Educational Case

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Educational Case: *Staphylococcus aureus* Bacteremia: Utilization of Rapid Diagnostics for Bloodstream Pathogen Identification and Prediction of Antimicrobial Susceptibility

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.[1]

Keywords
pathology competencies, diagnostic medicine, microbiology, antimicrobials, mass spectrometry, molecular testing, susceptibility

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Primary Objective

Objective M2.10: Mass Spectrometry in Microbiology: Explain how the application of Matrix-assisted Laser Desorption/Ionization time of Flight (MALDI-TOF) mass spectrometry in the clinical microbiology laboratory can impact patient care.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic M: Microbiology; Learning Goal 2: Antimicrobials

Secondary Objectives

Objective M1.3: Identification: Give examples of the types of testing, and their optimal usage, performed in microbiology to identify an infectious disease.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic M: Microbiology; Learning Goal 1: Pathogenesis, Diagnosis, and Treatment of Infectious Disease

Objective M2.9: Molecular Testing in Microbiology: List examples of molecular tests that are commonly used in clinical microbiology and explain how they have an important impact on clinical care.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic M: Microbiology; Learning Goal 2: Antimicrobials

Objective M2.5: Genetics of Susceptibility: Name the genetic element detected by extrapolate cefoxitin and oxacillin susceptibility tests and describe how the results for *Staphylococcus* subspecies are used to predict activity of other β-lactam antibiotics.

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Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic M: Microbiology; Learning Goal 2: Antimicrobials

Patient Presentation

A 54-year-old woman with a past medical history of diabetes mellitus is brought into the emergency department (ED) due to lethargy, slurred speech, and witnessed collapse at home. Family members report that no head trauma or seizure activity occurred and that the patient was in her usual state of health until 3 days prior to evaluation when she developed a "cold" with malaise, fatigue, cough, and fever.

Diagnostic Findings, Part 1

Physical examination in the ED reveals a well-developed, unresponsive female. Vital signs are as follows: pulse: 149/min, blood pressure 76/53 mm Hg, respirations 39/min, and temperature 105.6 °F/40.9 °C. Pulmonary auscultation reveals bilateral rhonchus sounds with faint rales and rhonchi on the right lung. Cardiac auscultation demonstrates tachycardic rate, normal S1 & S2 sounds, and no murmurs/gallops/rubs. Distal pulses are diminished. The patient is admitted to the intensive care unit on mechanical ventilation with the impression of acute hypoxic respiratory failure.

Questions/Discussion Points, Part 1

What Is the Differential Diagnosis Based on Clinical Presentation and Physical Examination Findings?

Community-acquired pneumonia (CAP) should be high on your differential diagnosis for a patient with a history of fever and respiratory symptoms such as cough and shortness of breath. Other systemic symptoms that are commonly observed in patients with CAP include chills, fatigue, malaise, pleuritic chest pain, and anorexia. On physical examination, there are some signs that can point to CAP including tachypnea, increased work of breathing, and breath sounds like rales or crackles and rhonchi.[2]

A bloodstream infection (BSI) should be highly considered in this patient who presents with history of malaise, fatigue, altered mental status, suspected infection, and organ dysfunction with respiratory failure requiring mechanical ventilation. Additional evaluation and laboratory workup are necessary to determine whether the patient meets sepsis or septic shock criteria.[3]

Metabolic derangements such as hyperglycemic hyperosmolar state or diabetic ketoacidosis should be considered in the differential diagnosis in a patient presenting with acute altered mental status, especially if there is a known history of diabetes mellitus and laboratory results are consistent with metabolic acidosis.[4]

Table 1. Defining Criteria of Severe Community-Acquired Pneumonia (CAP).[5]

Severe CAP	
One MAJOR criteria:	Or 3 MINOR criteria:
• Septic shock requiring administration of vasopressors • Respiratory failure requiring mechanical ventilation	• Respiratory rate $\geq$30 breaths/min • Pao_2/Fio_2 ratio $\leq$250 • Multilobar infiltrates • Altered mental status • Uremia, BUN $\geq$20 mg/dL • Leukopenia, WBC <4000 cells/μL • Thrombocytopenia, Plt <100 000/μL • Hypothermia, temperature <36.8 °C • Hypotension requiring aggressive fluid support

Abbreviations: BUN, blood urea nitrogen; Pao_2/Fio_2, ratio of arterial oxygen partial pressure to fractional inspired oxygen; Plt, platelet count; WBC, white blood cell count.

What Diagnostics Tests Should be Performed?

A chest X-ray and testing for respiratory infectious diseases is needed. The Infectious Diseases Society of America (IDSA) and College of American Pathologists guidelines strongly recommend obtaining pretreatment respiratory tract secretion Gram stain and culture along with blood cultures only when there is severe CAP (Table 1) or if there is concern for methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa*. For adults with CAP managed in the outpatient setting, obtaining sputum Gram stain and culture is not recommended by the IDSA.[5] *Streptococcus pneumonia* (*S pneumoniae*) and *Legionella pneumophila* (*L pneumophila*) urine antigen testing should also be ordered if available. These assays detect an antigen common to all strains of *S pneumoniae* and an antigen for serogroup 1 of *L pneumophila.* Respiratory pathogen panel molecular testing can also be considered. Commercially available real-time and multiplex-panel polymerase chain reaction (PCR) assays can detect certain bacteria causing atypical CAP such as *Mycoplasma pneumoniae* and *Chlamydophyla pneumoniae*. These assays can also detect several respiratory viruses, including influenza A and B, respiratory syncytial virus, parainfluenza, human metapneumovirus, human rhinovirus, enterovirus, adenovirus, human bocavirus, and seasonal coronaviruses. Some newer panels also include the pandemic coronavirus, SARS-CoV-2. Viruses are increasingly recognized as causes of pneumonia, which may be in part due to the increased use of molecular diagnostics.[6] Guidelines for when to use these panels for upfront testing are lacking, but they may be appropriate for critically ill patients.[7]

