INTRODUCTION

One of the most important functions of the clinical microbiology laboratory is the detection and characterization of organisms causing bloodstream infections. Several preanalytical considerations have a considerable impact on downstream results for blood cultures. The laboratory, with input from key stakeholders, selects blood culture media types and provides guidance on collection methods and collection site sterilization, the volume of blood to be collected, and downstream testing options for positive blood cultures. This article provides an update on recent data and developments in each of these areas.

SELECTION OF MEDIA AND ADDITIVE TYPES FOR BLOOD CULTURE

Most modern media formulations are similar, a base of soybean casein digest (trypticase soy broth) with sodium polyanethol sulfonate (SPS) as an anticoagulant. The

KEYWORDS

- Blood culture
- Bloodstream infections
- Contamination
- Sepsis

KEY POINTS

- Considerable improvements can be made in preanalytical aspects of blood culture collection that impact patient care.
- There are newer methods developed in recent years to improve time to reporting blood culture isolate identification and antibiotic susceptibility patterns.
- The successful utilization of rapid identification and antimicrobial susceptibility results requires coordination with antimicrobial stewardship programs.
headspace of the bottles consists of CO₂ and N₂ for anaerobic bottles and ambient air supplemented with CO₂ for aerobic bottles. Anaerobic bottles also include reducing agents. Two major decisions are whether to utilize bottles with polymeric resin beads to neutralize antibiotics, and if one’s laboratory serves a pediatric population whether to use pediatric-specific bottles (aerobic only, modified media formulation, lower media volume, and lower SPS concentration). Resins can neutralize select antibacterial agents (including common empirically utilized antibiotics such as piperacillin-tazobactam, vancomycin, and some cephalosporins), but have lower to no ability to neutralize other agents (eg, carbapenems and fluoroquinolones). Resins improve the yield of blood cultures for patients who are on antibiotics at the time of blood collection; this has clear advantages for initial blood cultures but also results in more positive cultures for patients subsequently on therapy, and thus more laboratory expense. The clinical significance of subsequent positive cultures in bottles with resins is unclear. Pediatric bottles have been compared with standard aerobic bottles in vitro, and also compared with aerobic and anaerobic bottles in clinical evaluations. Bloodstream infections in children are rarely caused by strict anaerobes. In the largest study to date that included aerobic and anaerobic culture in a pediatric population, only 15 of 723 clinically significant isolates (2.1%) were strict anaerobes isolated from anaerobic bottles. Bacteremia with strict aerobes may be more common in this population. This does not mean, however, that utilizing anaerobic bottles could not contribute to increased sensitivity for pediatric cultures. A more recent study demonstrated improved yield with 1 mL of blood divided evenly between aerobic (BACTEC Peds Plus/F) and anaerobic bottles (68/72, 94.4%) compared with 1 mL added to 1 pediatric bottle (56/72, 77.7%). The additionally detected organisms using the combination of aerobic and anaerobic bottles were mostly facultative anaerobes rather than strict anaerobes. Studies to date have either not specifically evaluated fastidious organisms (such as *Neisseria*) or are too small to expect a meaningful number of such isolates to occur in clinical evaluations; thus it is not clear if the modified media formulation or reduced SPS would offer advantages for these types of organisms in a larger study or clinical use. It seems that loading the entire volume into a pediatric bottle or dividing it evenly between a pediatric aerobic bottle and a standard anaerobic bottle are both reasonable and justifiable approaches to pediatric blood culture.

