Use of rapid diagnostic techniques in ICU patients with infections

Almudena Burillo\textsuperscript{1,2,3} and Emilio Bouza\textsuperscript{1,2,3,4*}

Abstract

Background: Infection is a common complication seen in ICU patients. Given the correlation between infection and mortality in these patients, a rapid etiological diagnosis and the determination of antimicrobial resistance markers are of paramount importance, especially in view of today’s globally spread of multi drug resistance microorganisms. This paper reviews some of the rapid diagnostic techniques available for ICU patients with infections.

Methods: A narrative review of recent peer-reviewed literature (published between 1995 and 2014) was performed using as the search terms: Intensive care medicine, Microbiological techniques, Clinical laboratory techniques, Diagnosis, and Rapid diagnosis, with no language restrictions.

Results: The most developed microbiology fields for a rapid diagnosis of infection in critically ill patients are those related to the diagnosis of bloodstream infection, pneumonia –both ventilator associated and non-ventilator associated–, urinary tract infection, skin and soft tissue infections, viral infections and tuberculosis.

Conclusions: New developments in the field of microbiology have served to shorten turnaround times and optimize the treatment of many types of infection. Although there are still some unresolved limitations of the use of molecular techniques for a rapid diagnosis of infection in the ICU patient, this approach holds much promise for the future.

Keywords: Rapid diagnosis, Clinical laboratory techniques, Intensive care unit, Microbiology

Review

Background

Although intensive care units (ICUs) have fewer than ten percent of the total number of beds in most hospitals, more than 20 percent of all nosocomial infections are acquired in ICUs and carry substantial morbidity, mortality, and expense [1-4]. The most common clinically significant infections observed in the ICU are intravascular catheter-related bloodstream infection (CR-BSI), ventilator associated pneumonia (VAP), and catheter associated urinary tract infection (CA-UTI).

In addition, multidrug-resistant (MDR) pathogens are evermore frequently isolated in ICUs [5,6] and this hinders the initiation of appropriate, effective antibiotic therapy, which correlates with excess mortality [7-9].

In this setting, a rapid etiologic microbiological diagnosis is mandatory. This paper reviews some of the rapid diagnostic techniques available for ICU patients with infections.

Main text

Rapid diagnosis of catheter-related bloodstream infections (CR-BSI)

A diagnosis of CR-BSI should be based on microbiological identification of the catheter as the source of bloodstream infection, and may be performed with or without catheter removal [10].

Attempts to establish the role of the catheter in episodes of BSI are justified by the following: a high proportion of the suspicious of CR-BSI are note confirmed after catheter removal and culture [11], and many CR-BSI can be managed empirically without immediately removing the catheter [12-14]. Central venous catheter (CVC)
removal limits vascular access, and diagnostic methods exist that do not require catheter removal [15].

**Rapid procedures that do not require catheter removal**

The conservative approach to CR-BSI diagnosis (i.e., without catheter withdrawal) is highly convenient. Conservative procedures include differential paired quantitative blood cultures (comparison of colony counts in peripheral vein blood versus catheter hubs), superficial cultures (semiquantitative cultures of skin around the portal of entry and of catheter hubs), and a method comparing time to positivity between concurrent blood cultures of peripheral vein and catheter hub samples, named “differential time to positivity” (DTTP) [16-18].

**Paired central/peripheral cultures**

A ratio or differential colony count ≥3:1 cfu/mL of bacteria from the catheter-drawn blood cultures compared to percutaneously-drawn blood cultures is usually accepted as a prove of CR-BSI. This cutoff shows a sensitivity (Se) of around 80% and specificity (Sp) of 90-100% [19].

Blood should be drawn from all hubs, representing the different catheter lumens [20]. This technique is usually performed with lysis-centrifugation tubes. Blood is inoculated in tubes containing the cell-lysing agent saponin, followed by vortexing and centrifugation. Then, after removing the supernatant (lysate), the concentrate is plated on agar medium and the plates incubated overnight before counting. The tubes need to be processed within 8 hours of inoculation [21]. Drawbacks of this technique include: the manual and individual processing of each individual sample, the risk of contamination, the risk of exposure of laboratory technicians to blood and the high cost [19].

