pathogens, can cause central line-associated BSIs and have been associated with hospital-associated outbreaks in hematologic-oncology wards. Mycobacterium mucogenicum and Mycobacterium canariense were identified in patients with acute myeloid leukemia, acute lymphocytic leukemia, and aplastic anemia. In this case report, environmental surveillance cultures detected genetically similar M. mucogenicum and M. canariense in the water supply of the hematologic-oncology ward (35).

Depending on local geography and CD4 count, HIV patients commonly present with mycobacteremial infections ranging from Mycobacterium avium-intracellulare to Mycobacterium tuberculosis (36, 37). Infections caused by NTM are rapidly emerging among all hosts with altered immune responses and are often associated with an intravascular catheter (33, 38).

Fungi

Yeasts

Candidemia is frequently observed among immunocompromised hosts receiving broad-spectrum or prolonged antibiotic therapy, parenteral nutrition, intravascular catheters, or renal failure, and in those with prolonged stays in an intensive care unit (39–41). Candida spp. rank fourth among common causes of BSIs in some groups of hospitalized patients (42, 43). Over 95% of all candidemia is caused by 5 species: C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei (43). Other yeasts include Cryptococcus spp., Trichosporon spp., Geotrichum spp., Rhodotorula spp., and Hansenula spp., all of which have emerged as important bloodstream pathogens in the immunocompromised host (44,
All yeasts, except Cryptococcus spp., are often related to the presence of indwelling devices (45).

Standard blood culture bottles will recover many medically important yeasts without the need for special fungal blood bottles (46); however, performance studies show that traditional blood cultures are able to identify medically important yeasts with only varied sensitivity, which can be as low as 38 to 80% when compared to molecular methods (47). For those reasons, special broth formulations, adapted for optimal fungal recovery, are warranted for patients at high risk for candidemia and fungemia.

Nosocomial candidiasis is a life-threatening condition in both critically ill and immunocompromised patients (48). The frequency of infection and the associated mortality have not decreased over the past two decades (48). These findings highlight the difficulties encountered when diagnosing Candida infection in high-risk patients and the challenges in administering adequate and appropriate antifungal therapy despite the introduction of several extended-spectrum triazole and echinocandin antifungal agents (48). Moreover, the criteria for initiating antifungal therapy are not completely standardized and often result in administration of antifungal therapy with the associated risks of toxicity, development of antifungal resistance, and high costs (48).

Many yeasts are emerging as new causes of disease. Kodameae ohmeri (formerly Pichia) is a yeast that is an emerging pathogen in patients with compromised immunity, like cancer patients and premature neonates, with fatal outcomes (49, 50). A rare case of fungemia due to Trichosporon mucoides in a diabetes mellitus patient adds to an emerging list of trichosporonosis infections (51). Rhodotorula spp. are emerging opportunistic pathogens found in immunocompromised hosts (52, 53). Within the C. glabrata complex, Candida nivariensis and Candida bracarensis are important, since both species have lower susceptibility to fluconazole, but they may be difficult to identify (54).

Molds
Aspergillus spp. (A. fumigatus, A. flavus, A. niger, A. terreus), hyaline molds (Fusarium spp. and Scedosporium spp.), and a wide variety of dematiaceous fungi can cause invasive BSIs (55–58) and are associated with high mortality (59, 60). Most patients with invasive aspergillosis have underlying hematological diseases or have undergone hematopoietic stem cell transplantation. Recognized risk factors for infection are graft-versus-host disease, receipt of steroids, secondary neutropenia, age >40 years, and a stem cell source (61). In contrast, invasive mold infections are unusual in patients with human immunodeficiency virus (62).

Invasive mold infections are especially challenging among patients receiving chemotherapy for hematological malignancies, hematopoietic stem cell transplant recipients (57), HIV patients (63) and solid organ transplant recipients (64, 65). Even Acremonium spp. are implicated in systemic fungal diseases, with a case of Acremonium kiliense fungemia with proven involvement of the lungs in an allogeneic hematopoietic stem cell patient (66). Saprochaete capitata (formerly known as Geotrichum capitatum and Blastoschizomyces capitatus) has been known to produce serious opportunistic infections in patients with hematological malignancies (67).

Traditional blood culture methods can recover some molds (68), but depending on the species, broth culture has low sensitivity for recovering fungi when compared with molecular methods. The recovery of Aspergillus spp. from specific fungal blood culture bottles is infrequent despite disseminated disease (including endocarditis), owing to tissue tropism and limitations in traditional broth culture technology. Vessel invasion and skin infarcts can limit mold recovery from the bloodstream. In contrast, some molds and dematiaceous fungi such as Fusarium spp. (69), Paecilomyces spp. (70), and Wangiella (Exophiala) dermatitidis (71) have been successfully cultivated from patients, especially those with indwelling catheters.

Viruses
Many viruses are well-known sources of sepsis in immunocompromised hosts; however, these syndromes and their associated test methods are well covered elsewhere and therefore will not be reiterated here; rather, we provide a summary table with a list of relevant publications and rare or miscellaneous pathogens (Table 1).

Other fastidious pathogens
Immunocompromised patients are susceptible to invasive infections with fastidious pathogens that may require unique incubation procedures. Microorganisms such as HACEK (species of Haemophilus, Actinobacillus, Cardiobacterium, Eikenella, and Kingella) bacteria can be recovered within 5 days of incubation in currently available automated blood culture systems; extended incubation is not deemed necessary (72, 73).

Legionella spp. grow poorly in routine blood culture media; they require blind subculture from blood culture bottles and special supplementation for growth on solid media (74). Legionella bozemanae has been reported to cause pneumonia in immunocompromised patients (75). Some Campylobacter spp., Helicobacter spp., and...
Capnocytophaga spp. may also grow poorly or not at all but are important in the immunocompromised population (76, 77). Laboratories may consider performing acridine orange staining for bottles that flag positive but do not show microbes via the Gram stain (78).

Care must be taken when interpreting the Gram stain to avoid false positives that may occur due to Gram stain precipitate. Telemicroscopy is becoming feasible in microbiology and can drastically reduce the number of errors for Gram stain interpretation made by nonmicrobiologists during night shifts or by generalists, for whom competency remains a challenge due to limited exposure to the practice (79).

With improved blood culture systems, the ability to detect Bartonella spp. has also improved, but it still requires a prolonged incubation period of up to 6 weeks followed by subculture to freshly prepared media (80). For other microbes, the addition of supplemental agar or different incubation conditions can help support the growth. These additional considerations are well worth
the effort for immunocompromised patients and should be included in standard culture algorithms (Fig. 3).

**PREDISPOISING FACTORS FOR BLOODSTREAM INFECTION**

**Risk Factors and Outcomes**

Immune changes are the main risk factors for developing sepsis. It is important to assess the patient’s predisposition for infection and how likely it is for a patient to develop organ dysfunction. Much is known about immunosuppression-related sepsis risk factors, and they are discussed in greater detail later in this article. Although less is known about the risk factors leading to organ dysfunction, it is important to document the patient’s baseline organ function (overall health status) and any inherited risk factors (3). Age, sex, and race contribute to and influence the prevalence of sepsis. For example, severe sepsis occurs more frequently in infants and elderly individuals, is more common among males than females, and has a greater incidence rate in African Americans than in Caucasians (81). Genetic characteristics may contribute to the host’s susceptibility to sepsis and are currently of great interest (82–87).

As with all medical conditions that suppress immunity, the major risk factors for acquiring a life-threatening infection, such as BSI, include two major concepts: pathogen exposure and the immune status of the individual (33). Depending on the predisposing factor (HIV, organ transplant, diabetes, etc.) different risk factors can occur. In addition to underlying immunosuppression, immunocompromised patients have increased risk for infections from unusual or multidrug-resistant (MDR) microorganisms, owing to chemoprophylaxis regimens, extended hospitalizations, or prolonged stays in intensive care units.

Improvements in the management of patients with sepsis and septic shock have increased the survival rate from 20% to over 70% in some populations (3, 88); however, as septic shock is the most severe facet of sepsis, it is not surprising that it has the highest mortality rate, still approximately 50% in some studies (88). Mortality seems to be associated with a poor prognosis when
attributed to Gram-negative organisms or yeast, as opposed to Gram-positive organisms (88).

Even after discharge, patients who survive sepsis are less likely to live long-term, and sequelae such as impaired biological functions, exacerbation of chronic conditions (chronic kidney disease or cardiovascular disease), emergence of new chronic conditions, and neurocognitive dysfunction lower their overall quality of life (3). Refer to Table 2 for a summary of risk factors.

Critical care and surgery
Sepsis can be a fatal outcome for postsurgical patients in a variety of settings, including transplantation, wound surgery, splenectomy, intra-abdominal surgery, and cancer surgery (89–91).