**PREVENTING CONTAMINATION OF BLOOD CULTURES**

Contamination of blood cultures has a big impact on patient care and hospital resources. Several key factors that can reduce contamination rates have been clearly demonstrated. Skin sites for collection should be disinfected with an alcohol-containing disinfectant, and blood should not be collected from an intravascular device unless specifically requested out of concern it is the source of bacteremia. Additionally, ongoing education should be provided to phlebotomy staff and other blood collectors, and methods or devices that divert an initial volume of blood can significantly reduce blood culture contamination rates. In fact, it is possible to make so much progress relative to historic baselines that a group of experts on the topic recently proposed changing the longstanding performance standard for US health care institutions, currently set at less than 3% of blood cultures contaminated, with the proposal to consider lowering this expectation to less than 1% contamination. As noted, the use of commercial diversion devices reduced contamination rates, which in models is projected to lead to considerable cost savings and reductions in patient length of stay. In 2 recent studies, blood culture contamination was
reduced without using expensive commercial diversion devices, by diverting a small amount of blood using vacutainer tubes by changing the test draw order, which could also be accomplished for blood cultures alone by simply discarding the diverted portion.\textsuperscript{15,16}

**VOLUME OF BLOOD COLLECTED FOR CULTURE**

The volume of blood collected for blood culture is the single most important variable in the ability of the microbiology laboratory to detect microorganisms causing bloodstream infections.\textsuperscript{17,18} The American Society for Microbiology (ASM) and the Infectious Diseases Society of America (IDSA) jointly recommend 2 to 4 collections per septic episode, with each collection consisting of 20 to 30 mL divided among 2 or 3 bottles.\textsuperscript{19} Thus, at a minimum, 40 mL total volume should be cultured per septic episode, with 2 important exceptions. The IDSA recommends that for neutropenic patients, blood collected for culture should be limited to 1% of total blood volume (usually approximately 70 mL/kg) in patients weighing less than 40 kg.\textsuperscript{20} The other exception is pediatric patients, for whom there are various recommendations for blood culture volumes based on age or weight, with those from IDSA cited here.\textsuperscript{19} The volume of blood collected and number of bottles collected can be increased without increasing the number of collections by increasing the volume at each collection to 30 mL in 3 bottles (2 aerobic bottles and 1 anaerobic bottle in this study) instead of 20 mL, which could improve causative microorganism recovery without significantly increasing costs.\textsuperscript{21}

Although blood collection happens outside of the laboratory, ensuring that adequate volume is collected is the direct responsibility of laboratory administration. The College of American Pathologists (CAP) requires that accredited laboratories have a system in place to evaluate adult blood culture volume and communicate these data at regular intervals to clinical staff responsible for blood collection. Laboratory-initiated education and quality improvement projects can lead to marked improvements in blood volume submitted. One group found that targeted education in intensive care units (ICUs) led to 30% to 60% sustained improvement in blood volume submitted on average.\textsuperscript{22} Another recent study found that a rigorous quality improvement program that included group education, targeted communications, and bottle marking led to not only considerably increased blood volume (increased from 2.3 mL average per bottle before implementation to 8.6 mL after implementation) but most importantly a 20% improved yield of true pathogens in blood culture.\textsuperscript{23}

**BLOOD CULTURE SYSTEMS AND ALTERNATIVE BLOOD CULTURING METHODS**

Numerous improvements in blood culture incubation and monitoring have occurred over time, culminating in the current continuous-monitoring blood culture system (CMBCS). Three US Food and Drug Administration (FDA)-cleared CMBCS are available, including BACTEC (Becton-Dickinson, Sparks, MD, USA), BacT/Alert (bio-Mérieux, Inc., Durham, North Carolina) and VersaTREK (Thermo Scientific, Waltham, Massachusetts). Key features are summarized in Table 1. All 3 systems detect microbial growth via some form of gas detection but utilize distinct methods. Delays in getting bottles onto the CMBCS can result in delayed positive results or false-negative results. In a 30-month retrospective study of 50,955 blood cultures, where cultures were only loaded onto the CMBCS during the morning to late afternoon or otherwise held on the hospital floor at room temperature, 13.0% were positive while the laboratory was open, but only 10.8% were positive when the laboratory was closed.\textsuperscript{24}
Table 1
Summary of current continuous-monitoring blood culture system features