**Differential time to positivity (DTTP)**

DTTP supporting CRBSI diagnosis is defined as a difference in time to positivity of ≥2 h between a CVC blood culture and a peripheral blood culture, or between 2 CVC blood cultures from different lumens of a multilumen catheter [10,22,23]. The DTTP test is conducted using a continuous-monitoring automated blood culture system. This method requires inoculating the same amount of blood in each culture bottle. For multiple lumen catheters, blood should be drawn from all ports [20,24]. To ensure accurate results, the first milliliters of blood drawn from the catheter should be used for culture. Then, bottles must be sent to the laboratory and incubated as soon as they arrive there. Depending on the type of catheter (short- vs. long-term) and the patient, the test shows a Se of 86-93%, Sp of 87-92%, positive predictive value (PPV) of 85-88% and negative predictive value (NPV) of 89-95% [22,24,25].

DTTP is nowadays the main technique to assess CR-BSI used in most microbiology departments. Caution in interpretation should be applied in patients receiving antimicrobial agents [22]. The validity of DT, however, has been recently questioned by Kaasch et al. [26], that found a poor diagnostic performance (Se 37%, Sp 77%, PPV 46%, NPV 70%, validity 63%) in patients with CR-BSI caused by *Staphylococcus aureus*. However, they failed to adhere to instructions of utmost importance related to the protocol. The microbiology service was not available on a 24/7 basis, suggesting long pre-incubation periods before introducing the bottles in the automated blood culture machine, possibly leveling times to positivity of paired cultures, thus invalidating the diagnostic procedure [27,28].

Our group recently demonstrated that the DTTP threshold applied to bacterial CR-BSI is not applicable in cases of CR-BSI caused by *Candida* spp. [29].

**Superficial cultures (combined exit-site and hub cultures)**

We call “superficial cultures” to the combination of semiquantitative cultures independently obtained from the 2 cm of skin surrounding the catheter insertion site and the various hubs.

The threshold for positivity of these semiquantitative cultures is 15 cfu per plate. Growth of <15 cfus per plate of the same microbe from both the insertion site culture and catheter hub/s culture/s strongly suggests that the catheter is not the source of the BSI. Superficial cultures are justified only in cases of suspected CR-BSI (targeted cultures) in which they serve to rule out CR-BSI owing to their high sensitivity and good negative predictive value [16].

Gram staining of skin and hub swabs may also be helpful for the rapid diagnosis of CR-BSI [30].

Recently Bouza et al. compared the use of paired blood cultures, superficial cultures and DTTP for the diagnosis of CR-BSI without catheter removal [31]. DTTP showed a better sensitivity and negative predictive capacity than paired blood cultures to detect catheter tip colonization (96.4% and 99.4% vs. 71.4% and 95.6%, respectively) (Table 1). However, central/peripheral paired blood cultures showing a ratio >5:1 provided the best specificity (97.7%) for a diagnosis of CR-BSI. The three tests showed a high negative predictive capacity. If a negative result was obtained in any of the three tests, it was possible to rule out catheter colonization and CR-BSI reasonably well.

**Rapid diagnosis of sepsis**

The diagnosis of BSI among critically ill patients is a major challenge. Blood cultures are still considered the gold standard diagnostic procedure since pathogens may be isolated and subjected to antibiotic susceptibility testing (AST). In effect, the use of blood cultures in septic shock
Table 1 Validity indices (95% confidence interval) for three commonly used methods of detecting catheter-related bloodstream infection

| Measure            | Semiquantitative superficial cultures | Differential quantitative blood cultures | Differential time to positivity |
|--------------------|---------------------------------------|------------------------------------------|--------------------------------|
| Sensitivity        | 78.6 (59.0-91.7)                       | 71.4 (51.3-86.8)                         | 96.4 (81.7-99.9)               |
| Specificity        | 92.0 (87.0-95.6)                       | 97.7 (94.3-99.4)                         | 90.3 (85.0-94.3)               |
| Positive predictive value | 61.1 (43.5-76.9) | 83.3 (62.6-95.3) | 61.4 (45.5-75.6) |
| Negative predictive value | 96.4 (92.4-98.7) | 95.6 (91.4-98.1) | 99.4 (96.6-99.9) |
| Accuracy           | 90.2 (85.3-93.9)                       | 94.1 (90.0-96.9)                         | 91.2 (86.4-94.7)               |

From reference [31].

patients as part of the compliance with six or more interventions of the 6-hour resuscitation bundle of the “surviving sepsis campaign” has been related to a reduction in mortality [32].