Cancer
An estimated 5% of cancer patients acquire severe sepsis, and hospitalized patients with cancer are more than 5 times more likely to die (37.8%) than cancer patients without sepsis. The use of cytotoxic agents is largely responsible for immune suppression in these patients, and their therapies predispose them to BSIs. In addition, malignant neoplasms can provide entry for bacteria into the bloodstream. Even more risk comes for patients with hematologic cancers, who are 15 times more likely than the average person to suffer from severe sepsis (92).

Long-term immunosuppressive therapy for cancer is linked to development of life-threatening complications, including BSIs. Central line-associated BSIs are common among these patients with febrile neutropenia following cytotoxic chemotherapy due to translocation of intestinal organisms (E. coli, E. faecium, and Streptococcus spp.) (93). Further studies on enterococcal bacteremia show a high global mortality rate (42%) in patients with hematological malignancies (94). Poor outcomes are associated with age >50 years, underlying disease (leukemia, lymphoma, myeloma, etc.), pneumonia, and shock (94). Other risk factors include long hospital stays (average of 25 days), the presence of a central venous catheter, and the urinary tract as a source of infection (94). One study established that previous use of carbapenem antibiotics increased the risk of acquiring hospital-associated vancomycin-susceptible E. faecium BSI, which can be problematic if vancomycin resistance emerges, as that will limit therapy options (95).

Age
Age over 40 is a risk factor for sepsis (7, 96), and the poorest outcomes occur in patients older than 85 years, with an associated mortality rate of over 38.4% (81). People over 65 years old account for only 1/8 of the U.S. population but account for 66% of all sepsis cases. At the other end of the age spectrum, children, particularly neonates, share elevated mortality rates (97). Infections are more frequent identified in elderly patients (aged ≥65 years) due to preexisting comorbidities, an impaired integumentary system, reduced mechanisms of clearance, and diminished immune response (i.e., immunosenescence, which is more marked in women than in men). Because elderly patients have a decreased immune response, the clinical presentation of infection can be atypical or subtle, which often makes infection difficult to diagnose in those with increased age. Persons over the age of 85 have the highest risk for hospitalization and mortality (98). The increased risk of hospitalization alone puts them at risk for infection (99).

In 2012, the number of elderly persons in the U.S. was 43.1 million (13.7% of the total population), mostly due to the aging of the baby boomer generation; it is estimated that that number will increase to nearly 84

TABLE 2 Predisposing factors that contribute to immunosuppression

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<td>Surgical and/or invasive procedure</td>
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<tr>
<td>Catheterization (i.e., urinary tract, central venous, central line-associated BSIs)</td>
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millions by 2050 (20.9% of the total population) (100). As the elderly population increases, health care providers should not only be prepared for increased hospitalization rates in the years to come, but they should also focus efforts to reduce infection rates in the elderly. Survival rates do appear to be positively influenced by public health campaigns and behavioral changes, e.g., reductions in smoking and obesity (100).

The most frequent cause of BSIs in the elderly population is urinary tract infection, especially with MDR uropathogens; depression of immunity plays a role in their predisposition to infection (101–103). Bacteremic urinary tract infection is defined as the isolation of the same organism from both the blood and urine within 48 h (104). The majority of urosepsis cases in the elderly are community-acquired (approximately 80%), of which nearly 90% of the Gram-negative infections are due to *E. coli* (104). Non-*E. coli* Gram-negative organisms are more common among hospital-acquired infections. Gram-positive microbes are associated with male patients with chronic urinary catheter insertion.

At the other end of the age spectrum, at birth, infants have little immunity of their own and are susceptible to infections (105–107). Similar to elderly sepsis, the diagnosis of neonatal sepsis is difficult due to nonspecific signs and symptoms. In 2015, 45% of neonatal sepsis-related deaths (died within 28 days of birth) were due to infectious etiologies (108).

**Hospitalization**

Risk factors for health care-associated infections (recent hospitalization, extended-care facility, dialysis, or regular home care or hospital clinic visits) predispose patients to BSIs and sepsis. Common etiologies of health care-acquired infections are methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, extended-spectrum β-lactamase-producing *K. pneumoniae*, and extended-spectrum β-lactamase-producing *E. coli* (109). Patients receiving prophylaxis with fluoroquinolones, common in hospitals, are at risk for BSIs with methicillin-resistant *S. aureus* (110) and MDR Gram-negative bacilli (111).

**HIV**

Advances in antiretroviral therapy (ART) have extended the life expectancy of people with HIV, and because infection is related to immunosuppression, the incidence of opportunistic infections in HIV-infected persons has been reduced. Moreover, just as the epidemic has evolved, so has the spectrum of HIV-associated opportunistic infections. For example, people with HIV are more likely to be admitted to the ICU for sepsis than for *Pneumocystis* pneumonia, as was more common in the past (112). The incidence of a community-acquired bacterial BSI is higher in HIV-positive patients (20% in adults and 30% in children) than in HIV-negative adults and children (9%) (113). Although causative agents vary by geographic region, HIV-infected persons worldwide are particularly susceptible to developing nontyphoidal salmonellae and *S. pneumoniae* bacteremia. Other pathogens such as *E. coli* and *S. aureus* are common causes of BSI among HIV-positive and HIV-negative individuals. Moreover, patients who receive ART are less likely to be hospitalized for a BSI, with an incidence rate of 0.63 preART and 0.2 post-ART (113).

Regardless, a low CD4 count is the main risk factor for the development of BSIs in HIV-infected persons. Mortality rates vary from 7% to 46% worldwide, with Europe and the U.S. at 16% to 18% and 25% to 43%, respectively, and Africa at 7% to 46% (113). Although data are limited, no studies have yet shown a difference in mortality rates between HIV-positive and HIV-negative patients with community-acquired BSI (113).

**Transplant**

BSIs are a major cause of morbidity and mortality after solid organ transplant and hematopoietic stem cell transplantation, with the highest incidence rate (7.4% to 14%) within the first 30 days (114–116). When coupled with septic shock, the mortality rate of posttransplant BSI is approximately 50% (117). The transplantation type (i.e., kidney, kidney–pancreas, liver, etc.) also plays a role in incidence rate, and liver transplantation was associated with the highest risk of BSI (118). Although etiologic agents vary between studies, most Gram-negative BSIs are caused by *E. coli*, and the Gram-positive BSIs are largely due to *Staphylococcus* spp. (115, 116). Moreover, the Gram-negative isolates mainly consist of multidrug-resistant organisms (115, 116), thus necessitating the use of a more aggressive empiric agent, such as a carbapenem.

Risk factors for BSI posttransplantation include age >50 years, nosocomial infection, pulmonary source (117), urinary tract sources (115), and central venous catheters (119). In one study, for 27% of liver transplant BSIs, urosepsis began with catheter-related infections (115). Certain causative agents are also associated with poor outcomes. For example, *Candida* spp., enteric Gram-negative bacteria other than *E. coli*, and MDRO non-lactose-fermenting Gram-negatives (e.g., *P. aeruginosa*) (117, 119) are associated with increased mortality. Moreover, the prophylactic antimicrobials used to mini-
mize opportunistic infection in high-risk patients inadvertently increase the rate of resistant microorganisms isolated. Increasing resistance rates of MDR organisms complicate treatment options and continue to be a concern in transplant patients.

**Steroid use**

Glucocorticoids, also known as corticosteroids, play an important role in regulating the immune response, and as one would expect, disruption in their levels can destabilize a sepsis patient. Glucocorticoids function to inhibit the inflammatory response, thus creating an immunosuppressive effect. During sepsis, cortisol and glucocorticoid production are reduced, which causes individuals to experience exaggerated and uncontrolled inflammatory responses. Therefore, glucocorticoids are frequently used to treat patients with severe sepsis or septic shock as a means to stabilize their immune imbalances. New data suggest that glucocorticoids serve to reprogram immune cells, which balances homeostasis and restores organ function for improved patient outcomes (120). On the other hand, high random cortisol levels are a marker of disease severity and a poor prognostic marker (120, 121). Not surprisingly, an uncontrolled immune response during sepsis disrupts homeostasis, causing metabolic chaos. For example, hyperglycemia is one clinical characteristic of sepsis that becomes an important therapeutic factor to manage in septic patients. The use of corticosteroids, such as hydrocortisone, during sepsis has the ability to stabilize blood glucose levels. However, current studies show limited to no benefit of the use of corticosteroids as sepsis therapy, and the lack of evidence-based data keep this a controversial topic (121).

**Nutrition (malnourished, diabetic)**

Many metabolic and nutritional deficiencies are linked to increased risk of infections and to increased severity and mortality of BSIs (122, 123). A variety of metabolic and vitamin deficiencies predispose hosts to infections and are linked to higher incidence and severity of BSIs. Vitamin D, selenium, iron, amino acids, and hormone deficiencies have all been linked to higher risks and severity of disease (124–136).

**B-cell depletion**

B lymphocytes produce antibodies, which function to inhibit infections by eliminating microorganisms. B-cells also regulate the immune response by producing specialized signaling molecules called cytokines. In BSIs, proinflammatory cytokines, such as TNF, interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8), function to provide response to human infection. When produced and released, the proinflammatory cytokines induce a systemic inflammatory response. Anti-inflammatory cytokines, such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and INF-γ, function to limit tissue injury.