<table>
<thead>
<tr>
<th>Blood Culture System</th>
<th>Method for Monitoring Growth</th>
<th>Bottle Types</th>
<th>Additional Information</th>
<th>FDA-Cleared Indications</th>
<th>Max Fill Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>BacT/ALERT</td>
<td>Colorimetric change caused by drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT FA PLUS</td>
<td>Aerobic media with adsorbent polymeric resin beads</td>
<td>Blood or SBF</td>
<td>10 mL</td>
</tr>
<tr>
<td>BacT/ALERT FA PLUS</td>
<td>Colorimetric change caused by drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT FN PLUS</td>
<td>Anaerobic media with adsorbent polymeric resin beads</td>
<td>Blood or SBF</td>
<td>10 mL</td>
</tr>
<tr>
<td>BacT/ALERT PF PLUS</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT SF PLUS</td>
<td>Pediatric, standard aerobic</td>
<td>Blood</td>
<td>4 mL</td>
</tr>
<tr>
<td>BacT/ALERT SA</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT SN</td>
<td>Standard aerobic</td>
<td>Blood or SBF</td>
<td>10 mL</td>
</tr>
<tr>
<td>BacT/ALERT BPA</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT BPN</td>
<td>Aerobic media</td>
<td>Platelets</td>
<td>10 mL</td>
</tr>
<tr>
<td>BacT/ALERT BPA</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BacT/ALERT BPN</td>
<td>Anaerobic media</td>
<td>Platelets</td>
<td>10 mL</td>
</tr>
<tr>
<td>BACTEC</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BACTEC Plus Aerobic</td>
<td>Aerobic media with adsorbent polymeric resin beads</td>
<td>Blood</td>
<td>10 mL</td>
</tr>
<tr>
<td>BACTEC Plus Aerobic</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BACTEC Plus Anaerobic</td>
<td>Anaerobic media with adsorbent polymeric resin beads</td>
<td>Blood</td>
<td>10 mL</td>
</tr>
<tr>
<td>BACTEC Peds Plus</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BACTEC Lytic Anaerobic</td>
<td>Anaerobic media with detergent to lyse RBCs and WBCs</td>
<td>Blood</td>
<td>10 mL</td>
</tr>
<tr>
<td>BACTEC Lytic Anaerobic</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BACTEC Standard Aerobic</td>
<td>Standard aerobic</td>
<td>Blood</td>
<td>10 mL</td>
</tr>
<tr>
<td>BACTEC Standard Anaerobic</td>
<td>Change in fluorescence caused by a drop in pH from increased CO₂ levels</td>
<td>BACTEC Myco/F Lytic</td>
<td>Mycobacterial/fungal media</td>
<td>Blood</td>
<td>5 mL</td>
</tr>
<tr>
<td>VersaTREK</td>
<td>Measures pressure changes caused by gas consumption or production</td>
<td>REDOX 1</td>
<td>Aerobic</td>
<td>Blood or SBF</td>
<td>10 mL</td>
</tr>
<tr>
<td>VersaTREK</td>
<td>Measures pressure changes caused by gas consumption or production</td>
<td>REDOX 2</td>
<td>Anaerobic</td>
<td>Blood or SBF</td>
<td>10 mL</td>
</tr>
<tr>
<td>VersaTREK Myco</td>
<td>Measures pressure changes caused by gas consumption or production</td>
<td>REDOX 1 EZ Draw</td>
<td>Aerobic, direct blood inoculation</td>
<td>Blood or SBF</td>
<td>5 mL</td>
</tr>
<tr>
<td>VersaTREK Myco</td>
<td>Measures pressure changes caused by gas consumption or production</td>
<td>REDOX 2 EZ Draw</td>
<td>Anaerobic, direct blood inoculation</td>
<td>Blood or SBF</td>
<td>5 mL</td>
</tr>
<tr>
<td>VersaTREK Myco</td>
<td>Measures pressure changes caused by gas consumption or production</td>
<td>VersaTREK Myco</td>
<td>Mycobacterial media</td>
<td>Blood, SBF or processed specimen</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Abbreviation: SBF, sterile body fluids.
The current BACTEC CMBCS model is the BACTEC FX, which is available in different sizes depending on the capacity required. Bottles for the BACTEC system contain fluorometric sensors that fluoresce in the presence of CO₂ via acidification. Specialized media (BACTEC Myco/F Lytic) for improved recovery of Mycobacteria and fungi are available.