Testing as part of the "sepsis bundle"[8] should be immediately initiated. Blood cultures should be collected prior to administration of broad-spectrum antibiotics to increase the likelihood of isolating a pathogen. Lactate level, which is an indirect measurement of tissue perfusion, should be measured. Elevated levels represent tissue hypoxia. It is recommended to remeasure if the initial lactate is >2 mmol/L.

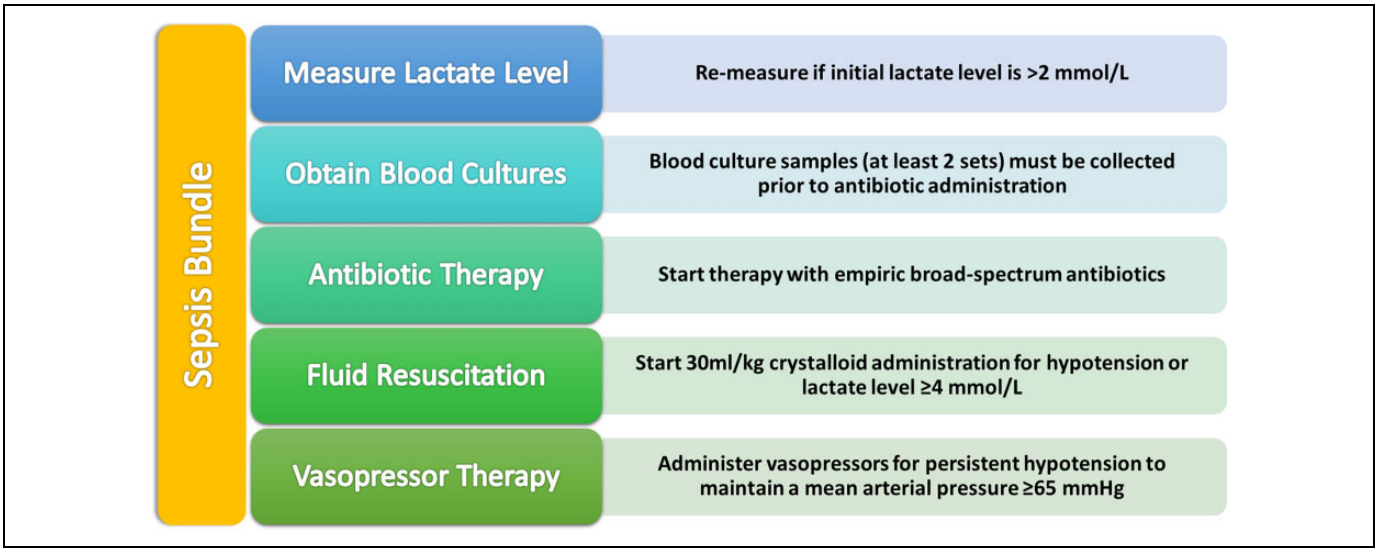

Figure 1. The surviving sepsis campaign bundle[8] recommends the hour-1 bundle upon recognition of sepsis and septic shock. The goal of implementing this bundle is to complete all elements quickly after recognition of sepsis in order to minimize time to initial resuscitation and treatment, and to improve outcomes in patients with sepsis and septic shock.

What Is the "Sepsis Bundle"?

The "sepsis bundle" is based on evidence-based guidelines that are included in the Surviving Sepsis Campaign with the final goal of improving outcomes in patients with sepsis and septic shock. This campaign reiterates that sepsis is a medical emergency that requires early recognition and proper clinical assessment. It focuses on starting the management immediately at the time of presentation with rapid restoration of perfusion and early administration of antibiotics in patients meeting the criteria. An "hour-1 bundle" is recommended which includes measuring lactate levels, obtaining blood cultures, and administering antibiotic therapy, intravenous fluid, and vasopressors (Figure 1).[8]

Diagnostic Findings, Part 2

A portable chest X-ray examination reveals multilobar pneumonia (Figure 2). Remarkable laboratory findings include a glucose level of 900 mg/dL (reference range: 70-140 mg/dL), white blood cell count of 1.9 k/μL (reference range: 4.8-10.8 k/μL), lactate level of 8.5 mmol/L (reference range <2.5 mmol/L), and 15.0 mmol/L (reference range <2.5 mmol/L) upon remeasurement. *Streptococcus pneumoniae* and *L pneumophila* urine antigens are negative.

After 14 hours, growth is detected in the aerobic blood culture bottles of 2 blood culture sets. Gram stain reveals Gram-positive cocci in pairs and clusters. Two hours later, *Staphylococcus aureus* (*S aureus*) is identified by Matrix-assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (MS) directly from the positive blood culture bottle.