B-cell dysfunction obviously alters the host’s immune response, but so does B-cell depletion, a type of therapy utilized for the treatment of autoimmune diseases (i.e., lupus, rheumatoid arthritis, multiple sclerosis) and B-cell malignancies (i.e., Hodgkin lymphoma, chronic lymphocytic leukemia). Depletion therapy alters the host immune response, and treatment with rituximab, an anti-CD20 antibody, is associated with induced hypogammaglobulinemia, which predisposes patients to increased infections.

**Neutropenia**

Neutropenia predisposes patients to BSIs and sepsis, and febrile neutropenia represents one of the biggest diagnostic challenges (137–146). Like depletion therapy, treatment-related neutropenia can have severe consequences and is associated with a variety of common and rare microbes (141, 147). The situation is similar for autoimmune cytopenia (148) and other causes of granulocytopenia (107, 143, 149–153).

**Intensive care unit**

In critical care settings, the definitions of sepsis and septic shock were last revised in 2012. Experts from a variety of fields (154, 155) introduced changes in sepsis definitions, because previous definitions were thought to include an excessive focus on inflammation and the misleading model that sepsis follows a continuum through severe sepsis to shock. In addition, the inadequate specificity and sensitivity of systemic inflammatory response syndrome criteria was addressed and led to removal of the term “severe sepsis,” which was deemed to be redundant (154, 155). In an ICU setting, sepsis is now defined as life-threatening organ dysfunction, which is caused by a dysregulated host response to infection. Organ dysfunction can be represented by an increase of 2 points or more in the SOFA score, which is associated with an in-hospital mortality of greater than 10% (154, 155). These updated definitions and clinical criteria now replace previous definitions and facilitate earlier recognition and more timely management of patients with sepsis or patients at risk of developing sepsis (154, 155).

Septic shock is now defined as a subset of sepsis in which profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mor-

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Mortality than with sepsis alone (154). The presence of septic shock is associated with hospital mortality rates greater than 40%. Patients with septic shock are identified by the need for use of vasopressor therapy in order to maintain their mean arterial pressure at ≥65 mm Hg, and a serum lactate level greater than 2 mmol/liter (>18 mg/dl) in the absence of hypovolemia, a decreased volume of circulating blood.

In outpatient settings, emergency departments, or general hospital ward settings, adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes typical of sepsis if they have at least 2 of the following clinical criteria, which together constitute a new bedside clinical score termed “quickSOFA” (qSOFA): respiratory rate of ≥22/min, altered mental status, or systolic blood pressure of ≤100 mm Hg.

Nosocomial BSI
Besides the classically known health care-associated infection BSIs, such as *Staphylococcus* spp. (156), *Enterococcus* spp., and MDRO Gram-negative infections, emerging pathogens are becoming more prevalent in health care-associated infection transmissions and outbreaks (156–168). Indeed, due to hospital-associated drug-resistant infections, the simple act of hospitalization increases risk for BSIs and sepsis. Therefore, clinicians and microbiologists must stay keenly aware of novel pathogens and their incidence among their patient populations to serve as a warning system for potential outbreaks and for device- and infusion-based contamination events.

Other
Introduction of novel monoclonal antibody therapy, such as anti-TNF for common conditions such as Crohn’s disease, rheumatoid arthritis, and invasive procedures are all risk factors for development of sepsis (60, 88). Other conditions that can be successfully controlled with iatrogenic immunosuppression, such as diabetes, splenectomy, and congenital immunodeficiency, also impart higher risk (60, 88). In addition, rheumatic or congenital heart disease, surgery, cirrhosis, and chronic diseases (including diabetes, heart failure, chronic renal failure, and chronic obstructive pulmonary disease) add risk. Addictions, such as intravenous drug abuse and alcoholism, also represent risk factors for sepsis. Finally, other infections, such as septic abortion, pelvic infection, severe community-acquired pneumonia, abdominal infection, urinary tract infection, meningitis, and cellulitis will predispose patients to sepsis (60, 88).

**DIAGNOSTIC APPROACHES AND INTERPRETATION**

Unfortunately, despite the enormous human and financial impact of sepsis, this diagnosis remains largely a clinical one (169), due to the lack of rapid, sensitive, and specific laboratory tests to detect the causative pathogens. In order to provide a more accurate diagnosis, there is a significant need to improve the speed and diagnostic breadth of laboratory detection methods for the causative agents of bacteremia and sepsis and the immune aspects of bloodstream infections. There is a wide variety of diagnostic approaches for the identification and treatment of bacteremia and sepsis.

**Radiographic Techniques: MRIs, CT Scans**
It is important to determine the source of the infection to eliminate reseeding of the bloodstream from regions that are difficult to penetrate with antimicrobials or that may require drainage or removal. Source control is recommended within the first 12 h after a BSI is diagnosed (4) in order to prevent severe downstream morbidity and mortality. For this purpose, magnetic resonance imaging (MRI), routine radiographs, ultrasound, or computed tomography (CT) scans are useful.

**Histopathology**
Skin or end-organ biopsy for histopathological and microbiological investigation may be helpful in making early diagnosis of fungemia, particularly in disseminated *Fusarium* spp. or *Candida* spp. infections. *In situ* hybridization methods can be useful to identify pathogens in tissue specimens (170). Surgical pathology, autopsy services, and microbiology sections of the laboratory must work closely together and communicate findings with each other.

**Microbiology Procedures**
Blood cultures are among the most important cultures performed by the clinical microbiology laboratory (171). During the initial evaluation of an immunocompromised patient with a suspected BSI, blood should be routinely cultured for both aerobic and anaerobic bacteria, as well as for commonly encountered yeasts. If the clinician has a high suspicion of a dimorphic fungus or mycobacteria, then blood bottles specifically designed to recover these microorganisms should be obtained; molds may require additional test methods (172).

Routine blood cultures are collected as a set (one aerobic and one anaerobic bottle), in which the patient's blood (optimally 16 to 20 ml, and in some cases 30 ml) is split and inoculated into the broth medium of each
bottle. The blood culture is incubated at 35°C and remains in the instrument until growth is detected, usually up to 5 days, or less if the bottle flags positive in the instrument.

Blood from the positive bottle is used to prepare a smear, and a Gram stain or other stains are performed. A variety of agar plates are then inoculated and incubated until microbial colonies can be identified and AST can be performed. The Gram stain is reviewed and resulted by a medical technologist, and this critical result is notified to the providers as soon as possible (optimally within 30 min of the blood culture flagging positive) (173). The laboratory diagnosis of bacteremia or fungemia requires urgent attention so that appropriate antimicrobial therapy can be initiated or targeted once the stain results are known (174).

Rapid detection of BSIs in the critically ill, followed by appropriate antimicrobial therapy, can have a life-saving impact (6, 173). According to a review of more than 2,600 cases from 15 intensive care units in five U.S. and Canadian cities, the risk of death from sepsis increases by 6 to 10% with every hour that passes, from the onset of septic shock until the start of effective antimicrobial therapy (2, 175). Thus, the development of rapid, sensitive, and accurate molecular diagnostic laboratory methods to identify bacterial and fungal pathogens and characterize associated antimicrobial resistance can benefit diagnosis and therapy for septic patients and can save many lives.

While a variety of historical methods were grandfathered into laboratory protocols prior to CLIA regulations, and new methods are implemented by validating them as laboratory-defined tests, the clinical utility and accuracy of these off-label assays must be reestablished in each laboratory where the method is used. In an ad hoc survey conducted with members of the American Society for Microbiology’s LISTSERV communities, the clinmicronet and divC in May 2009, the survey participants reported use of the following rapid methods, extrapolated from the published literature: the direct tube coagulase (176, 177), directly inoculated cefoxitin disk test with reduced zones to predict MRSA (176, 178, 179), rapid OxiD PBP2a latex agglutination test (Thermo Fisher Scientific, Waltham, MA) (176, 180), latex agglutination for streptococci (181), direct germ tube for Candida albicans (182), MRSASelect Agar (BioRad, Hercules, CA) (183), and Pneumoslide for S. pneumoniae (Becton Dickinson, Cockeysville, MD) (184). All were performed directly from positive blood culture bottles. In addition, direct susceptibility testing for bacteria (185, 186) and yeast (187) has been reported.

Despite attempts to rapidly identify pathogens and their associated antimicrobial susceptibilities, these techniques are still not a common practice; patients and their physicians continue to wait for confirmatory results from blood cultures, the gold standard for diagnosis of bacterial sepsis, which can take several days before a pathogen is identified, limiting their usefulness in the management of the acutely ill patient. Moreover, blood cultures frequently remain negative even in severe cases of sepsis. For example, routine methods are relatively ineffective for detection of certain pathogens, such as Coccidioides spp., and some Brucella spp., which are examples of causes of culture-negative fungemia and bacterial endocarditis, respectively.