The current BacT/Alert system models include the BacT/Alert 3D and the recently introduced BacT/Alert VIRTUO. Both systems detect CO₂ production via a colorimetric system present in the bottom of the bottles that changes color with pH. The BacT/ALERT VIRTUO system automates bottle loading and unloading, helping to maintain temperature stability. During the loading process, the instrument scans the bar-coded labels, determining the fill-level in the bottles, while on the instrument an improved algorithm shortens the time to positive detection. In 1 study comparing the BacT/ALERT VIRTUO with the BacT/ALERT 3D, 115 clinical isolates were inoculated into blood cultures, and although both systems demonstrated similar detection rates, the BacT/ALERT VIRTUO reduced time to detection by approximately 20%.25 Similarly, a reduced time to detection was observed with BacT/ALERT VIRTUO in a multicenter study with clinical specimens, which was statistically significant for gram-negative bacilli and enterococci.26 The type of BacT/ALERT bottle used determines the maximal volume inoculated. Additionally there are BacT/ALERT bottles FDA-cleared for sterile body fluids and platelet sterility testing (see Table 1).

Unlike the other CMBCS, microbial growth detection by the VersaTREK system requires an adapter to be affixed onto the bottles before instrument loading. This adapter allows the instrument to monitor pressure changes in CO₂, O₂, H₂, and N₂ caused by gas production or consumption by growing microorganisms. Additionally, the VersaTREK system is FDA-cleared for culturing mycobacteria from blood, processed specimens, and sterile body fluids, and for Mycobacterium tuberculosis susceptibility testing.

There are numerous studies comparing the CMBCS and media types. Recent examples will be highlighted. Overall, the CMBCS perform similarly in many aspects with some differences. One study compared the BacT/ALERT VIRTUO and BACTEC FX system using contrived bottles with differing (125 CFU/mL, 30 CFU/mL, and 5 CFU/mL) microorganism concentrations, for a total of 405 comparisons.27 Although both systems flagged positive for all cultures at 125 CFU/mL and 30 CFU/mL, the BacT/ALERT VIRTUO demonstrated a lower false-negative rate (5.2%) at 5 CFU/mL compared with the BACTEC FX (8.1%), but no statistically significant difference was noted in the time to detection.27 In contrast, another simulated study inoculated 2610 bottles with 30 CFU/mL of 330 clinical relevant species and compared the BacT/ALERT Virtuo, BacT/ALERT 3D and the BACTEC FX system. Although all 3 systems flagged positive for 99.6% of all cultures, the median time to positivity for the BacT/ALERT VIRTUO was significantly shorter.28 A previous study comparing the BacT/ALERT 3D and VersaTREK using patient specimens found no overall difference in time to positivity or isolation of microorganisms, but there was a higher false-positive rate with the VersaTREK system (ie, 1.6% vs 0.7% for aerobic bottles).29 However, the VersaTREK demonstrated a statistically significant higher recovery of streptococci and enterococci.29

As noted, monitoring and feedback to collectors of inadequate blood culture volumes is a CAP requirement, and the BacT/Alert and BACTEC can assist in volume determination. The BacT/Alert Virtuo scans the sample level to determine the inoculated volume. An evaluation of the BacT/Alert Virtuo volume monitoring of 1141 bottles was compared with weight-based volume determination.30 Although the results between the BacT/Alert Virtuo and weight-based volume strongly correlated (r = 0.87),
the BacT/Alert Virtuo tended to overestimate volumes (median difference of 1.4 mL for the aerobic bottle and 0.2 mL for the anaerobic bottle). Software for the BACTEC FX, BD EpiCenter, determines the mean blood volume from at least 25 culture-negative aerobic bottles by measuring red blood cell metabolic activity. This measurement method means that low hematocrit levels and delays in getting bottles on the instrument can give inaccurate results. One study compared the BACTEC FX to weight-based volume determination, initially found that the BACTEC FX had a mean underestimation of 1.4 mL but this was reduced to a mean underestimation of 0.3 mL with a software upgrade. However, this improved software demonstrated a mean overestimation of 2.8 mL with plastic BACTEC bottles, but further updates were planned to adjust for plastic bottles.