Blood cultures, however, are time-consuming and slow. They only detect viable microorganisms and show a low sensitivity for slow growing, intracellular and fastidious microorganisms. Overall positivity may be as low as 30-40% despite proper implementation of standard procedures, adequate blood volume collection and a high clinical suspicion of BSI.

Molecular techniques are ever-evolving to provide faster and more sensitive results along with the direct identification of responsible pathogens [33-36]. These techniques are likely to impact soon clinical decision-making and antibiotic treatment.

Existing commercial nucleic acid testing (NAT) diagnostic tests are all based on a similar procedure: pathogen lysis, nucleic acid extraction and purification, amplification of nucleic acids by PCR, and identification by various methods, such as ELISA-based hybridization, fluorescence-based real-time detection, liquid or solid phase microarray detection, sequencing and database recognition [34]. The reader is referred to Afshari et al. [34] for a comprehensive review of the tests commercially available today.

Pathogen-specific assays are even capable of detecting genes encoding resistance to antibiotics, such as mecA in staphylococci or van genes in enterococci.

A recent meta-analysis on the use of LightCycler SeptiFast revealed a Se and Sp of 80% and 95%, respectively, for this technique to detect bacteremia, and of 61% and 99%, respectively, to detect fungemia [36]. However, the bacteremia outcome subgroup showed high variation. The turnaround time of the technique was 6 hours.

In general terms, there are still important shortcomings of molecular techniques. For instance, the lack of an appropriate gold standard since blood cultures are unable to detect many true cases of infection; emphasis on microbiological rather than clinical assessment; no guidance for targeting appropriate clinical situations; and the potential for wrong interpretation of results if no expert assistance is available [37,38].

Ideally, tests should provide relevant information 2–6 hours after samples are taken on which to base the choice of treatment. Under real-life conditions, there are often considerable delays due to practical issues, such as availability of staff outside daily routines or batch analysis of samples [39]. Test sensitivity needs to be improved to detect clinically relevant low bacterial loads and fastidious microorganisms. They should be able to distinguish between living and dead bacteria, especially for patients on antibiotics. They should also be able to clarify the impact of DNAemia in cases of clinical signs of BSI. For instance, in a recent paper on the combined use of blood cultures and SeptiFast to predict complicated BSI in cases of staphylococcal or Candida infection, the authors found that patients with a positive SeptiFast result between days 3 and 7 after a positive blood culture had an almost 8-fold-higher risk of developing a complicated bloodstream infection [40].

At present, molecular tests are used to complement the results of traditional culture, especially in serious clinical situations such as ICU patients with severe sepsis [37]. They also have the potential to be a cost-effective strategy to manage sepsis [41]. However, conventional blood cultures remain necessary because of the high incidence of multidrug-resistant bacteria in ICU patients and the need for AST to establish adequate treatment.

Other helpful rapid tests for the diagnosis of sepsis

Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) serves to identify isolated colonies of bacteria and fungi, and can also be used directly on positive blood culture broths in under one hour after the technician has been alerted of growth. This procedure is now replacing biochemical and gene sequencing methods for organism identification because it is easily implemented, highly accurate, inexpensive and fast [42-44]. Some 5 to 10 ml of broth from a single positive blood culture bottle are needed for this technique. However, in most reports to date, identification yields are greater for Gram negative organisms than Gram positives or yeasts. To improve diagnosis, different sample preparation methods for positive blood
The peptide procalcitonin is synthesized by monocytes that are in the process of adhesion. PCT levels rise when there is local or systemic bacterial infection but not in the presence of a virus or autoimmune disease. Thus, PCT is more specific than CRP for detecting bacterial infection.

In a recent prospective study, on day 1 after admission to a medical-surgical ICU, a cut-off PCT >1.39 ng/ml showed the best area under the curve (AUC) for diagnosing sepsis (87%) and levels were found to significantly drop from day 1 to day 2 in survivors [54]. In addition, high PCT levels have been linked to an increased risk of mortality. As an example, in a recent prospective multicentre observational study performed in 1156 Greek in-patients, a PCT > 0.85 ng/ml was associated with 45% mortality in ICU patients [55]. It would appear that as for CRP, trends in PCT observed over time are more useful than single measurements [56].