Clinicians should cautiously interpret the clinical relevance of recovering molds or NTM from blood cultures drawn through catheters, because of environmental contamination with mold spores or asymptomatic colonization at the site of an indwelling line. Features in the clinical history (e.g., extensive immunosuppressive therapy, prolonged fever, embolic events) may aid clinical differentiation between infection and contamination.

Semiautomated systems as well as manual blood culture systems are still available but have largely been replaced by automated continuous monitoring systems that enhance the detection of microorganisms and improve laboratory work flow (188).

Types of blood culture systems

Automated systems

There are several fully automated, continuous-monitoring broth-based blood culture systems on the market (e.g., BD Bactec FX, bioMérieux’s BacT/Alert, and Trek Diagnostics VersaTrek). Aerobic and anaerobic broth formulations allow for recovery of the most common causes of BSIs. The broth contains an anticoagulant (sodium polyanethol sulfonate, SP5), an antiphagocytic agent, and an antimicrobial neutralizing agent (charcoal or resin). Using a variety of indicator systems, the bottle(s) are incubated and continuously monitored by the blood culture instrument for growth. Resin-containing media increases microorganism recovery from patients who are receiving antibiotics at the time of collection (189), and technological advancements include monitoring fill volumes.

Positive growth is detected by changes in volatiles and detected as increased CO2 (BacT/Alert, Bactec) or by changes in pressure (VersaTrek). The bottles are monitored every 10 minutes. Blood cultures are incubated for up to 5 days (or longer for some fastidious microbes). Most, but not all, pathogen growth will trigger a positive
instrument reading, which typically indicates the presence of viable microorganisms (false flagging can occur in some systems due to CO₂ metabolism of high white blood cell density in the bottles).

The most common Gram-negative microorganisms isolated are *E. coli* and *Klebsiella* spp.; common Gram-positive microorganisms include staphylococci (*S. aureus* and coagulase-negative staphylococci), streptococci, and enterococci (113, 190).

**Interpretation of positive blood cultures**
The two most useful parameters that aid in the interpretation of a positive blood culture result include the type of microorganism detected (191) and the number of sets of blood cultures from which a potential pathogen is recovered (192). Collection of two sets of blood cultures from peripheral blood before initiating antimicrobial therapy is the optimal approach for determining clinical relevance of recovered microorganisms. False-positive blood cultures, growing skin contamination introduced during collection, can lead to diagnostic confusion and unnecessary antibiotic use and costs. Therefore, blood cultures should be collected from two different sites, peripherally if possible, prior to the initiation of antimicrobial agents; however, the administration of antimicrobials should not be delayed, as early administration is critical for proper treatment.

False-positive blood cultures can lead to diagnostic confusion and unnecessary antibiotic use for immunocompromised as well as immunocompetent patients. As previously described, the common contaminating microorganisms (e.g., coagulase-negative staphylococci or viridans group streptococci) can be implicated in invasive disease, thus confounding the diagnosis. Conversely, positive blood cultures for yeasts must be assumed to represent disease, whereas negative results do not exclude it. Similarly, because of autolysis of *S. pneumoniae*, negative cultures do not exclude it as a cause of BSI (184, 193). Commercially available blood culture bottles, such as the Bactec Myco/F Lytic medium (Becton Dickinson, Franklin Lakes, NJ) and the BacT/Alert MB medium (bioMérieux, Durham, NC) provide fast and sensitive methods to detect mycobacteria (71).

**Isolator**
Although continuous-monitoring systems have emerged as the new gold standard, the Isolator system (Wampole Laboratories, Cranbury, NJ) is still optimal for isolating some fastidious microorganisms (*Brucella, Bartonella*, mycobacteria, filamentous fungi, *Histoplasma capsulatum*) by lysis and centrifugation (188, 194–196). After centrifugation, the resulting pellet is used to inoculate agar media. Contamination is a known problem due to multiple manual processing steps (197).

**Principles of Blood Culture Collection**
The importance of rigorously following guidelines for the proper collection of blood cultures such as those published by the Clinical and Laboratory Standards Institute cannot be overemphasized. At the initial evaluation for patients with signs and symptoms suggestive of bloodstream infection, an adequate blood volume must be cultured to achieve overall test sensitivity. For nonfastidious bacterial pathogens in patients with no prior history of antibacterial therapy, one set of blood cultures (i.e., 20 ml of blood) has been shown to detect a bacterial or yeast bloodstream infection in approximately 65 to 91% of cases; two or three sets of blood cultures improve the overall yield of detecting the pathogens, raising the yield to 80 to 99% and 96 to >99% of cases, respectively (198, 199). Medical urgency often dictates prompt initiation of empiric antibiotic therapy in immunocompromised hosts, but two to three sets of blood cultures should be obtained within 5 minutes of each other before initiating therapy. The optimal volume of blood for children can vary based on body weight, but similar to adults, higher volumes of blood increase the likelihood of recovering microorganisms.

There are many factors that contribute to clinically effective blood culture data, including disinfection of draw site and culture bottle tops prior to collection, disinfecting agent (iodine versus chlorhexidine), site of collection (peripheral versus line draw), timing of collection (transient versus persistent bacteremia and before antibiotic initiation), volume of collection (negative versus positive growth), and number of bottles/draws (contamination versus true bacteremia). Because blood cultures are susceptible to contamination, proper sample collection is essential in order to avoid culture misinterpretation and unnecessary antibiotic therapy. If the skin is not adequately cleansed prior to blood collection, then commensal skin microorganisms, such as coagulase-negative staphylococci, are likely to contaminate the culture. Coagulase-negative staphylococci are common causes of bacteremia and often associated with endocarditis (200); therefore, it is important to recognize when a positive blood culture is due to improper collection technique versus infection. One way to differentiate between a skin contaminant and a true pathogen is to begin by collecting two sets of blood cultures, each from different sites (e.g., right and left peripheral draws). Assuming proper collection technique, a contaminant
will likely only grow in one bottle. If poor collection technique is the issue, then it is likely that more than one bottle will be positive, thus elevating the blood culture contamination rate. Data show that blood culture contamination rates decrease when draws are limited to phlebotomy staff versus nonphlebotomists (5). Likewise, if the septum of the blood culture bottle is not sufficiently decontaminated, then environmental organisms, such as Bacillus, can be inadvertently inoculated into the blood culture and grow, triggering a false-positive culture. Therefore, it is not only necessary to disinfect the skin prior to collection, but it is also crucial that the septa of the blood culture bottles are thoroughly disinfected prior to collection.

Aseptic technique in blood culture collection is imperative to prevent contamination from skin and other environmental microbiota. In the U.S., the positivity rate for blood cultures is approximately 10%, and many of these positive results represent contaminating skin microbiota due to inadequate skin preparation or from colonized indwelling vascular catheters (198). Surface dirt and oils should be removed by thoroughly cleansing the skin with 70% alcohol (isopropyl or ethyl). Chlorhexidine gluconate in alcohol can then be used to disinfect the area prior to venipuncture and has become the new standard in blood culture collection. One must not contaminate the area by touching it after skin preparation (171).

Best practices and chlorhexidine skin preparation can drop the contamination rate to <1% for both phlebotomist and nursing collection rates (DM Wolk, personal communication) (201), as can use of a new skin contaminant diversion device, the SteriPath System (Magnolia Medical Technologies, Seattle, WA). One must remember that assuming a 10% positivity rate, a 3% overall contamination rate would apply to 30% of all positive cultures; therefore, successful use of new products makes a contamination rate of near 0% possible. We recommend <1% contamination as the current goal to optimize patient care and minimize needless costs.

Despite the American College of Physicians recommendation that blood cultures should be obtained through fresh venipunctures rather than indwelling intravascular devices (202), blood cultures frequently are collected from an indwelling central venous catheter in immunocompromised hosts because of restricted access to robust veins in those patients. Several studies have shown that blood cultures drawn from the indwelling catheters are less specific and have lower positive predictive value and higher rates of contamination than those obtained from a peripheral venipuncture (203, 204). The exception is a newly inserted catheter, for which investigators have shown no increase in contamination rates for these catheter-drawn blood cultures (205). The clinical significance of blood cultures collected through an indwelling catheter, particularly when only one set of blood cultures is obtained, cannot be determined reliably; therefore, true bacteremia cannot be distinguished from contaminating microbiota. For example, in a patient presenting with hypotensive shock, physicians may mistakenly infer causality from a single positive blood culture, and that may result in misuse of antibiotics and failure to investigate alternative diagnoses.

**Peripheral collection**

Peripheral blood is the preferred specimen for blood cultures. One set of blood cultures should be drawn from a single venipuncture, consisting of approximately 20 ml (10 ml per bottle) or such that the specimen provides a blood-to-broth ratio of 1:5 to 1:10 (171). Larger blood-to-broth ratios improve microorganism recovery (206). Additionally, microorganism recovery increases when blood cultures are collected before or during a fever spike (171). Two sets of blood cultures drawn consecutively from different anatomic sites prior to antimicrobial therapy are recommended for the diagnosis of acute sepsis (pneumonia, osteomyelitis, meningitis) (4, 171). If continuous bacteremia (endocarditis or fever of unknown origin) is suspected, then an additional set of blood cultures may improve recovery. For instances in which antimicrobial therapy was initiated, cultures should be collected when the concentration of antimicrobial agent is at its lowest.