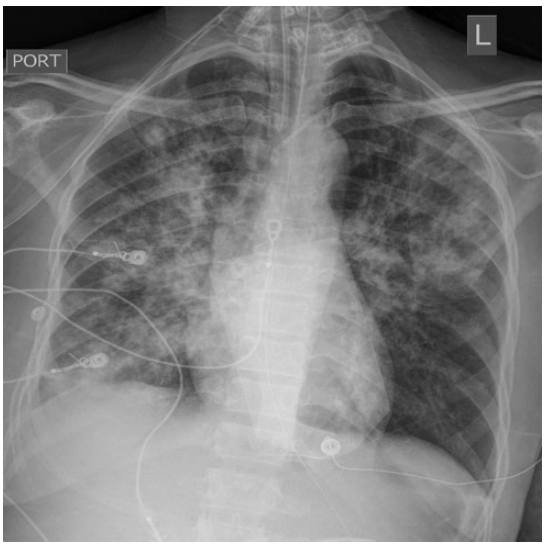

Figure 2. Portable pulmonary chest X-ray imaging reveals bilateral pulmonary infiltrates with more confluent opacity in the right lung, consistent with multilobar pneumonia.

Questions/Discussion Points, Part 2

How Do the Additional Laboratory Findings Help You Narrow the Differential Diagnosis?

Our patient presented with more than 2 severe inflammatory response syndrome (SIRS) criteria (temperature >100.4, respiratory rate >20 rpm, heart rate >90 bpm) and was hypotensive. After initial presentation, the patient developed respiratory failure requiring mechanical ventilation. In addition, this patient had an elevated lactate and a positive blood

culture with *S aureus* identified rapidly by MALDI-TOF MS. Previous criteria defined sepsis as at least 2 SIRS criteria plus confirmed or suspected infection, and our patient meets this definition. The most current definition of sepsis does not incorporate SIRS and requires that patients exhibit organ dysfunction which is determined by a scoring system that is based on many clinical parameters, including liver function, renal function, coagulation markers, Pao_2/Fio_2, mean arterial pressure, Glasgow Coma Scale score, and urine output. A more simplified metric, called the quick sequential organ failure assessment (qSOFA), can be used to identify patients at risk for life-threatening sepsis and includes meeting at least 2 of 3 criteria: altered mental status, respiratory rate >20 rpm, and systolic blood pressure $\leq$100 mm Hg.[9] Our patient meets qSOFA suggesting a final diagnosis of sepsis and a risk of poor outcome due to *S aureus* bacteremia secondary to CAP.

How Are Organisms Causing BSIs Isolated and Identified?

Blood culture analysis is considered the reference gold standard for the diagnosis of BSI. Two or 3 sets of blood cultures containing 20 to 30 mL of blood per set are required to achieve a sensitivity of 90% to 99% for the detection of bacteremia.[10] Adequate blood volume is the most critical factor for recovery of organisms in the bloodstream because the number of circulating organisms can be as low as 1 CFU/mL. Blood culture sets should be collected by peripheral venipuncture at 2 different sites, which aids in distinguishing contamination verse infection when skin colonizers are recovered. Blood cultures should be collected prior to the administration of antimicrobials, if possible, but the sets can be collected at the same time in high-acuity patients, since timing holds less significance for organism recovery.[11] Blood culture bottles are incubated in automated instruments that detect organism growth by various methods, including measurement of CO_2 production over time.[12] When growth is detected, samples from positive cultures are examined by Gram staining and plated onto agar for organism recovery. Even when rapid identification is performed by MALDI-TOF MS or molecular assays, recovery of the organism is needed for phenotypic susceptibility testing and storage in case additional testing is needed.

Gram stain examination in our case demonstrated Gram-positive cocci arranged in pairs and clusters consistent with *S aureus*. Growth on selective and differential media is used to identify pathogenic bacteria. Staphylococci grow well on sheep blood agar plates within 24 hours with colonies appearing as smooth, white or yellow-pigmentated, and usually exhibiting β-hemolysis. A variety of selective agars for the isolation of Gram-positive organisms are available including Columbia colistin-nalidixic acid agar and phenylethyl alcohol agar. These agars inhibit the growth of most Gram-negative organisms. Mannitol salt agar can be used to distinguish *S aureus* from other staphylococci. This media contains high concentrations of salt that inhibit the growth of many other bacteria and a pH indicator that turns yellow when reduced by growth of the mannitol-fermenting *S aureus*. In addition, several chromogenic agars containing cefoxitin are available to selectively isolate and differentiate MRSA.[13]

Biochemical-based identification of staphylococci should include a catalase test. The catalase test, which detects the presence of cytochrome oxidase enzymes, differentiates catalase-positive staphylococci and other members of genus *Micrococcacea* from streptococci and enterococci. Coagulase production can further differentiate the organisms, with most *S aureus* strains having a coagulase or clumping factor on the surface that reacts with fibrinogen in plasma to cause agglutination. Latex beads coated with fibrinogen and antibody that binds to the protein A on the surface of *S aureus* can be used in place of the more laborious and time-consuming tube-based coagulase assay.[14] In contrast, most staphylococci species are coagulase negative (CoNS) including *Staphylococcus saprophyticus* (*S saprophyticus*), *S epidermidis*, *S capitis*, *S hemolyticus*, *S hominis*, *S lugdunensis*, *S saccharolyticus*, and *S warneri*.[13] Although identifying *S aureus* by phenotypic methods is relatively easy, these tests require recovery of isolated colonies on solid media which can take 6 to 24 hours. Matrix-assisted Laser Desorption Ionization Time-of-Flight MS or molecular assays performed directly on a sample from the blood culture bottle can provide an identification prior to the growth of the organism on the media plates.