However, we have yet to find a marker specific enough to provide a true diagnosis of BSI. The Surviving Sepsis Campaign 2012 guidelines state that the utility of PCT levels or other biomarkers to differentiate acute inflammatory patterns of sepsis from other causes of generalized inflammation (e.g., postoperative, other forms of shock) remains to be demonstrated [57].

**Rapid diagnosis of ventilator-associated pneumonia**

Hospital-acquired pneumonia (HAP), especially ventilator-associated pneumonia (VAP), is one of the leading causes of infection and death in the ICU [58-62]. The incorrect or delayed treatment of HAP within a few hours gives rise to a worse prognosis and a higher mortality rate [63-65]. Useless antibiotics are also a cause of adverse events and unnecessary expense [66]. Thus, the etiologic diagnosis of VAP is a microbiological emergency because of its impact on the morbidity and mortality of this disease.

Bacterial identification and AST take 2 or 4 days, so there is a need for rapid diagnostic procedures. Rapid information is clearly more beneficial to the patient than more complete but delayed information. Gram staining, quantifying microorganisms in polymorphonuclear cells in bronchoalveolar lavage samples, and antibiograms conducted directly on clinical samples may provide information that correlates with subsequent culture results.

New diagnostic techniques, such as real-time PCR assays and “in situ” hybridization of bacteria, have been developed to speed up the identification of the pathogens responsible for this disease [67,68].

**Lower respiratory tract samples for microbiology**

All patients suspected of having VAP should undergo lower respiratory tract (LRT) sampling followed by a microscopy examination and culture of the specimen [69]. Deciding upon the best type of sample for diagnosing
VAP is controversial and at present no sampling procedure has proved meaningfully superior to the rest [70-72]. Culture samples should ideally be transferred to the Microbiology Department within 30 minutes of collection to avoid a delay in processing and bacterial overgrowth [73,74]. Storing LRT specimens refrigerated or frozen for 24 hours is an acceptable alternative when culturing cannot be performed immediately [75-77]. Despite this possibility, we would warn against this practice since any delay in receiving information will have devastating clinical consequences.

**Laboratory processing of samples upon arrival. Gram stain**

There is still much controversy over the value of the Gram stain for anticipating the microbiological diagnosis of VAP. The medical literature is replete with varying data on the sensitivity (57-95%), specificity (48-87%), positive predictive value (PPV) (47-78%), negative predictive value (NPV) (69-96%) and accuracy (60-88%) of the Gram stain in the management of patients with VAP [78-82].

Some authors claim that a negative endotracheal aspirate (EA) Gram stain is of great negative predictive value for the diagnosis of VAP and may guide the decision to not initiate or to limit antibiotic treatment until culture results become available [78,80,83,84]. Our opinion is that immediate reporting to the responsible clinicians of the result of a Gram stain on LRT secretions obtained by tracheal aspiration may help guide early treatment. At our Microbiology Department, the diagnostic validity of the Gram technique on EA in patients with suspected VAP has been estimated at: sensitivity 91%, specificity 61%, PPV 50.5%, NPV 94%, test accuracy 70%, positive likelihood ratio (PLR) 2.3, negative likelihood ratio (NLR) 0.14, and a post-test probability of a negative result of 6% [85]. This means that a negative Gram stain makes it highly unlikely that a positive culture result will be obtained the next day.

As a complement to the Gram stain, quantifying the proportion of cells containing intracellular organisms has also been proposed as a rapid method for the diagnosis of VAP. A cut-off of >1-2% of “infected” cells in bronchoalveolar lavage (BAL) specimens rendered a sensitivity of 79–93.6% and a specificity of 82-100% [86-88]. Thus, the detection of intracellular organisms in BAL specimens can be described as a rapid specific test with a high positive predictive value, and is recommended by the British Society of Antimicrobial Chemotherapy to guide initial therapy (grade A recommendation) [89]. In addition, this test does not seem to be affected by antibiotic therapy given up to 72 hours prior to sampling [90]. Along these lines, the European care bundle for the management of VAP recommends immediate reporting of Gram stain findings in respiratory secretions, including “infected” cells [91].