**Collection via indwelling port**

Cultures drawn though an indwelling catheter are not recommended; however, if limited vein access requires the use of an indwelling catheter, then a peripheral site draw must accompany that culture in order to fully evaluate the culture results (207, 208). Comparing the time to detection, also known as time to positivity (209–218), or performing quantitative blood cultures (219, 220), may be used to diagnose catheter-related sepsis. Catheter tip cultures may also be useful in the diagnosis of a catheter-related infection when paired with blood cultures, but these have fallen from favor as a standard practice.

**ANTIMICROBIAL SUSCEPTIBILITY TESTING**

Laboratories utilize a combination of solid agars to promote the growth of microorganisms from positive blood cultures. There are different methods for cul-
turing. A Gram stain-directed approach utilizes media-driven algorithms. For example, if Gram-negative rods are observed on Gram stain, then a MacConkey plate will be inoculated. When a clinically significant microorganism has been isolated from a patient with bacteremia, susceptibility testing should be performed according to the methods described by the Clinical and Laboratory Standards Institute.

In the 24 to 48 h when susceptibility results are not available, clinicians are encouraged to rely on their institution’s antibiograms to help guide their selection of antibacterial therapy. In some cases, when there are enough isolates to substratify the data, a location-specific antibiogram for ICU and emergency department may be useful, as is a source-specific substratification of the data (DM Wolk, personal communication). Although some organisms such as viridans-group streptococci have predictable patterns and susceptibility testing may not be performed, clinicians should be aware that unusual resistance patterns can occur among immunocompromised hosts. For example, *Streptococcus mitis* and *Streptococcus salivarius*, two microorganisms considered to be very susceptible to penicillin, have been observed to have high MICs to penicillin when recovered from patients with febrile neutropenia (111). Multidrug-resistant Gram-negative bacillary infections are also becoming a serious problem in immunocompromised hosts (24). Depending on the institution’s frequency of resistance owing to extended-spectrum beta-lactamases or *ampC*-mediated mechanisms, empirical therapy with a carbapenem may be warranted. For infections associated with intravascular devices, removal of the catheter often is necessary. Microorganisms can be embedded within a biofilm layer and thereby can be resistant to the activity of antibiotics (59, 221), which thwarts eradication of bloodstream infection without catheter removal (222). The presence of persistent bacteremia should raise suspicion of an endovascular source such as endocarditis or a metastatic focus (e.g., vertebral osteomyelitis or renal abscess). Endocarditis in granulocytopenic patients is unusual, because inflammatory-mediated processes and platelet deposition are critical for bacterial colonization of the endocardium. In other immunocompromised hosts, however, clinical suspicion for bacterial endocarditis should remain high. Further evaluation with transesophageal echocardiography may be required to assess presence or absence of valvular vegetations. Depending on the clinical context, investigations of other metastatic foci may warrant radiological imaging such as a CT scan or an MRI.

Susceptibility testing is appropriate for candidemia, particularly for questions of treatment failure or as supporting evidence that patients may be converted safely from intravenous to oral therapy. While awaiting testing results, susceptibility patterns can often be predicted based on the species of *Candida* isolated and the institution’s specific antibiogram profiles. Fluconazole is appropriate as empiric therapy for institutions with low rates of non-albicans candidemia, except for patients with prior triazole exposure. Indications for voriconazole and echinocandins as empirical therapy are dictated by institution-specific trends for recovery of non-albicans yeast. Echinocandins are often very effective for patients with candidemia, but notable exceptions include *Candida guilliermondii*, the non-*Candida* yeast, and *Trichosporon* spp.

CLSI has developed and published approved methods for broth-dilution testing of caspofungin, fluconazole, and voriconazole (CLSI approved standard M27-A3) and for disk diffusion testing (CLSI approved guideline M44-A) of yeast. These methods are accurate and reproducible and are being used in clinical laboratories. Interpretative breakpoints are available for select *Candida* spp. with certain azoles and echinocandins, and the clinical relevance of testing any other organism–drug combination remains uncertain. A “susceptible–dose-dependent” category exists for species with elevated MICs to triazoles and denotes that higher doses may provide clinical efficacy. Susceptibility testing for filamentous fungi has been more challenging to standardize. CLSI has developed methods for MIC testing of filamentous fungi (CLSI document M38-A2), but more clinical data for correlating *in vitro* results with *in vivo* responses are needed. Generally speaking, for filamentous molds, the term “resistance” testing is informally used as a means to predict therapeutic failures.

For fungemia, all central venous catheters should be removed when feasible. All patients should undergo at least one ophthalmologic examination to exclude endophthalmitis, and patients with fungal endocarditis require immediate cardiothoracic surgical evaluation. Breakthrough or persistence of candidemia (or other fungi) on therapy suggests an infected intravascular device, profound immunosuppression, or microbiologic resistance. Duration of antifungal therapy is highly variable, depending on several factors, including the type of microorganism recovered (e.g., *C. albicans* versus *Fusarium* spp.), the degree of underlying immunosuppression, and the presence of metastatic foci.
Rapid Antibiotic Therapy Saves More Lives than Any Other Intervention

In cases of sepsis, rapid intervention with appropriate antimicrobial therapy can be critical to patient survival (223, 224). For aerobes, anaerobes, and fungi, appropriate antibiotic therapy increases survival by approximately 25 to 45%. Eliminating delays in appropriate antibiotic administration increases survival by ~7 to 10% per hour (9, 175). Optimized antibiotic care requires intravenous broad-spectrum antibiotics with daily reevaluation to optimize efficacy, prevent resistance, avoid toxicity, and minimize costs, with the ultimate goal to discontinue broad-spectrum therapy within 3 to 5 days and to continue antibiotics targeted to the causative pathogen (10).

COMMERCIAL AVAILABLE MOLECULAR METHODS

Recent developments in molecular diagnostics allow for rapid identification of microorganisms directly from positive blood culture bottles, and some can be used on samples directly from the patient.

Peptide Nucleic Acid Fluorescence In Situ Hybridization

Peptide nucleic acid fluorescence in-situ hybridization (PNA FISH) is a technology to test blood smears made directly from positive blood culture bottles. Results are provided within 2.5 h for the direct identification of Gram-positive organisms (staphylococci and enterococci), Gram-negative organisms (E. coli, K. pneumoniae, P. aeruginosa) and Candida spp. from positive blood cultures. Probe kit utilization is driven by the Gram stain result; thus, Gram-positive cocci in clusters would utilize the Staphylococcus probe, yeast would utilize the Candida probe and Gram-positive cocci in pairs, and chains would utilize the dual Enterococcus probe.

PNA molecules contain the same nucleotide bases as DNA and follow standard base-pairing rules for hybridization for the following base pairs: adenine (A), thymine (T), cytosine (C), guanine (G). Differences in the PNA hybridization probes allow for stronger and more specific binding. Furthermore, PNA FISH probes target rRNA in microbial chromosomes, offering the following advantages of a small subunit rRNA (ssRNA) target: 1) sequences are known and unique between species; 2) ssRNA targets are highly abundant, multicopy targets; and 3) PNA probes, due to their small size, can bind in highly conserved regions that are not accessible to larger DNA probes. In addition, the PNA probes confer very low background, which allows the fluorescent signal to be visualized and confers greater sensitivity.

According to product inserts and clinical publications, PNA FISH performance data have demonstrated accuracy. Sensitivity and specificity are as follows: for S. aureus, 98.8% and 99.6%, respectively; for C. albicans, 99.3% and 100%; for E. faecalis, 96.3% and 98.3%; for other Enterococcus spp., 93.1% and 99.3% (225–229); for E. coli, 100% and 100%; for K. pneumoniae, 60% and 100%; for P. aeruginosa, 100% and 100%; and overall accuracy for Enterobacteriaceae is 94.3% and for non-Enterobacteriaceae is 100% (230). The compatibility of these probes has been proven with demonstrated accuracy for various blood culture media, including FAN and resin bottles (226, 227, 231–233).

Several studies have applied PNA FISH as an adjunct to the Gram stain from positive blood culture bottles, and it is clear that when used appropriately with a team involving laboratory, pharmacy, and physicians, PNA FISH can direct therapy, but it also reduces antibiotic and hospital costs and saves lives. Optimal outcomes for rapid intervention with PNA-FISH have been best documented when there is a strong collaborative effort between the laboratory and pharmacy. Holtzman et al. found no impact when their pharmacy was not involved (234). Others found success with physician intervention, including substantial decreases in turnaround time, mortality, and hospital expenditures (235).