How Is MALDI-TOF MS Used in Clinical Laboratories for Identification of Bloodstream Pathogens?

Matrix-assisted Laser Desorption Ionization Time-of-Flight MS identification is performed through acquisition of the unique protein spectrum of a sample and then comparing this spectrum to a library of known organisms. Most species have a unique enough "fingerprint" at the protein level that allows for differentiation from other species (Figure 3). To perform MALDI-TOF MS, the organism can be directly added to an assay target or a protein extraction can first be performed. A matrix is then overlayed on top of the sample to help ionize proteins and prevent their fragmentation. Matrix-assisted Laser Desorption Ionization Time-of-Flight MS instruments use a laser to cause desorption and ionization of the sample from the target plate. These desorbed, charged analytes then accelerate within the vacuum of the mass spectrometer toward a detector. Each analyte has a specific charge and mass, and each takes a specific amount of time to hit the detector. Using these variables, a mass to charge ratio is calculated for each analyte and a spectrum, or "fingerprint" of all the analytes is generated. An analysis is then performed to determine how well the unknown fingerprint matches the most closely related organism in the reference library.[15] The number and types organisms that can be identified by MALDI-TOF MS is vast. For example, the Bruker MALDI Biotyper library contains 334 species/groups covering 425 clinically relevant bacteria and yeast (covering 98% of organisms seen in the clinical laboratory).[16] Furthermore, there are thousands of research databases that can be validated and then utilized for identification.[17]

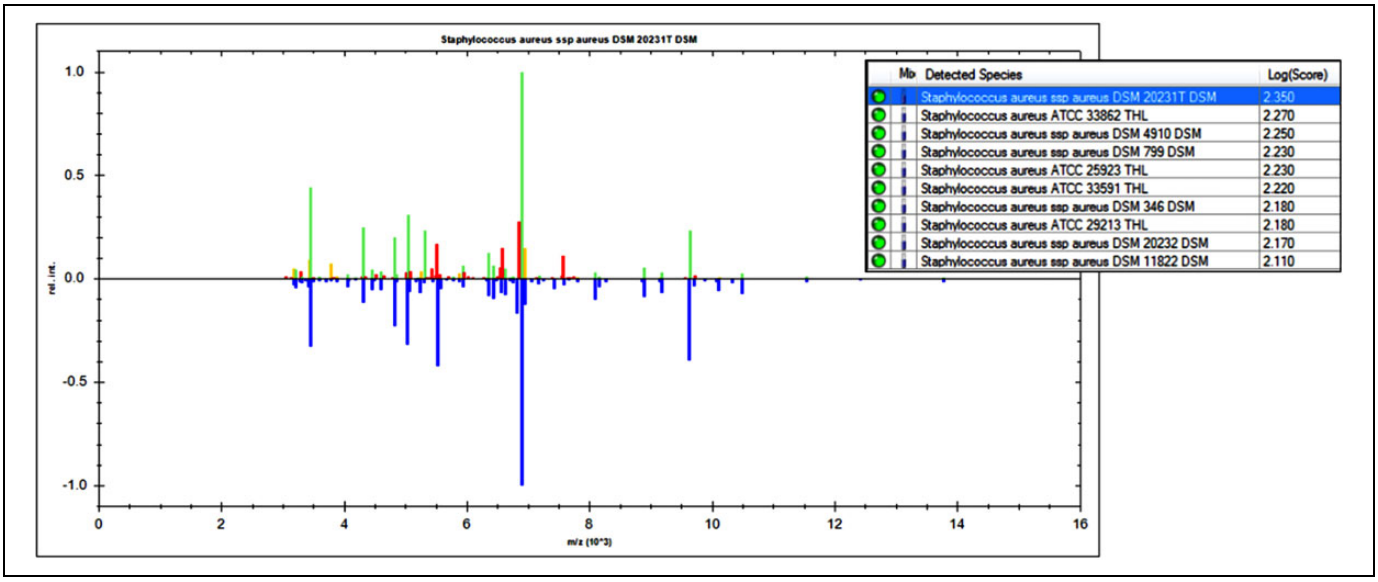

Figure 3. Matrix-assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (MS) spectrum collected on a Bruker MALDI Biotyper instrument. The spectrum illustrates the analytes detected in the sample prepared from the blood culture bottle (top panel) compared to the best matching reference organism in the library (blue, bottom panel). The peak colors in the top panel represent the degree of matching of the unknown compared to the best matching reference organism (green, full match; yellow, partial match; red, no match). The x-axis indicates the mass/charge (m/z) ratio of the detected analytes and the y-axis indicates relative abundance. The degree of peak matching and the relative abundance are used to determine the log(Score). A log(Score) >2.0 indicates a high likelihood of a correct species-level identification while a score <1.7 is considered unreliable.[15]

The benefits of MALDI-TOF MS over conventional identification are many. Overall, the time to identification can be decreased by 6 to 48 hours in comparison to conventional culture and biochemical identification methods. Further, identification by MALDI-TOF has been shown to decrease hospital stays by 2 to 6 days, decrease time to effective and optimal antimicrobial therapy, decrease mortality rates by 4% to 9%, and some studies have also shown reduced hospital costs.[18,19]

Of importance, identification of a microbe can be performed directly from the blood culture bottle, prior to isolation on solid media. Direct identification from a positive blood culture bottle has a much quicker resulting time, typically 30 minutes compared to 24 to 72 hours after growth is detected on agar plates.[20] There are, however, limitations to identification straight from the blood culture bottle. Species level identification may not be achieved if the blood culture bottle contains multiple species or excessive nonpathogen proteins originating from the patient's blood.[21] Matrix-assisted Laser Desorption Ionization Time-of-Flight MS can be performed from the culture plates in these scenarios, which may still offer a great reduction in time to identification for many organisms.

