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Utility of rapid microbiological techniques for the diagnosis of severe infections

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ABSTRACT

Rapid diagnostic microbiological techniques and antimicrobial susceptibility testing are necessary for early and adequate treatment. The utility of old (Gram stain, antigen detection, direct antimicrobial susceptibility testing, chromogenic media) and new techniques (molecular assays, MALDI-TOF) is summarized for the rapid diagnosis of bacteraemia and fungaemia, catheter-related bloodstream infections, pneumonia, meningitis, skin and soft-tissue infections, urinary tract infection, *Clostridium difficile* infection, viral infections, and tuberculosis.

Key words: rapid diagnosis, rapid microbiological techniques, severe infections, intensive care unit.

Utilidad de las técnicas rápidas de Microbiología en el diagnóstico de los grandes síndromes

RESUMEN

Las técnicas microbiológicas de diagnóstico rápido y de sensibilidad a antimicrobianos son necesarias para instaurar un tratamiento precoz y adecuado. Se resume la utilidad de las viejas técnicas (tinción de Gram, detección de antígenos, antibiograma directo en muestras clínicas, medios cromogénicos) y las nuevas (métodos moleculares, MALDI-TOF) para el diagnóstico rápido de la bacteriemia y la fungemia, bacteriemia relacionada con el catéter, neumonía, meningitis, infecciones de piel y tejidos blandos, del tracto urinario, infección por *Clostridium difficile*, infecciones víricas y tuberculosis.

Palabras clave: diagnóstico rápido, técnicas microbiológicas rápidas, infecciones graves, unidad de cuidados intensivos.

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INTRODUCTION

Rapid diagnostic microbiological techniques and antimicrobial susceptibility testing allow early identification of microorganisms and resistance patterns, which are necessary for adequate clinical and therapeutic management of patients. In patients with severe infections, timely antimicrobial therapy is even more critical to outcomes, and in this setting rapid etiologic microbiological diagnosis is mandatory. The most common and severe infections are bacteraemia and fungemia, intravascular catheter-related bloodstream infections, pneumonia, meningitis, acute skin and soft-tissue infections, urinary tract infections, and *Clostridium difficile* infection. In addition, early diagnosis of tuberculosis and viral respiratory infections allows rapid treatments and avoids the use of unnecessary antimicrobials. This manuscript summarizes some of the rapid techniques used for the diagnosis of these infections.

RAPID DIAGNOSIS OF BACTERAEMIA AND FUNGAEMIA

Bloodstream infections remain a major clinical challenge with a high attributable mortality and are associated with elevated costs. Rapid identification of patients with bacteraemia or fungaemia is critical in influencing antimicrobial therapy[1]. Several molecular assays (SeptiFast®, SeptiTest®, Plex-ID®, among others) based on real-time PCR have been developed and commercialized for the detection of bacteria and fungi directly from blood specimens[1]. These assays include the detection of different series of Gram-positive cocci, Gram-negative bacilli, different species of yeasts and filamentous fungus. Some also include the detection of methicillin and vancomycin resistance genes (*mecA, vanA, vanB*). Recently, a new nanodiagnostic approach, the T2 magnetic resonance assay, is being used for the rapid diagnosis of candidemia in whole blood, and represents a new era of molecular diagnostics[2].