The PNA FISH assay (AdvantDx, Woburn, MA) can be utilized to reduce the time required to administer targeted antibiotics therapy (153, 156, 236), to curtail unnecessary antibiotics when blood cultures are contaminated (58, 237), and to support antifungal selection for candidemia (231, 238).

GeneXpert, Xpert MRSA/SA BC Assay

The rapid detection of Staphylococcus aureus bacteremia and a swift determination of methicillin susceptibility have serious clinical implications affecting patient mortality. In a multicenter preclinical evaluation, Wolk et al. evaluated the performance of two Cepheid Xpert MRSA/SA assays for detection of MRSA and methicillin-susceptible S. aureus (MSSA) (239). Using an integrated DNA extraction process coupled to real-time PCR, MSSA and MRSA are identified directly from positive blood culture bottles in <1 h. A total of 114 wound specimens and 406 blood culture bottles were tested from study sites in the United States and Europe in order to characterize assay performance of these assays in a clinical setting. Newly positive blood culture broths with Gram’s stains showing Gram-positive cocci in clusters
were tested. Broths from each of the following continuous monitoring blood culture instruments were included: bioMérieux BacT/Alert SA standard aerobic, SN standard anaerobic; Bactec Peds Plus/F Medium, Plus Aerobic/F Medium, Plus Anaerobic/F Medium; Standard Anaerobic/F Medium, Standard/10 Aerobic/F Medium, Lytic/10 Anaerobic/F Culture Vials; and VersaTREK Redox 1 (aerobic), Redox 2 (anaerobic). BacT/Alert FAN aerobic and anaerobic broths were not included. The primers and probes in the Xpert MRSA/SA assays detect sequences within the staphylococcal protein A (spa) gene, the gene for methicillin resistance (mecA), and the staphylococcal cassette chromosome (SCCmec) inserted into the SA chromosomal attB insertion site (240, 241). Inclusion of both the attB insertion site and the mecA gene targets enables the assays to identify the presence of SCCmec cassette variants with mecA gene deletions, thus reducing false-positive results that occur in molecular tests that only target the SCCmec cassette (242, 243). For the Xpert MRSA/SA assay performed on the Cepheid GeneXpert system, sensitivity was 97.1% and 98.3% for MRSA in wound and blood culture specimens, respectively. Sensitivity was 100% for S. aureus from both specimen types (244).

**SmartCycler, BD GeneOhm StaphSR Assay and BD Max**

The BD GeneOhm StaphSR assay on the SmartCycler is used for detection of *Staphylococcus aureus* and MRSA directly from positive blood culture bottles. In a study by Stamper et al., a real-time PCR assay, the StaphSR assay (BD GeneOhm, San Diego, CA), was used for the identification of MSSA and MRSA from positive blood culture bottles (n = 300) (245). The sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm StaphSR assay for MSSA detection were 98.9, 96.7, 93.6, and 99.5%, respectively, when compared to phenotypic identification with a variety of reference standards, including oxacillin and cefoxitin Etests (AB Biodisk, Solna, Sweden), penicillin-binding protein 2 (Denka Seiken Co., Tokyo, Japan), and mecA PCR. For the detection of MRSA, the BD GeneOhm StaphSR assay was 100% sensitive and 98.4% specific. Inhibition was seen with only one sample, and the issue was resolved upon retesting.

Recently, Grobner et al. evaluated the BD GeneOhm StaphSR assay for its use in the detection of *S. aureus* and MRSA (n = 134). Of 134 samples, five discrepant results arose with this assay due to the presence of methicillin-susceptible, revertant MRSA strains (3/45) and MRSA strains that were not detected by the BD GeneOhm StaphSR assay (2/45) (246).

**BioFire Film Array**

The FilmArray system (BioFire; Salt Lake City, UT) is a novel and completely integrated real-time multiplex PCR instrument that incorporates multiple singleplex PCR reactions in a disposable pouch (58, 189). New additions to their FDA-cleared menu include a blood culture identification panel for the detection of relevant bacteria and yeasts, as well as antimicrobial resistance bacterial genes. The blood culture identification panel multiplexes 27 different genetic targets. All nucleic acids are extracted and purified directly from the unprocessed sample. During the nested PCR, the FilmArray performs a single, large-volume, multiplexed reaction with primers specific to several targets. Following the multiplex PCR reaction, an individual singleplex second-stage PCR reaction further amplifies the products that amplified during the first round of multiplex PCR. The FilmArray software analyzes and interprets the endpoint melting curve of the amplicons.

**GenMark eSensor**

The eSensor technology (XT-8) (GenMark; Carlsbad, CA) promotes high assay specificity and sensitivity owing to competitive DNA hybridization and electrochemical detection of the post-PCR target sequence. The technology is not based on fluorescence or optical detection as are the first-generation multiplex assays; thus, the protocols do not require as many of the time-consuming washing and preparation steps required by earlier multiplexing technologies. The XT-8 systems can be used in tandem with up to 3 modules per computer for a throughput of 1 to 24 samples in random access mode.

The eSensor cartridge is preprogrammed with a specific test protocol, lot number, and expiration date on an internal memory chip. In the cartridge, the preamplified target DNA is mixed with the signal probe solution and then analyzed via electrochemical detection. The GenMark pipeline includes a multiplex panel for rapid detection of common bloodstream pathogens. New adaptation of the system toward one requiring less than 60 minutes of hands-on time will make the GenMark system more easily adaptable to routine laboratory settings, and it is expected to be a fully integrated cartridge system with sample-to-answer workflows.

**Nanosphere Verigene**

Nanosphere’s Verigene (Northbrook, IL) system uses a gold nanoparticle probe technology, typically 13 to 20 nanometers in diameter that carries a defined number of target-specific oligonucleotides or antibodies utilized in
two methodologies, array and amplification, depending on the specific assay. The nanoparticles support increased sensitivity compared to fluorophores. Light scattered from one nanoparticle is reported to be equivalent to the light emitted from one-half million fluorophores.

Automated nucleic acid purification and amplification is performed on the Verigene Processor SP. Inside the Verigene test cartridges, nucleic acid targets are hybridized to capture oligonucleotides on a microarray containing specific oligonucleotides and gold nanoparticle probes. Finally, signal amplification of probes occurs via a silver staining process, with automated qualitative analysis performed on the Verigene Reader. Products for identification of select Gram-positive and Gram-negative pathogens from positive blood cultures, which are probe-based without target amplification (2, 213), are available.

**Molecular Testing for Drug Resistance**

Rapid and accurate determination of microbial drug susceptibility is actually the most critical challenge; it is essential to facilitate successful antimicrobial therapy for any person, and it is particularly useful for those with immune-function impairment. Rapid testing for genetic resistance markers is an emerging clinical practice that can not only identify the potential for drug resistance, but also help distinguish ambiguous breakpoints associated with susceptibility testing. The application of molecular diagnostic methods to detect drug resistance is evolving for some laboratories. Future methods should facilitate educated choices for therapy, which can be initiated early in diagnosis to impact patient outcomes. Evidence-based studies will be key to the adoption of these new antimicrobial testing paradigms.

Targets for genetic testing require relative genetic stability of the target sequence, a requirement that demands national and international efforts for monitoring genetic mutations in these target regions. Together, MRSA and vancomycin-resistant enterococci are the two most important resistant bacterial pathogens in U.S. hospitals, and their rapid detection remains a critical necessity as antimicrobial resistance continues to increase in the U.S. and worldwide (247, 248). Among bacteria, other useful antimicrobial resistance targets include resistance genes for β-lactams, aminoacyl-tRNA synthetases, chloramphenicol, fluoroquinolones, glycopeptides, isoniazide, macrolides, mupirocin, rifampin, sulfonamides, tetracyclines, and trimethoprim (249–251). For a list of PCR primers used to target such resistance markers, see Tenover and Rasheed, 2004 (251). For fungi, rapid detection of antifungal resistance is useful, primarily due to the increase in fungal infections among immunocompromised patients. Current antifungal assays rely on fungal susceptibility testing, which is dependent on growth. The practical application of antifungal molecular testing is yet to be seen, as there is still much to learn about the genetic markers which mediate resistance. The genetic information needed to examine fungal resistance at the molecular level is complex; a review of molecular mechanisms of antifungal resistance has been published (252).

The full potential of molecular diagnostics for drug resistance testing in microbiology has not been reached—its application is still in its infancy. As the molecular mechanisms of antimicrobial resistance are described, newer technologies may enhance the utility of such an approach. Furthermore, microarray technology has the promise to impact the rapid and accurate detection of multiple mutations associated with resistant bacteria, mycobacteria, viruses, and fungi. Until the full potential of drug-resistant markers is understood, rapid molecular antimicrobial testing must still be combined with traditional microbial cultivation (247, 248).