Although MALDI-TOF MS is highly accurate for the identification of organisms, some identifications are difficult to obtain because of similarities to other species at the protein level. Ribosomal proteins make up a significant portion of the spectra so species with similar 16S genes (ie, *Escherichia coli* and *Shigella*) are nearly identical and cannot be differentiated.[17]

What Other Rapid Diagnostic Tools Can Be Used for Pathogen Identification?

Molecular assays can be implemented in the diagnosis of BSIs to speed up pathogen identification. These methods are primarily performed using aliquots taken from the positive blood culture bottles; therefore, significant time is saved since the organism identification can occur prior to growth on culture plates. Molecular approaches include in situ hybridization, DNA microarrays, and nucleic acid amplification technologies. In situ hybridization assays use molecular probes that are complimentary to pathogen nucleic acid sequences. The probes are fluorescently labeled, so the signal can be measured or observed using a fluorescent microscope. Microarray-based assays use oligonucleotides that are attached to a solid surface or particle to capture complimentary nucleic acid sequences present in the positive blood culture bottle. The captured oligonucleotides are detected using chemicals or fluorescent molecules that bind to the captured complexes. Nucleic acid amplification technologies are based on PCR and can be multiplexed to detect a broad range of pathogens. The amplified product can be detected in real time using a variety of methods, but one of the most common methods is by using oligonucleotide probes that fluoresce as amplification occurs.[22,23]

Genes mediating antimicrobial resistance can be detected using the same molecular techniques described above. However, if a resistance marker is not detected by a genetic assay, susceptibility cannot be assumed because there may be other

mechanisms resulting in phenotypic resistance.[22] Rapid phenotypic-based assays are also being developed. One assay that is commercially available can be performed directly from aliquots of the positive blood culture bottle. This assay uses automated microscopy to measure organism growth over time in the presence of antimicrobials.[22,23]

Are There Rapid Diagnostic Tools That Can Detect Bacteremia Directly From a Blood Sample?

There is one commercial assay which allows for the identification of a small number of pathogens directly from a 5-mL whole blood specimen. The system utilizes PCR followed by magnetic resonance to detect the amplified products. Results are available in 3 to 5 hours, which is often earlier than the detection of growth by the automated blood culture instrument.[22]

How Does MALDI-TOF Compare to These Other Rapid Diagnostic Methods?

An advantage of MALDI-TOF MS compared to molecular assays is the wider range of pathogens that can be identified because of the size of the reference libraries.[24] Nucleic acid–based identification is limited by the targets contained on the panel. Another advantage is the low cost per test and cost savings compared to standard identification methods.[25,26] Although the initial MALDI-TOF MS instrument cost can be greater than that of instruments needed for molecular assays, the reagent costs to identify an isolate from a culture plate can be less than one dollar.[25] Cost of identification performed directly from a blood culture bottle is higher at ~$5 per specimen,[27] but molecular panels are significantly more expensive.[24]

Disadvantages of MALDI-TOF MS compared to other rapid methods include increased setup time and lack of susceptibility information. The protocols to perform identification directly from blood culture bottles require at least 30 minutes hands-on time to perform,[27] whereas molecular assays require only minutes of setup time.

Does the Utilization of Rapid Diagnostics Improve Patient Outcomes?

Implementation of rapid identification and susceptibility assays reduces time to result reporting, but multiple studies and meta-analyses have demonstrated that the rapid results must be paired with interventions by an antimicrobial stewardship clinician or pharmacist to improve patient outcomes.[28-30] In one prospective, randomized control trial, positive blood cultures were processed using standard of care methods, multiplexed PCR, or multiplexed PCR with antimicrobial stewardship intervention. Time to antimicrobial de-escalation was only reduced for the group of patients whose blood cultures were processed using multiplexed PCR with antimicrobial stewardship intervention.[29] Although length of stay or mortality were not reduced in this study, antimicrobial de-escalation is important for limiting the development of antimicrobial resistance, which may have long-term benefits for individual patients and for reducing the spread of resistant organisms in the hospital environment. Other studies that have examined the impact of MALDI-TOF MS and stewardship compared to a preintervention period have demonstrated reductions in length of stay[31,32] and recurrent bacteremia.[32] In one of these studies, the antimicrobial stewardship intervention was associated with a trend toward reduced mortality,[32] which is consistent with the need to rapidly establish appropriate therapy to reduce mortality.[33]

For treatment of *S aureus* bacteremia, rapid diagnostics that detect the *mecA* gene can be used for rapid de-escalation of MRSA-directed antibiotics when *mecA* is not detected. For *S aureus*, de-escalation is supported because ~95% of resistance is mediated by the *mecA* gene.[34] Multiple studies have demonstrated reductions in time to de-escalation for treatment of methicillin-susceptible *S aureus* (MSSA) BSIs upon implementation of molecular assays to detect *mecA*.[35-37] The ability to rapidly differentiate *S aureus* from CoNS has also been shown to reduce unnecessary antimicrobial administration to patients whose isolates represent contaminants.[35]

Taken together, rapid diagnostics with antimicrobial stewardship interventions reduce time to appropriate therapy, but not all studies have been able to demonstrate reduced length of stay or mortality which may be due to limitations in study design or differences in patient populations. Other theoretical downstream effects of rapid change to appropriate, narrow spectrum antibiotics include limiting the number of antibiotic-associated, *Clostridium difficile* infections, but this remains to be thoroughly evaluated after rapid diagnostic implementation.