Although molecular assays can reduce the time to detection of specific pathogens in blood to 4 h, blood culture techniques and Gram staining, are still necessary. These techniques are simple and available in all Microbiology laboratories, and despite their shortcomings, blood cultures remain the standard laboratory tests for the diagnosis of bacteraemia and fungaemia. At present, molecular assays lack sufficient sensitivity to be used as standalone tests, and no molecular assay can be a substitute for blood cultures, but can be useful as adjunts to blood cultures[1]. In addition, different systems have been developed for the detection of microorganisms directly from blood cultures. Among these, the microarray-based nucleic acid assays (Verigene®, FilmArray ®) for the detection of bacteria and resistance markers have shown high positive-predictive values, ranging from 95% to 100%, and are potential adjuvant tools for improving the microbiological diagnosis of sepsis[1]. Other nucleic acid amplification tests (Gene Xpert®) can be used for the detection of the *mecA* gene in methicillin-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* and for the detection of the *vanA* gene in vancomycin-resistant strains of enterococci. Antigen detection is another alternative than can be used directly from blood cultures. The BinaxNOW® *S. aureus* test is an immunochromatographic assay that detects an *S. aureus*-specific protein, allowing for differentiation between *S. aureus* and other Gram-positive cocci with 97.6% sensitivity and 100% specificity[1]. In recent years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) has been widely used for the rapid microbial identification from blood cultures. The use of MALDI-TOF reduces considerably the time to identification and results are concordant to the genus level for more than 95% of blood cultures in comparison with conventional identification methods[1]. The peptide nucleic acid-fluorescent *in situ* hybridization (PNA-FISH) method (AdvanDx®) has also been shown to reduce the time to identification of microbial pathogens present in blood cultures, however, this method has only been developed for the detection of a limited number of bacterial and yeast species[1]. Finally, the use of chromogenic media for diagnosing (the colours of the colonies help in the identification) are available for detection of many microbial species and for the detection of extended-spectrum-beta-lactamases-mediated resistance and other mechanisms of resistance. These media also allow rapid detection of polymicrobial bacteraemia or fungaemia when used directly from blood cultures and they are cost-effective by reducing both time and reagents used to identify the organisms[1]. Direct antimicrobial susceptibility testing from blood cultures by the disk diffusion or by the gradient diffusion methods also decreases the time needed for susceptibility test results. Although these methods are not standardized, they are commonly used in many laboratories and provide a rapid and useful information to clinicians.

RAPID DIAGNOSIS OF CATHETER-RELATED BLOODSTREAM INFECTIONS (CR-BSI)

Catheter-related infections are among the most important nosocomial infections, causing at least 20% to 40% of all hospital-acquired bacteraemias. In patients with suspected bacteraemia, a substantial number of tip cultures are negative. The diagnosis of CR-BSI can be performed with or without catheter removal. Semiquantitative cultures of superficial structures (catheter hubs and skin around the insertion point of the catheter) are an easy, rapid, safe, and conservative method for ruling out CR-BSI. With a negative predictive value of 96.7%, this method avoids many unnecessary catheter withdrawals[3]. Other conservative procedures that do not require catheter removal include differential paired quantitative blood cultures using lysis-centrifugation tubes: to compare colony counts in peripheral vein blood versus blood drawn from the catheters (a ratio of ≥3/1 cfu/ml, catheter/peripheral, indicates CR-BSI), and the method named differential time to positivity using a continuous-monitoring automated blood culture system: ≥2 h between a catheter blood culture and a peripheral blood culture indicates CR-BSI[4].

RAPID DIAGNOSIS OF PNEUMONIA

Rapid microbiological diagnosis of hospital-acquired pneumonia and especially ventilator-associated pneumonia (VAP) has a high impact in the prognosis of the disease. Expectorated and induced sputa and tracheal aspirates are the most common specimens submitted for diagnosis of lower respiratory tract infections. Microscopic examination and culture of these samples remain the mainstays of the laboratory diagnosis of pneumonia despite controversy concerning their sensitivity and specificity. Microscopy (e.g., Gram stain, acid-fast stains, calcofluor white stain, specific fluorescent antibody tests for *Pneumocystis jirovecii*) can provide a rapid diagnosis if positive of bacterial and fungal infections, but have low sensitivity, and alternative test methods should also be used when negative. Infections with some respiratory pathogens (*Streptococcus pneumoniae, Legionella pneumophila*) can be diagnosed by detecting specific antigens in urine within 10 to 20 minutes. The nucleic acid assays are becoming the diagnostic tests of choice for the rapid diagnosis of some pathogens including *P. jirovecii*, *Mycoplasma pneumoniae*, and respiratory viruses, including influenza and respiratory syncytial virus[5]. In the case of *Mycobacterium tuberculosis* commercialized molecular tests detect the organism and resistance genes in less than 2 hours. For the specific case of VAP, molecular techniques allow a rapid identification of methicillin-resistant or susceptible *S. aureus* (MRSA and MSSA) by directly subjecting clinical samples to PCR (GeneXpert®) in 1 hour[6]. In addition, the performance of direct gradient susceptibility testing (E-test) on lower respiratory tract samples has proven to be a rapid and accurate procedure for antimicrobial susceptibility testing. By using this method, susceptibility results are available in 18 to 24 h with

a correlation of 96.1% with the standard method[7]. Another approach is a modification of the direct E-test technique using a chromogenic agar medium (Mueller-Hinton base) to generate both rapid antimicrobial susceptibility and organism identification results. Full agreement with the standard procedure was observed in 94.9% of cases[8].