The guidelines of the Society for Healthcare and Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) published in 2008 recommend a Gram stain directly on the sample and the quantitative culture of an EA or a BAL sample [92].

Are there any other rapid direct methods that provide useful information before culture results become available? Although it is widely accepted that the prognosis for a patient with VAP depends on the antibiotic susceptibility of the causative pathogen and on the time elapsed since its diagnosis and the first dose of effective antibiotic received [65,93], there is presently no rapid procedure other than those mentioned whose efficacy in the management of VAP has been reliably proven. In the specific field of VAP, there is a clear need to address new molecular techniques that can detect one or several microorganisms [94] or rapidly identify certain resistance mechanisms directly on clinical samples. We recently obtained excellent results for the rapid diagnosis of VAP due to methicillin-resistant or susceptible *Staphylococcus aureus* (MRSA, MSSA) by directly subjecting clinical samples to PCR (GeneXpert, Cepheid® Inc., Sunnyvale, CA) [95]. This simple procedure shows a high diagnostic efficiency and can shorten the time to adequate antibiotic treatment. These results have also been validated by other authors [96,97]. However, the GeneXpert kit has not yet received CE mark approval for this purpose. The ideal VAP molecular diagnostic assay should target various microorganisms and resistance genes, including *S. aureus, Pseudomonas aeruginosa, Acinetobacter baumannii*, a DNA sequence common to all *Enterobacteriaceae*, and the resistance genes *mecA*, *blaKPC*, *blaIMI*, *blaVIM* and *blaOXA* [98].

**Rapid preliminary cultures and susceptibility testing**

(VAP E-test)

Conventional processing of a secretion sample for microbiological investigation usually takes from 2 to 4 days. After inoculation and incubation for 24–48 hours, bacterial counts are performed and strains are isolated for pure culture. This is followed by pathogen identification and AST, which delays the results at least a further 24 hours. To this process, we would need to add the time of delays in transmitting information and in making therapeutic decisions.

In a study conducted at the Hospital Gregorio Marañón (Madrid, Spain), we compared the results of a direct E-test antibiogram for 6 antibiotic agents conducted on clinical LRT samples to those obtained by the standard AST. The E-test antimicrobial susceptibility procedure is a quantitative method for AST that consists of a plastic strip with a predefined gradient of antibiotic. The stable gradient provides inoculum tolerance where a 100-fold variation in
Other diagnostic markers of VAP

The use of biomarkers such as CRP to more objectively and specifically diagnose VAP has also been assessed. Lisboa et al. used CRP as a diagnostic and prognostic marker, as well as to assess antibiotic treatment appropriateness [104]. These authors noted that the CRP coefficient (defined as the ratio between CRP levels on follow-up and CRP levels at baseline) decreased in patients receiving adequate treatment and that a coefficient of 0.8 at 96 hours post treatment onset was a good indicator of the appropriateness of antibiotic treatment (Se 77%, Sp 87%, area under the ROC curve 86%, 95% CI 75-96%). Unfortunately, CRP is a nonspecific biomarker of inflammation and may also be elevated in the presence of pulmonary infiltrates of non-infectious cause [74].

Regarding PCT, it is not a good marker for the diagnosis of VAP [105]. However, in VAP, this marker has been described as prognostic with elevated levels indicating a more severe clinical course and sustained high levels during the first week of illness indicating a worse outcome [106]. Some studies have also correlated a drop in PCT with a favorable outcome [107,108] and reduced antibiotic consumption [109], although in other studies, neither PCT threshold values nor their kinetics were able to predict VAP survival [110,111].

Despite these discrepancies, PCT seems to be a good indicator of bacterial load in patients with VAP. Most importantly, a low level of PCT is thought to accurately reflect controlled bacterial infection [74].

Other proposed biomarkers are the soluble triggering receptor expressed on myeloid cells-1 (Strem-1) [112] and interleukin-1beta and interleukin-8 in BAL fluid [113]. Chastre et al. recommend that PCT and Strem-1 should only be used to complement standard microbiological diagnostic tests. However, knowledge of serum PCT and Strem-1 levels may prompt a change in treatment early in the course of VAP and such findings have been used to step-up treatment when levels remain high or to avoid long courses of antibiotics when the levels of these markers rapidly fall [114]. Whether PCT and/or Strem-1 guidance can reduce antibiotic use in such a setting is yet to be seen, but the strategy appears promising [112,115].