What Is Recommended Next for Management and Evaluation?

According to IDSA guidelines, empirical therapy for severe CAP and bacteremia caused by *S aureus* includes agents that cover MRSA. These guidelines recommend the administration of daptomycin or vancomycin for management of bacteremia.[38] However, before daptomycin administration, the clinical team needs to be aware that it is not effective in the management of MRSA when the source is pulmonary. The decrease in its efficacy in the lungs is explained by daptomycin being inactivated by its interaction with pulmonary surfactants which results in the inhibition of its antibacterial activity.[38,39] For our patient, empiric therapy should include vancomycin instead of daptomycin.

After bacterial identification of *S aureus* by MALDI-TOF MS, rapid molecular assays can be performed directly on a sample from the positive blood culture bottle to determine whether de-escalation of MRSA coverage is appropriate. Routine antimicrobial susceptibility testing must also be performed to confirm the molecular results and to determine susceptibility

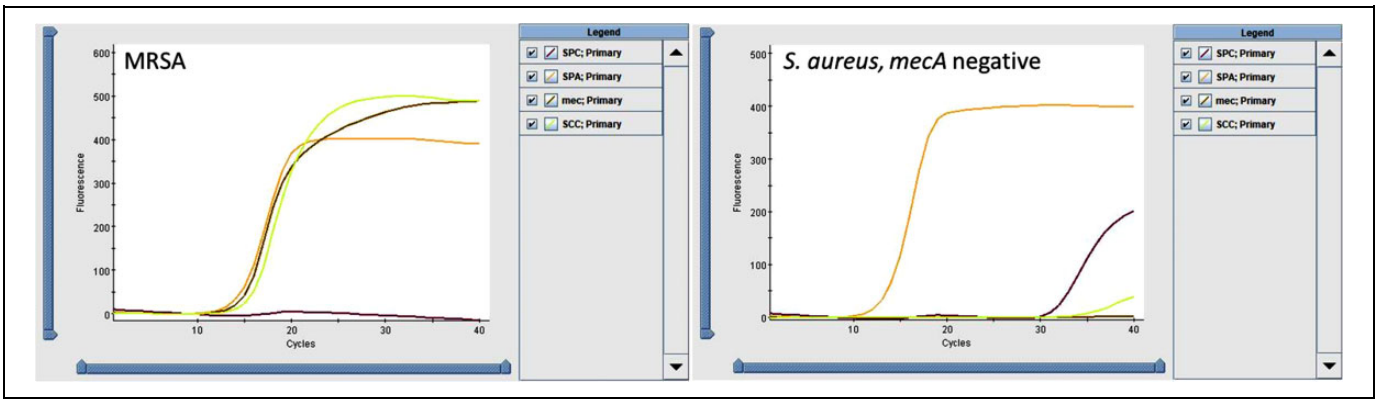

Figure 4. Real-time RT-PCR amplification curves of methicillin-resistant *Staphylococcus aureus* (MRSA) and *S aureus* lacking the *mecA* gene. The assays were performed using aliquots from positive blood culture bottles. The *S aureus*–specific *spa* gene region was amplified in both the MRSA and *S aureus*, *mecA* negative specimens. The SCC and *mecA* targets, which together indicate the presence of the SCC*mec* chromosomal cassette, were amplified in the specimen containing MRSA. The specimen processing control (SPC) was amplified only in the *S aureus*, *mecA* negative specimen; however, both assays are valid because SPC must only be detected when no other targets are amplified to indicate that DNA extraction was successful.

to other antimicrobials that may be used over the course of treatment.

Diagnostic Findings, Part 3

A rapid molecular PCR assay is performed which identifies *S aureus*, lacking the *mecA* gene. Phenotypic susceptibility testing confirms methicillin-susceptibility (oxacillin and cefoxitin minimum inhibitory concentration (MIC) of 0.5 μg/mL). The isolate is also susceptible to clindamycin (MIC $\leq$ 0.5 μg/mL), gentamicin (MIC $\leq$ 2 μg/mL), and tetracycline (MIC $\leq$ 0.5 μg/mL) but resistant to trimethoprim/sulfamethoxazole (MIC > 2/38 μg/mL).

Questions/Discussion Points, Part 3

How Do Rapid Real-Time PCR-Based Assays Detect Methicillin Resistance?