In addition to the CMBCS, some laboratories use lysis centrifugation methods to culture blood for molds and mycobacteria. Note that CMBCS are the preferred method for recovery of *Candida* species and *Cryptococcus* species. In a 10-year retrospective study, the Isolator system recovered the lipid requiring *Malassezia* species and isolated dimorphic and filamentous fungi not recovered in CMBCS. However, this same study found that the Isolator rarely provided results not available by other methods, and there were many instances of fungal isolates deemed to be clinically insignificant.

**INCUBATION TIME**

Blood cultures are routinely incubated for 4 to 7 days with CMBCS. Studies indicate that 98% to 99% of true pathogens are detected within 5 days of incubation relative to longer incubation times. Five days of incubation appear to be optimal for balancing increased recovery versus occupying incubator space unnecessarily and delaying final reporting for negative cultures. It may be reasonable, however, to shorten incubation times to 4 days or even 3 days if equipment failure or a surge of demand for blood cultures (eg, during the COVID-19 pandemic) leads to a sudden demand for more CMBCS space than laboratory infrastructure can accommodate. Several studies demonstrated only modest reductions in true pathogen recovery with 3 to 4 days of incubation relative to longer times. Although routine incubation beyond 5 days is not necessary with CMBCS, even for HACEK organisms, it may be justified to hold cultures for a longer period of time on request in select cases.

**PROCESSING POSITIVE BLOOD CULTURES: STAINING METHODS AND IMPACT OF GRAM STAIN CALL TIME**

The first analytical step in processing a positive blood culture is probably the most impactful step for patient care: the Gram stain. Studies have demonstrated that for therapeutic interventions, the reporting of Gram stain results to the patient care team was more impactful than reporting final antimicrobial susceptibility testing (AST) results. The timing of Gram stain reporting matters too. In many clinical microbiology laboratories it can be difficult to provide highly skilled Gram stain readers on off shifts for rapid turnaround of positive blood culture Gram stains. Gram stain turn-around time for blood cultures was shown in a relatively small study to correlate with mortality, with increased mortality among patients with delayed Gram stain reporting. Infrastructure can also be developed to allow for remote consultative review of blood culture Gram stains so that more experienced readers can assist technologists at remote laboratory sites if needed to improve accuracy. Some microorganisms stain poorly or not at all with Gram reagents, and for positive blood cultures with negative Gram
stains there are alternative stains, such as acridine orange, which can be employed in addition to repeating Gram stains.

**RAPID IDENTIFICATION FROM POSITIVE BLOOD CULTURES: MOLECULAR TESTING**

There are several commercial, FDA-cleared, molecular tests available for the rapid identification (<2 hours) of organisms in positive blood culture bottles. These tests may target a single organism (eg, *Staphylococcus aureus*), or large panels of organisms tested simultaneously (gram-negative and gram-positive bacteria on the same test) or organized by Gram stain results “Panels and Syndromic Testing in Clinical Microbiology”. Molecular testing direct from blood culture bottles is sometimes negatively impacted by residual microbial nucleic acid in commercial blood culture media (sterile, but not always genetically inert), leading to false-positive results; this has led to product recalls for issues related to false-positive *Enterococcus* and *Proteus* identification in recent years. Blood culture molecular identification panels generally also include genetic markers of resistance for such targets as *mecA* when *Staphylococcus* is detected, *vanA/vanB* when *Enterococcus* is detected, and ESBL or carbapenemase genes such as KPC when gram-negative rods are detected “Panels and Syndromic Testing in Clinical Microbiology”.