The rapid diagnosis of urinary tract infection

The turnaround time for microbiological confirmation of a urinary tract infection (UTI) in a urine culture is not usually as critical as in life-threatening diseases like sepsis. Still, microbiological confirmation of a UTI takes 24–48 hours. In the meantime, patients are usually given empirical antibiotics, sometimes inappropriately.

Rapid UTI screening methods. The Gram stain

The usefulness of Gram staining of fresh uncentrifuged urine to detect significant bacteriuria was first demonstrated in 1968 [116], and it has since been used as a screening test for UTI [117-119]. The accuracy of Gram staining for the diagnosis of UTI has been reported in the literature as: sensitivity 82.2–97.9%; specificity 66.0–95.0%; PPV 31.6–94.3%, and NPV 95.2–99.5%, varying with the different counts of microorganisms in the sample [118-122]. As with other rapid screening tests, accuracy is higher for greater bacterial counts.

The benefits of direct Gram staining of urine samples sent for culture are clear: it shortens the turnaround time for reporting negative culture results and guides empirical antibiotic treatment when microorganisms are seen. In addition, when compared to alternative rapid screening tests, the Gram stain has a higher accuracy [119,123] and lower cost [122].

The use of the Gram stain has not been generalized because it needs more equipment and time than dipstick analysis, and is unlikely to replace dipstick testing across all health-care settings [123]. Skilled laboratory personnel are needed to correctly evaluate smears [124]. Yet, in laboratories where stained smears are part of the routine microbiological examination of urine samples, the time necessary to perform the stain and examine the slide under the microscope is relatively short [124].

MALDI-TOF mass spectrometry has been successfully used to rapidly identify culture-isolated microorganisms [42,43] but has been little used directly on clinical
We recently assessed the capacity of subjecting urine samples to sequential Gram staining and MALDI-TOF MS to anticipate clinically useful information [129]. From May through June 2012, 1,000 random urine samples from patients with a suspected UTI were Gram stained, and those returning bacteria of a single morphotype were subjected to MALDI-TOF MS. This procedure was correlated with standard semiquantitative urine culture results and the outcomes recorded as: match (information anticipative of culture result), minor error (information partially anticipative of culture result), or major error (information incorrect and potentially leading to inappropriate antibiotic therapy decisions). Results were available in 1 hour. Information anticipative of culture results was provided in 83% of cases, information with minor errors in 13% and information with major errors in 4%. For 96% of urine samples from patients with suspected UTI, the sequential procedure provided information that was consistent or showed minor errors. In future work, the clinical impacts of this rapid UTI diagnosis strategy need to be assessed in terms of factors such as a reduced time to appropriate empirical treatment or earlier withdrawal of unnecessary antibiotics.

**Anticipation of antibiotic susceptibility with direct testing**

The practice of performing direct AST of urine specimens has the advantage of next-day reporting of antimicrobial susceptibilities. Direct AST of urine samples has proved to be as effective as standard methods, providing results 24 hours in advance with similar costs [130]. However, this method is criticized because the inoculum is not standardized and because sometimes a mixture of microorganisms can be found in the sample. Nevertheless, it has been used for many years with excellent results [131-136] and correlates well with reference methods. The fact that this method can confirm the appropriate antibiotic treatment in only 24 hours translates to the reduced use of wide-spectrum antibiotics with the consequence of diminishing antibiotic resistance.

**Rapid diagnosis of skin and soft tissue infections**

According to the IDSA 2013 guide to the diagnosis of infectious diseases [137], cultures are not indicated for uncomplicated common forms of skin and soft tissue infections (SSTIs) (e.g., cellulitis, subcutaneous abscesses) treated in the outpatient setting. Whether cultures are beneficial for managing cellulitis in the hospitalized patient is uncertain and the sensitivity of blood cultures in this setting is low. Cultures are however recommended for the patient who requires operative incision and drainage because of the risk of deep structure and underlying tissue involvement [138]. The IDSA guide includes recommendations for sampling and processing specimens for a microbiological diagnosis of the most frequent SSTIs. Basically, the quality of the sample and the number of potential pathogens to be considered is first established in a Gram stain, and this is followed by a conventional culture procedure. These still traditional procedures are not rapid.