Real-time PCR assays usually detect regions of the staphylococcal chromosomal cassette SCC*mec* and the *mecA* gene contained within this region (Figure 4). This cassette is contained on a mobile genetic element and is the primary cause of methicillin resistance in *S aureus*. The *mecA* gene encodes an alternative penicillin-binding protein, PBP2a, which maintains peptidoglycan cross-linking in the cell wall during treatment with β-lactams because of low affinity for these antimicrobials.[40] Some PCR assays also detect *mecC*, a *mecA* homolog that emerged recently but remains a rarer cause of resistance.[41,42]

Polymerase chain reaction–based assays can yield false positive MRSA results.[43-45] For some assays, false positive results can occur if there is an MSSA isolate that contains an empty SCC*mec* cassette. This occurs when *S aureus* discards the *mecA* gene but leaves remnants of the region behind.[44,45] In addition, false positive results can occur for any molecular assay when the resistance gene is present but there are mutations conferring the gene product to be nonfunctional. For multiplexed molecular assays that are performed directly on positive blood cultures and use separate probes for the detection of *mecA* and *S aureus*, false positive results can occur when both CoNS and MSSA are present.[43] Coagulase negative *S aureus* often contains the *mecA* gene, so it is not possible to attribute the methicillin resistance to the CoNS or *S aureus* isolate when both organisms are in the blood culture bottle.[46,47]

False negative results can occur if the methicillin resistance is mediated by another mechanism of resistance. *S aureus* isolates that overexpress β-lactamases[48]or have modifications to native penicillin-binding proteins that reduce antibiotic binding[49] can be resistant to methicillin and result in false negative results if *mecA*/*mecC* PCR is performed. False negative results can also occur if there are alterations to the sequence that is targeted by the PCR assay.[50]

Which Antimicrobials Are Used to Confirm Methicillin Susceptibility or Resistance for S aureus*?*

Methicillin susceptibility testing for *S aureus* can be achieved by performing disk diffusion testing with cefoxitin (Figure 5) or microbroth dilution using either cefoxitin or oxacillin. Oxacillin disk diffusion is only accurate to determine susceptibility for the staphylococcal species *S epidermidis*, *S pseudointermedius*, and *S schleiferi*; therefore, it must not be used for *S aureus*. In addition, growth on oxacillin (6 μg/mL) salt agar (4% NaCl) can also be used to detect resistance.[51] Methicillin is not used for phenotypic susceptibility testing since it is not commercially available, and in vitro performance is not adequate. Oxacillin and cefoxitin are more stable under storage and are more sensitive for the detection of heteroresistant strains. Moreover, cefoxitin is more sensitive for the detection of *mecA*-mediated resistance.[52] *mecC*-mediated resistance is rare and strains with *mecC* are cefoxitin resistant and oxacillin susceptible.[41]

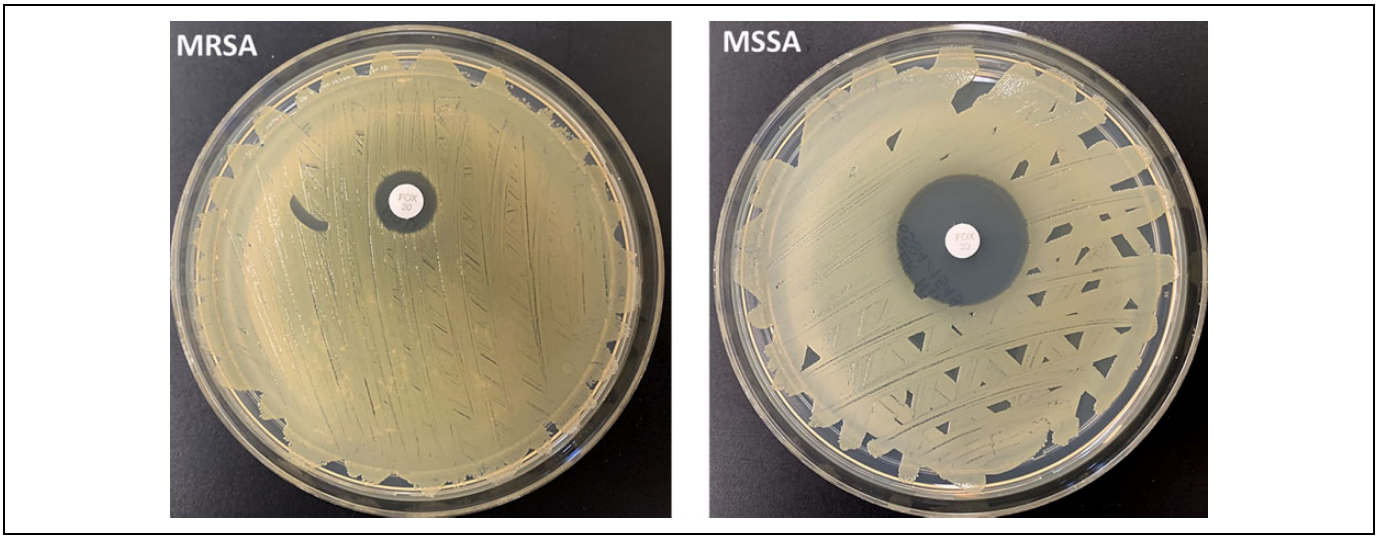

Figure 5. Cefoxitin disk diffusion assay to determine susceptibility to penicillinase-stable penicillins (eg, oxacillin, cloxacillin, dicloxacillin, nafcillin). A zone diameter size of ≤21 mm indicates resistance (shown MRSA, 11 mm; MSSA 25 mm).

Methicillin-resistant *S aureus* strains are resistant to all semisynthetic or penicillinase-stable penicillins including methicillin, oxacillin, nafcillin, and cloxacillin. Methicillin-resistant *S aureus* strains should also be reported as resistant to cephalosporins and carbapenems, with the exception of ceftaroline which is a fifth-generation cephalosporin.[51]

Is Continued Treatment With Vancomycin Optimal for Confirmed MSSA Bacteremia?