RAPID DIAGNOSIS OF MENINGITIS

Acute meningitis is a medical emergency that requires rapid identification of the aetiologic agent. Gram-staining of the cerebrospinal fluid (CSF) is generally positive for patients with bacterial meningitis (with the exception of infection with *Listeria monocytogenes*), and its sensitivity can be improved by concentrating the specimen by centrifugation when it is received in the laboratory. In recent years, the use of direct antigen tests for bacteria in CSF has little value due to the decrease in the incidence of *Haemophilus influenzae* meningitis and the lack of a reliable *Neisseria meningitidis* serotype B antigen test. However, antigent tests for *Cryptococcus neoformans* are rapid (results can be obtained in 15 minutes), sensitive and specific[5]. Cultures for the most common causes of meningitis are generally positive within 1 to 2 days and, as in the case of VAP, it is also useful the performance of direct gradient susceptibility testing on CSF when microorganisms are present in the Gram-stain. In these cases, susceptibility results are available in 18 to 24 h (unpublished data from the author). The use of multitarget PCR tests for the detection of all bacterial pathogens in CSF provide a rapid (less than 3 hours) and specific diagnosis of meningitis, and some of these are currently available commercially (RealCycler® MENELI). At present, the detection of enterovirus and other central nervous system viruses can also be performed in less than 2 hours by the use of different molecular methods and platforms.

RAPID DIAGNOSIS OF SKIN AND SOFT-TISSUE INFECTIONS

In severe skin and soft-tissue infections, a Gram stain must be performed to obtain some preliminary information of the infecting organism(s). Nowadays, the rapid detection of MSSA and MRSA can be performed directly in wound specimens in 1 hour by using a commercialized multiplex PCR assay (GeneXpert®). The agreement between this assay and the standard culture is >95%[9]. Direct antigen detection tests are highly sensitive and specific for the rapid diagnosis of severe infections caused by *Streptococcus pyogenes*[5].

RAPID DIAGNOSIS OF URINARY TRACT INFECTIONS

Rapid screening techniques for urinary tract infection include direct Gram stain and several commercially available products such as dipstick methods and bioluminescence, among others. Gram-staining of fresh uncentrifuged urine is a cheap, rapid and accurate method for the detection of significant bacteriuria. This method has a negative predictive value of >95%, which shortens the time for reporting a negative culture result. When positive, Gram stain guides antimicrobial treatment. The use of MALDI-TOF mass spectrometry performed directly in urine samples after a positive Gram stain for the identification of bacteria anticipates the culture results in 83% of cases and results can be obtained in 1 hour with 4% of major errors[10]. The use of chromogenic media also allows rapid identification of the most frequent microorganisms causing urinary tract infections. In addition, direct antimicrobial susceptibility testing of urine samples, although criticized because the inoculum is not standardized, has shown a good correlation with standard methods providing results in 24 hours[11].

RAPID DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

Clostridium difficile is the most common cause of hospital-acquired bacterial gastroenteritis. The appearance of hypervirulent strains, such as ribotype 027, has contributed to the increased incidence and severity of infection. A variety of diagnostic test methods are available for the rapid diagnosis of *C. difficile* disease. At present, multistep algorithms based on the initial results for glutamate dehydrogenase followed by a sensitive toxin test and/ or a nucleic acid amplification test are best options and results can be obtained in less than 2 hours[1].

BIOMARKERS OF ACUTE BACTERIAL INFECTIONS

Porcalcitonin (PCT), the prohormone of calcitonin, is synthesized by a variety of tissues in response to bacterial infection to a greater extent than to viral infections. A cut-off of PCT >1.39 ng/ml is accurate for diagnosing severe sepsis. Levels of serum procalcitonin are also useful to guide antimicrobial therapy in acute respiratory infections and to reduce the antibiotic use[5,12].

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