The recent availability of a rapid-detection assay to identify MRSA from wound specimens allows for better-informed therapeutic decisions. The Xpert MRSA/SA skin and soft tissue infection assay (GeneXpert, Cepheid* Inc., Sunnyvale, CA) is approved for rapid detection (within 1 h) of MRSA and MSSA in wounds. In a multicenter evaluation that included a total of 114 wound specimens, the MRSA/SA SSTI assay showed a Se of 97%, a Sp of 96%, a PPV of 92% and a NPV of 99% for MRSA detection; similar percentages were noted for MSSA [139]. Overall agreement between the assay and standard culture was 96.5%.

The GeneXpert kit directly applied to synovial fluid and tissue specimens (e.g., bone, muscle, fascia, etc.) has also proved useful for the diagnosis of osteoarticular and chronic prostatic joint infections due to staphylococci [140,141], though it has not yet received CE mark approval for this purpose.

The rapid identification and differentiation of MRSA in a wound specimen allows clinicians to more rapidly initiate appropriate antimicrobial therapy.

The steps recommended for the early diagnosis of a SSTI by *Streptococcus pyogenes* are: direct Gram staining of skin biopsies, tissues, fascia, muscle, purulent exudate or joint aspirates and the rapid detection of capsule and protein antigens in skin and/or tissues using available kits that show a Se of 60% to 91% and a Sp of 85% to 98% [142-144].

**Other rapid microbiological tests that may provide useful information in ICU patients**

Besides the tests already mentioned, other diagnostic tests used in Microbiology can expedite the diagnosis of infection in these patients.

Those most often used in clinical practice, which also show adequate diagnostic performance, are the detection of the antigens of *Streptococcus pneumoniae* [145] and *Legionella pneumophila* serogroup 1 in urine [146] for patients with pneumonia; the detection of some viruses such as influenza and other respiratory viruses, or enterovirus and other central nervous system viruses [147]; and the detection of *Mycobacterium tuberculosis*, which in some cases is accompanied by the identification of resistance genes [148]. The detection of respiratory viral agents includes single or multiple pathogens (multiplex panels), which is highly convenient since most of these agents cause similar symptoms.
Conclusions

Much progress has been recently made in the rapid etiologic diagnosis of infectious diseases. Some of the new approaches available are even able to detect antimicrobial resistances and this allows for treatment optimization, especially in the most vulnerable patients such as those admitted to the ICU. Current microbiology has shortened turnaround times in the treatment of many types of infection, such as sepsis, pneumonia, urinary tract infections, skin and soft tissue infections, viral infections or tuberculosis. Molecular techniques still have issues that need to be dealt with such as their limits of detection and sensitivity for certain samples and certain situations, their correlation with adequate diagnostic gold standards, their clinical validation and the correct interpretation of results, and the risk of contamination. Improvements are also needed in terms of widening the spectrum of pathogens and resistance mechanisms that may be identified or the sample types these procedures can be used on. Despite these limitations, the future of the field of molecular techniques for the rapid diagnosis of infections is highly promising.

Abbreviations

AST: Antibiotic susceptibility testing; AUC: Area under the curve; BAL: Bronchoalveolar lavage; BCB: Blood culture bottles; BSI: Bloodstream infection; CA-UTI: Catheter-associate urinary tract infection; CE: Conformité Européenne, meaning “European Conformity”; it is a mandatory conformity marking for certain products sold within the European Economic Area (EEA) since 1985; cfu/mL: colony forming units/millilitre; CR: Catheter-related; CR-BSI: Intravascular catheter-related bloodstream infection; CRP: C-reactive protein; CVC: Central venous catheter; DTTTP: Differential time to positivity; EA: Endotracheal aspirate; HAP: Hospital acquired pneumonia; ICU: Intensive care unit; LRT: Lower respiratory tract; MRSA: Methicillin-resistant Staphylococcus aureus; MSSA: Methicillin-susceptible Staphylococcus aureus; NAT: Nucleic acid testing; NLR: Negative likelihood ratio; NPV: Negative predictive value; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; PCT: Procalcitonin; PLR: Positive likelihood ratio; PPV: Positive predictive value; ROC curve: Receiver operating characteristic curve; se: Sensitivity; Sp: Specificity; SSTI: Skin and soft tissue infection; sTREM-1: Soluble triggering receptor expressed on myeloid cells-1; UTI: Urinary tract infection; VAP: Ventilator associated pneumonia.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

EB and AB took primary responsibility for the literature search, drafted the manuscript, wrote the manuscript, critically revised the manuscript, read and approved the final version.