**DNA Sequencing**

Broad-range gene PCR and sequencing from clinical material provides an alternative, culture-independent method for detecting pathogens. The promise of this technique has remained strong, especially for the bacterial domain, but its application has not been reliably reproducible, owing in large part to issues with contamination and inadequate sensitivity (253). The gene target most commonly used to detect bacteria from a blood sample is 16S ribosomal RNA (16 rRNA gene or 16S rDNA), an approximately 1500-base-pair gene that codes for a portion of the 30S ribosome. Although investigators have demonstrated the ability to detect bacteria directly from blood with 16S rRNA gene amplification and sequencing (254), it should not be performed routinely since culture remains a more sensitive diagnostic test. Also, broad-range PCR is associated with contamination, and a positive result must be correlated with clinical history. When fastidious pathogens (Bartonella spp. or Coxiella burnetii) are suspected as the cause of bacteremia, serology and species-specific PCR from blood are optimal. The advantage of this technology in immunocompromised hosts is its ability to rapidly detect and identify bacterial and fungal pathogens from whole blood samples using broad-range primers. Its primary limitation is sensitivity, and until multicenter clinical trials are performed, these assays should be used as adjunctive tools to routine blood culture.
Pyrosequencing (Qiagen, Germantown, MD) is a rapid method for sequencing based on the detection of pyrophosphate released during DNA synthesis. Advantages of this technology include speed and ease of use in comparison to traditional sequencing technology; disadvantages include the short lengths of sequences that can be currently analyzed. Pyrosequencing provides short sequence information of roughly 30 to 50 bases; it is useful for short-read DNA and mutation/single nucleotide polymorphism analysis. After PCR amplification and amplicon cleanup, run times approach 1 h for 96 samples, with approximately 30 to 45 minutes for sequence analysis applications. Jordan et al. evaluated pyrosequencing directly from blood culture bottles to assess the potential of pyrosequencing to differentiate between bacteria commonly associated with neonatal sepsis (255). An informative 15 bases within the 380-bp amplicon was targeted for pyrosequencing following enrichment culture and PCR amplification. A total of 643 bacterial isolates commonly associated with neonatal sepsis, and 15 PCR-positive, culture-positive neonatal whole blood samples were analyzed by pyrosequencing. Results of DNA sequencing and culture identification were compared and were successful at using PCR and pyrosequencing together to accurately differentiate among several bacterial groups, both Gram positive and Gram negative. The system had some difficulty with viridans-group streptococci and S. pyogenes (255). The same group evaluated specimens from isolates from neonatal sepsis events, to support species identification that could lead to rapid deescalation or targeting of antibiotic therapy. A total of 643 bacterial isolates and 15 PCR-positive, culture-positive neonatal whole blood samples were analyzed by pyrosequencing. Pyrosequencing was able to provide useful information on the identity of species based on the amplicon generated by PCR (255).

Proteomic Tests: Sepsityper and VitekMS

Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) methods leverage the high sensitivity of mass spectrometry with linkage to large microbial databases to provide users with species-specific spectra that can be used to reproducibly identify microorganisms, and these methods can be used for identification of pure isolates or pathogens from positive blood culture bottles (off-label use) (256). The protein biomarkers that are measured in mass spectrometry of microorganisms are highly expressed proteins responsible for housekeeping functions, such as ribosomal components, chaperones, and transcription/translation factors (38).

Relying on identification of protein profiles, (generally measuring mass-to-charge ratios \([m/z]\) between 2 and 20 kDa), MALDI-TOF MS derives its information from profiles of highly conserved bacterial proteins. For MALDI-TOF MS, the protein profiles are generated from direct ionization of an intact colony or a bacterial protein extract, after manual extraction. Identification occurs after correlating an isolate’s spectral signature to a database of spectra collected from reference strains. In its current form, MALDI-TOF MS requires cultivated microbes and a small quantity of matrix (typically less than 1 μg/sample). The matrix and target spotting plates/slides are the primary consumables and are relatively inexpensive in disposable or reusable formats. Ongoing costs include the equipment maintenance agreements. MALDI-TOF MS is widely used for its high accuracy, low consumable cost and speed of analysis. A typical process consists of growth of bacteria or yeast, colony selection and placement on a target, addition of matrix, and analysis with MALDI-TOF MS. Mass spectrometry identification has diverse possibilities as it is a fast and sensitive method to measure proteins, lipids, and other targets, and it does not require prior knowledge about the organism. For a thorough review of MALDI-TOF MS technology, we refer the reader to a recent review of the technology (257).

Commercial mass spectrometry systems exist that can integrate with traditional antimicrobial susceptibility systems. Among them, the MALDI Biotyper from Bruker Daltonics is one of the most widely tested and has proven accuracy in the identification of bacteria (135, 258). The system uses a 48-spot MALDI target plate for organism identification and can be interfaced with the BD Phoenix Epicenter software or the Siemens MicroScan instrument to link antimicrobial susceptibility results that pair with bacterial identification. A few reports show promise for the identification of microorganisms using MALDI-TOF MS without subculture from blood culture bottles (66, 160, 194, 217, 240), but the methods were limited by the need for a large number of cells. These adaptations showed successful identification for only approximately 80% of blood cultures. A promising new technology, the MALDI Sepsityper system (Bruker Daltonics), aims to identify bacteria and yeast directly from positive blood culture bottles (118, 145, 217, 259).

T2Dx, Magnetic Resonance

T2 Biosystems (Lexington, MA) offers the T2Dx instrument, a miniature magnetic resonance-based platform that measures water molecules reacting in the presence
of magnetic fields. The test is the first to use direct sample EDTA whole blood for direct detection of pathogens. The T2MR technology does not require sample preparation because the signal is not disrupted by background in the sample. For target-specific testing, nanoparticles with magnetic properties that enhance the magnetic resonance signals are used. According to the manufacturer, T2Candida detects five species of Candida in whole blood specimens in approximately 3 h with a limit of detection as low as 1 CFU/ml (260).

**Biomarkers**

Studies show that cytokines can serve as biomarkers for early diagnosis of sepsis in neonates (261, 262). Additionally, cytokines appear to be associated with disease severity. Because early diagnosis is critical for targeted therapy and better outcomes, cytokines seem like promising biomarkers for sepsis. Other biomarkers, such as C-C chemokine ligand 3 (CCL3), IL-8, heat shock protein 70 kDa 1B (HSPA1B), granzyme B, and matrix metallopeptidase 8 (MMP8), may function as reliable predictors of mortality in children with septic shock (263). C-reactive protein (CRP) and amyloid A represent other biomarkers, but their specificity is relatively poor as they are nonspecific acute-phase proteins (106).

**Procalcitonin**

While patients with sepsis are largely identified based upon clinical criteria, there are several biomarkers of inflammation that can help differentiate patients with true bacterial sepsis from patients who suffer from other conditions for which antibiotic therapy is not required. Traditional biomarkers such as the white blood cell count, erythrocyte sedimentation rate, CRP, and other acute-phase reactants are not sufficiently sensitive nor specific to reliably confirm or refute the diagnosis of sepsis, and in the case of erythrocyte sedimentation rate, are slow to rise. In contrast, biomarkers, such as procalcitonin (PCT), are more useful for the diagnosis of severe bacterial infections and sepsis. PCT is used to help support diagnosis, to predict complications, and for therapeutic monitoring. High plasma levels of PCT are observed in situations characterized by the onset of organ dysfunction and other symptoms of “severe sepsis” or “septic shock” (264–266).

PCT is a precursor peptide of the hormone calcitonin that varies with severity of bacterial infections, where its release is induced from parenchymal cells (lung, liver, kidney, adipocytes, and muscle) (267, 268) and contributes to the deleterious effects of systemic infections. PCT rises (1 to 10 ug/liter) early in sepsis (within 3 h) and increases in concentration for up to 6 h. It remains in the bloodstream for up to 48 h but will drop quickly when BSIs are properly treated, but not with administration of steroids, as is the case for CRP.

Procalcitonin has been extensively evaluated as a marker of infection in several populations. Fernandez-Lopez et al. demonstrated improved sensitivity and specificity of PCT compared to CRP in the emergency department to differentiate bacterial from nonbacterial illnesses in febrile infants (269, 270). Van Rossum et al. reviewed a series of studies that demonstrated superior performance of PCT over CRP for both diagnostic and prognostic applications in patients with urinary tract infection and sepsis (271). In addition, PCT has demonstrated superior performance over CRP, lactate, and IL-6 in identifying bacterial sepsis in medical ICU patients (259). Kruger and colleagues demonstrated that PCT could be used as both a diagnostic and prognostic indicator for patients with community-acquired pneumonia, with improved prognostic ability over the CRP and the WBC count (272).

Clinical use of these tests requires rapid turnaround time and places the microbiology laboratory in a position either to provide 24-7 support for these assays or to share medical guidance with a STAT laboratory where testing is performed. Uptake of this test is high in Europe but less so in the U.S., where is it more commonly used for antimicrobial stewardship than diagnosis. Since there are diagnostic limitations with all biomarkers, for example in cases where only a small quantity of PCT is induced and simultaneous induction occurs due to trauma, clinical consultation for interpretation of these results is critical. Therapy may be initiated or altered based on infectious disease biomarkers, and clinical microbiology laboratories will be challenged to expand test menus to accommodate new biomarker technology.