**RAPID IDENTIFICATION FROM POSITIVE BLOOD CULTURES: MASS SPECTROMETRY**

Bacteria and yeast in positive blood culture bottles may also be directly identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), a technology commonly employed for identification of bacteria and yeast colonies. Whereas colony growth can be tested directly, organisms in blood culture broth must be processed to separate the organism from blood cells for testing, and various methods have been evaluated. Identification accuracy for direct MALDI-TOF from blood culture bottles is typically better for gram-negative bacteria than for gram-positive bacteria, and can be improved by a short incubation on agar (smudge plate, 4–8 hours, 84%–97% with correct identification) prior to MALDI-TOF analysis.

**RAPID, DIRECT, PHENOTYPIC ANTIMICROBIAL SUSCEPTIBILITY TESTING OPTIONS**

The Accelerate PhenoTestBC kit used in conjunction with the Accelerate Pheno system (APS) is the first FDA-cleared in vitro diagnostic test for pathogen identification and quantitative AST directly from positive blood cultures. The APS uses multiplexed fluorescence in situ hybridization (FISH) to identify on-panel microbes based on the colocalization of target-specific probes with a universal bacterial probe in the same cell. The minimum inhibitory concentration (MIC) is calculated from analyzing the cellular morphokinetic growth pattern in the presence of antimicrobial agents. The APS assay can provide pathogen identity and quantitative AST phenotype within 2 and 7 hours, respectively. However, whether this rapid turnaround time (TAT) translates into more favorable clinical outcomes remains to be determined.

Studies using the APS assay have reported pathogen identification sensitivities ranging from 91.2% to 100% and specificities ranging from 97.3% to 100% for organisms on the panel. However, 1 study reported an overall false-positive rate as high as 9.5%, of which 42.1% is mitigated by having prior negative Gram stain. Therefore, confirmatory Gram stain should be performed before performing the APS assay. AST results have demonstrated essential agreements (EAs) ranging from 94.5% to 97.6% and categorical agreements (CAs) ranging from 86% to 97.9% compared with routine methods. Importantly, lower CA values have been observed.
for *Pseudomonas* species and *Acinetobacter* species. Because assay performance varies depending on the pathogen, laboratories should decide on which organisms to perform the APS assay to best complement existing techniques and improve resulting times. Although this assay has high sensitivity for target organisms, in 1 study only 58.1% to 88.7% of clinical samples analyzed by this assay received any pathogen identification. This low overall sensitivity highlights the need to perform routine methods in parallel with the APS assay.

There is much interest in the application of FDA-approved automated AST platforms intended for use on isolated colonies, such as the BD Phoenix and Biomérieux VITEK2 systems, directly on positive blood culture samples. The Phoenix and VITEK 2 systems are capable of performing colorimetric-based biochemical identification and turbidimetric-based quantitative AST with TATs of 3 to 16 hours. Studies report variable performance of the Phoenix system depending on the species involved and the method of microbial extraction. For instance, the identity concordance of direct inoculation compared with standard procedure ranges from 82% with saponin-based to 92.9% to 95.2% with serum separation tube (SST)-based extraction methods. AST results using the Phoenix system similarly vary, with an overall CA of 77% and 95.4% to 99% when using saponin- and SST-based methods, respectively. Studies of direct phenotypic AST using the VITEK 2 system compared with standard techniques have similarly reported high identity concordance rates of 82.2% to 95.8% and AST CAs of 94.6% to 99.2% for gram-negative bacilli (GNB). Performance for gram-positive cocci (GPC) may not be as reliable. However, parallel MALDI-TOF MS identification and VITEK 2 for AST can provide reasonably accurate results for GNB and GPC. Investigations comparing direct AST results using the VITEK 2 and Phoenix systems have also reported high CAs, ranging from 92.4% to 99.5%.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently published a rapid AST (RAST) method based on direct disk diffusion (dDD) testing of positive blood cultures on Mueller-Hinton (MH) or Mueller-Hinton Fastidious agar plates. Zone diameter breakpoints after 4, 6, and 8 hours of incubation for 8 common bacterial species and relevant antibiotics are provided. Typical workflow begins with a Gram stain of positive blood culture, followed by inoculating the appropriate subculture plates and a RAST plate with the antibiotic disks of choice. Inhibition zone diameters after 4, 6, and 8 hours on the RAST plate can then be interpreted. In general, the EUCAST RAST method has CAs of greater than 90% when compared with standard method. Similarly, a Clinical and Laboratory Standards Institute (CLSI) committee has recently released some promising preliminary results. Given their low costs and ease of use, standardized dDD methods may eventually allow more laboratories to perform direct phenotypic ASTs.