Vancomycin is the mainstay therapy for MRSA and is widely recommended for empirical treatment of *S aureus* bacteremia. However, for the treatment of confirmed MSSA bacteremia, vancomycin is less effective when compared with semisynthetic penicillins and first-generation cephalosporins.[53,54] According to IDSA, the antimicrobial activity of vancomycin against staphylococci works at a slower rate in vitro, especially when the bacteria are at a high inoculum. Infectious Diseases Society of America guidelines state that vancomycin is "clearly inferior to β-lactams for MSSA bacteremia."[38] Wong et al concluded that empiric management with β-lactam agents, when compared with vancomycin, was associated with earlier clearance of MSSA bacteremia, suggesting that a β-lactam agent should be added empirically in cases where the prevalence of MRSA is not as significant and in critically ill patients.[54]

Several studies have also demonstrated that vancomycin, when continued as treatment of MSSA, is associated with adverse outcomes due to nephrotoxicity, persistent bacteremia, and treatment failure with higher relapse rate. Moreover, vancomycin is significantly associated with a higher *S aureus* bacteremia-related mortality.[53,55] Schweizer et al reported that switching from vancomycin to nafcillin or cefazolin after confirmed results of MSSA can be protective against MSSA bacteremia-related mortality. In this study, the switch was associated with a 79% lower adjusted rate of mortality and a 69% reduction in risk of 30-day mortality in MSSA bacteremic patients when compared with patients on continued treatment with vancomycin.[55] These studies support that β-lactam agents should be the antimicrobial of choice for definitive treatment of MSSA bacteremia and vancomycin should be avoided when the use of β-lactam agents is possible.

Diagnostic Findings, Part 4

MSSA is isolated from the patient's bronchoalveolar lavage fluid and the resident physician taking care of the patient notices that the patient's Influenza A test result is also positive.

Questions/Discussion Points, Part 4

Describe the Source and Disease Pathogenesis of MSSA and Influenza A Coinfection

Staphylococcus aureus is a common cause of coinfection or post-viral respiratory infection. It is present persistently as a colonizer in the anterior nares in around 20% of all healthy individuals and can become invasive during or after a viral infection. Physiological changes in the host elicited by Influenza A virus (IAV) infection including disruption of ciliary activity, epithelial and secretory cell damage, nutrient availability, and febrile and stress responses promote bacterial infection. Moreover, these physiological changes are the causative factor of *S aureus* biofilm dispersal from the nasal cavity with dissemination into the respiratory tract and lungs leading to secondary active pneumonia. Influenza A virus can also elicit an immune response which, in turn, impairs the host ability to combat a bacterial infection. The production of type I interferons (IFNs) triggered by IAV to produce an antiviral response can inhibit neutrophil-recruiting chemokines causing a deficit in the neutrophilic responses against bacteria. Type I IFNs act by inhibiting the interleukin-23 (IL-23)-dependent

induction of Th17 immunity, decreasing the levels of IL-17 and IL-22 which normally, through neutrophilic response and production of antimicrobial peptides, control the *S aureus* load in the nasal cavity and lungs.[56]

Polymerase chain reaction testing demonstrates a viral etiology in 20% to 40% of adults with CAP and it is important to have in mind that a positive PCR for a respiratory virus does not rule out the possibility of a superimposed bacterial infection. A coinfection with *S aureus* can easily disseminate into the bloodstream causing rapid deterioration and death. McDanel et al concluded that patients with influenza whose respiratory cultures were positive for *S aureus* had a significantly increased risk of death when compared with influenza negative patients.[57]

Teaching Points

- One of the most important preanalytic factors in detection of BSIs is collection of an adequate volume of blood. For adults, a minimum of 20 mL of blood per culture set, and at least 2 sets should be collected prior to administration of antimicrobials.
- There are many emerging tools for the identification of pathogens from positive blood cultures, which include MALDI-TOF MS and molecular assays.
- An advantage of MALDI-TOF MS for pathogen identification is the broad range of pathogens that can be identified and the reduced cost compared to molecular assays. In contrast, molecular methods can take less hands-on time to perform and often include the detection of genes associated with antimicrobial resistance.
- The use of rapid diagnostics such as MALDI-TOF MS and molecular diagnostics can greatly reduce time to pathogen identification, but impact on patient outcomes is greatest when paired with other interventions such as real-time treatment recommendations provided by antimicrobial stewardship teams.
- Genotype does not always correlate with phenotype. Resistance can be mediated by multiple mechanisms, some of which may not be targeted by the assay used. Also, a gene may not be expressed or can contain mutations that alter the function of the protein product.
- Since *mecA* is the most common mechanism of methicillin resistance in *S aureus*, genotypic detection of resistance is often accurate, with molecular assays demonstrating high sensitivity and specificity. De-escalation of MRSA coverage may be appropriate when *mecA* is not detected.
- To determine phenotypic susceptibility to methicillin, disk-diffusion assays using cefoxitin can be performed. Microbroth dilution testing using either cefoxitin or oxacillin, or use of oxacillin salt agar is also appropriate. However, cefoxitin testing has greater sensitivity compared to oxacillin for *mecA* detection.
- Vancomycin is not the ideal treatment for MSSA infections. Nafcillin or cefazolin has been demonstrated to improve patient outcomes.
- *Staphylococcus aureus* is a common cause of co- or post-viral upper respiratory infections. Infection can disseminate to the bloodstream and cause rapid deterioration and death.

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