**EMERGING TECHNOLOGY**

The future of clinical diagnostics is anticipated to include a variety of rapid and multiplex methods. Review of all technologies is beyond the scope of this review; however, several will be discussed, including multiplex-PCR, DNA sequencing, liquid microarrays, and PCR-electrospray ionization mass spectrometry. It is known that blood culture bottles, positive for bacteria, do not always support cultivation of the pathogen to agar (174, 273); therefore, new molecular methods may allow microbiology laboratories to identify fastidious pathogens.
or those damaged by antibiotics more efficiently. More detailed information about the following techniques may be found in other reviews (274, 275).

Automated Microscopy: ID/AST
A novel combination of automated microscopy paired with electrophoresis of microbes and FISH genetic probes, the Accelerate instrument (Accelerate Diagnostics, Tucson, AZ) utilizes both genomic and phenotypic detection technologies to significantly decrease time to result while sustaining high sensitivity and specificity. This process will occur on a circular cartridge. In a recent report, susceptibility of S. aureus was characterized by assessing immobilized live bacterial cells and differentiating them as susceptible or nonsusceptible using as few as 10 growing clones after 4 h of growth, compared to overnight growth required for traditional culture-based methods (193). In this report, 77/77 MRSA phenotypes and 54/54 MSSA phenotypes were correctly identified. In addition, 53/56 clindamycin-resistant and 75/75 clindamycin-susceptible strains were detected, and heterogeneous and inducible resistance was characterized (193).

PCR Mass Spectrometry, Genetic Profiling Directly from Blood Culture Bottles
PCR/electrospray ionization mass spectrometry from Abbott/Ibis has recently been adapted and developed for rapid identification, strain typing, and antibiotic-resistance assessment (276–278), including S. aureus genetic targets (239, 279). In a recent publication, Baldwin et al. provided evidence for the technology’s ability to identify a broad range of pathogens, such as those that may be encountered in blood cultures (278). With accuracy listed as 91% for the genus level, there is room for improvement; however, the approach shows promise as a rapid method for identification of pathogens.

In this approach, broad-range primers are used to amplify PCR products from groupings of microbes, rather than primers targeted to a single target or species. Electrospray ionization time-of-flight mass spectrometry is used to determine the nucleic base composition (the numbers of A, C, G, and T) of the PCR amplicon, and this base composition signature is used to identify the pathogens present. Unlike nucleic acid probe assays or microarrays, mass spectrometry does not require prior knowledge of the products analyzed; rather, it simply measures the masses of the nucleic acids present in the sample. The platform is significantly different from previous technologies in its ability to detect virtually all microbes from a family and even microbes that have mutated significantly. This technology has the potential for identification of all known human microbial pathogens in 4 to 6 h from blood or sterile body fluids (280, 281). The newest version of this technology, the IRIDICA BAC BSI assay, is currently in FDA clinical trials.

Molzyme SepsiTest
Another PCR method is based on the detection of conserved sequences in whole blood for rapid diagnosis of bacteremia and fungemia. The SepsiTest is not commercially available in the United States. The test uses a universal PCR from the 16S and 18S rRNA genes, with subsequent identification of bacteria and fungi, respectively, from positive samples by sequence analysis of amplicons. The SepsiTest was evaluated in a prospective, multicenter study of 342 blood samples from 187 patients with systemic inflammatory response syndrome, sepsis, or neutropenic fever. Compared to blood culture, the diagnostic sensitivity and specificity of the PCR were 87.0 and 85.8%, respectively, achieving results with improved accuracy over the SeptiFast, and yielding results in a few hours. The concordance of PCR and blood culture for both positive and negative samples was 86.0% despite the indispensability of blood culture diagnostics (282, 283). In another study employing 20 patients with infective endocarditis and 10 patients without infective endocarditis, heart valves and preoperative blood samples were analyzed by blood culture and SepsiTest. The diagnostic sensitivity of the molecular test was 85% and that of blood culture was only 45%, remaining negative in many cases as a result of antibiotic treatment. Also, etiological agents were identified by SepsiTest in 5/10 patients without infective endocarditis, whereas blood cultures were negative (284).

Immunexpress
Immunexpress (Seattle, WA) has developed a multiplex, quantitative real-time PCR and RT-PCR combination (RT1qPCR) in a ready-to-use kit, now in clinical trials in the United States. Their SeptiCyte test was developed to be utilized in the microbiology laboratory using whole blood collected from patients soon after ICU admission. The test analyzes gene expression using RNA extracted from whole blood of patients suspected of having sepsis. The test determines the level of expression of four genes, resulting in an expression profile reflecting several key biological pathways linked to the host response to infection. These genes have been identified from a panel of previously discovered biomarkers identified in gene expression array and RT1qPCR studies. This test, with
other laboratory findings and clinical information, is intended to aid in the detection and clinical assessment of systemic inflammation due to infection (sepsis) in critical-care patients. The SeptiCyte Lab signature and associated score was validated in a Netherlands-based clinical study, in which sepsis and infection-negative systemic inflammation cohorts were separated with a negative predictive value of 95.5% and positive predictive value of 74.7% (285).

Q-linea
Q-linea’s (Uppsala, Sweden) fully automated, ASTrID instrument provides microbial identification of fungi and Gram-negative and Gram-positive bacteria together with resistance marker mapping within one working day. The technology is based on highly specific padlock probes and circle-to-circle isothermal nucleic acid amplification technology. The ASTrID platform is currently under development, with the goal to start CE-IVD validation in 2018 followed by FDA certification shortly thereafter.

SUMMARY
Bacteremia and sepsis are critically important syndromes with high mortality and associated costs. BSIs and sepsis are among the top causes of mortality in the United States, killing over 600 people per day. Many septic patients are treated in emergency medicine departments or critical care units, settings in which rapid administration of targeted antibiotic therapy drastically reduces mortality. Unfortunately, routine blood cultures are too slow to support rapid therapeutic interventions. As a result, empiric, broad-spectrum treatment is common—a costly approach that may fail to effectively target the correct microbe, may inadvertently harm patients via antimicrobial toxicity, and may contribute to the evolution of drug-resistant microbes. To meet these diagnostic challenges, laboratories must understand the complexity of diagnosing and treating septic patients, in order to focus on creating algorithms that help direct targeted antibiotic therapy and synergize with existing emergency medicine department and critical care unit clinical practices put forth in Surviving Sepsis Guidelines. Immunocompromised hosts are particularly impacted by BSIs and sepsis, and there are numerous conditions that compromise patients and increase their risk and decrease their potential for survival.

Several methods for rapid molecular identification of pathogens from blood culture bottles are available. Labs can integrate with overall care to support local Surviving Sepsis campaigns by providing rapid testing to facilitate targeted therapeutic interventions for infections with common pathogens. As a result, empiric, broad-spectrum antibiotic therapy can be shortened to improve survival, reduce health care costs, and decrease antibiotic resistance. More evidence and clinical utility studies are needed to justify the added expense of molecular methods and to determine an appropriate niche in patient populations for which the cost–benefit would be favorable. The PNA FISH methods show much promise for clinical utility and cost-effectiveness; however, the accuracy and cost–benefit of multiplex PCR remains to be fully evaluated. Pyrosequencing and PCR/ESI-MS have proven accuracy and await practical adoption to routine microbiology laboratories. MALDI-TOF MS shows real promise and is currently being adapted to rapid identification of pathogen isolates and potentially for direct detection from positive blood culture bottles. Molecular diagnostic assays for detection of single pathogens from blood culture bottles have already been shown to have an impact by reducing mortality and decreasing costs. In the future, tests for multiple pathogens that could characterize Gram-positive, Gram-negative, and fungal infections would enable more rapid and targeted antimicrobial interventions for those with severe disease. Targeted drug-resistance gene testing of blood culture bottles will enable risk assessment and guide treatment options for sepsis. Ultimately, early intervention by molecular detection of bacteria and fungi directly from whole blood would provide the most benefit to patients and would contribute to tailored antibiotic coverage of the patient early in the course of the disease, allowing for more effective treatment and better outcomes for patients with sepsis and septic shock.

Over a century has passed since the introduction of blood cultures for the diagnosis of bloodstream infections. Significant advances have been made in improving blood culture media; as yet, no molecular or antigen-based method has proven superior for the detection of bacteremia. Our ability to make a rapid and reliable diagnosis of fungemia, however, remains elusive. Laboratorians can continue to optimize the use of blood cultures for the diagnosis of BSIs and to encourage their clinicians to obtain at least two sets of blood cultures by separate venipuncture before starting antimicrobial therapy. When blood cultures are obtained through indwelling lines or from a single venipuncture, positive blood culture results can be misleading and can result in diagnostic confusion and injudicious use of antimicrobial therapy. Although blood cultures remain the best means of diagnosing bacteremia and candidemia,
complementary testing with antigen tests, microbiologic investigations from other body sites, and histopathology can often aid in the diagnosis of disseminated disease.

REFERENCES


Bloodstream Infections