**OTHER DIRECT ENZYME-BASED TARGETED ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS**

Detection of bacterial antimicrobial resistance enzymatic activity is widely used for the qualitative phenotypic AST on isolated bacteria. Because these assays are relatively cheap and simple, many have explored their use directly on positive blood cultures to test for the presence of β-lactamase and/or carbapenemase-producing gram-negative bacteria, which are of great clinical concern internationally. Colorimetric methods usually start with microbial extraction from positive blood cultures using lysis centrifugation or a short subculture on solid media. The ideal choice of hemolytic agent varies depending on the assay. For example, 10% SDS is more compatible with the β-CARBA test, while 5% saponin is with the Carba NP and NeoRapid CARB tests.
Variations of CLSI’s modified carbapenem inactivation method (mCIM) have also been used for bacteria with ESBL and carbapenemase activity directly from positive blood cultures. Variation of the method include incubation with either a meropenem or a cephalosporin disk at 35°C to 37°C for approximately 2 hours. Zinc supplementation can be used for metallo-β-lactamase activity detection. The impregnated disk is then applied on an MH plate that is inoculated either freshly or 2 hours previous with a 0.5 McFarland suspension of a standard antibiotic-susceptible strain. The inhibition zone is read and interpreted after 6 to 22 hours of incubation.

Overall, both colorimetric and mCIM-based methods demonstrate high specificities (>90%) and sensitivities (80%–100%) for the detection of β-lactamase and/or carbapenemase-producing Enterobacterales extracted directly from aerobic blood culture bottles. Resulting times between 20 minutes to 6.5 hours have been reported for colorimetric methods, and between 8 to 24 hours for mCIM-based methods. Although these early results are generally promising, more studies must be done to standardize methodology and result interpretation before clinical use.

STEWARDSHIP/UTILIZATION

By March of 2020, the US Centers for Medicare and Medicaid Services required all acute care hospitals to implement antimicrobial stewardship programs (ASPs). ASPs ensure that patients receive appropriate antimicrobial regimens, which improves patient outcomes, reducing adverse events (eg, toxicity, antibiotic resistance, or Clostridioides difficile infection), and allows for optimal resource utilization. To meet these goals, the US Centers for Disease Control and Prevention recommend that ASPs work with the microbiology laboratory on diagnostic stewardship and implementation of rapid diagnostic testing (RDT). In regards to blood cultures, the IDSA recommends that ASPs advocate for RDT from positive blood cultures to optimize therapy and improve outcomes.

Once blood cultures flag positive, it is through the use of RDTs, such as those described previously, that timely microorganism identification and potentially antimicrobial susceptibility/resistance information, via genotypic or phenotypic methods, can be quickly provided to clinicians. However, the impact of RDTs on patient outcomes requires ASP intervention. In a meta-analysis that included 31 observational studies (5920 patients) and various RDTs, it was found that RDTs had a mortality benefit but only in the presence of ASPs. This mortality benefit was found with both gram-positive and gram-negative infections. In addition to the mortality benefit of RDTs in combination with ASPs, this combination is also cost-effective. A cost-effectiveness evaluation of RDTs with or without ASPs was compared to conventional laboratory methods with ASP involvement. It was found that the use of molecular RDTs with ASP had an 80% chance of being cost-effective, while it was only a 41.1% change without an ASP. Thus it is imperative for laboratories to work with their ASPs to maximize the benefits of RDTs from positive blood cultures.

DISCLOSURE

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