Acknowledgements

The authors thank the Fundación Rafael del Pino for its contribution to research in the field of nosocomial infections and Ana Burton for editorial assistance.

Financial support

This study was funded in part by the Programa de Centros de Investigación Biomédica en Red (CIBER) de Enfermedades Respiratorias CIBERES 06/00058.

Author details

1Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Doctor Esquerdo 46, 28007 Madrid, Spain.
2Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.
3Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain. “CIBER de Enfermedades Respiratorias (CIBERES CIB06)/ 06/0058”, Madrid, Spain.

Received: 8 April 2014 Accepted: 28 October 2014
Published online: 28 November 2014

References

1. Burgmann H, Hiesmayr JM, Sawey A, Bauer P, Metnitz B, Metnitz PG: Impact of nosocomial infections on clinical outcome and resource consumption in critically ill patients. Intensive Care Med 2010, 36:1597–1601.
2. Cohen E, Feinglass J, Barsuk JH, Barnard C, O’Donnell A, McGaghie WC, Wayne DB: Cost savings from reduced catheter-related bloodstream infection after simulation-based education for residents in a medical intensive care unit. Simul Healthc 2010, 5:98–102.
3. Zarb P, Coignard B, Griskevičiene J, Muller A, Vankerckhoven V, Weist K, Goossens M, Vaerenberg S, Hopkins S, Catby B, Monnet D, Goossens H, Suëtens C: The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill 2012, 17:20316.
4. Olachea PM, Palomar M, Alvarez-Lemua F, Otal JJ, Insauti J, Lopez-Puyelo MJ: Mortality and morbidity associated with primary and catheter-related bloodstream infections in critically ill patients. Rev Esp Quimioter 2013, 26:21–29.
5. Hidron AI, Edwards JR, Patel J, Homan TC, Severt DM, Pollock DA, Fridkin SK: NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 2008, 29:996–1011.
6. Brusselsena N, Vogelaers D, Blot S: The rising problem of antimicrobial resistance in the intensive care unit. Ann Intensive Care 2011, 1:17.
7. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH: The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest 2000, 118:146–155.
8. Muscedere JG, Shorr AF, Jiang X, Day A, Heyland DK: The adequacy of timely empiric antibiotic therapy for ventilator-associated pneumonia: an important determinant of outcome. J Crit Care 2012, 27:522, e327–314.
9. Palmer HR, Palavecino EL, Johnson JW, Ohl CA, Williamson JC: Clinical and microbiological implications of time-to-positivity of blood cultures in patients with Gram-negative bacilli bacteremia. Eur J Clin Microbiol Infect Dis 2013, 32:955–959.
10. Raad I, Hanna H, Mak D: Intravascular catheter-related infections: advances in diagnosis, prevention, and management. Lancet Infect Dis 2007, 7:545–567.
11. Rello J, Coll P, Prats G: Evaluation of culture techniques for diagnosis of catheter-related sepsis in critically ill patients. Eur J Clin Microbiol Infect Dis 1992, 11:1192–1193.
12. Benezra D, Kiehn TE, Gold JW, Brown AE, Turnbull AD, Armstrong D: Prospective study of infections in indwelling central venous catheters using quantitative blood cultures. Am J Med 1980, 85:495–498.
13. Rynders BJ, Peereman WS, Vervaert C, Wilmer A, Van Wijngaerden E: Watchful waiting versus immediate catheter removal in ICU patients with suspected catheter-related infection: a randomized trial. Intensive Care Med 2004, 30:1073–1080.
14. Fortun J, Grill F, Martin-Davila P, Blazquez J, Tato M, Sanchez-Conral J, Garcia-San Miguel L, Moreno S: Treatment of long-term intravascular catheter-related bacteraemia with antibiotic-lock therapy. J Antimicrob Chemother 2006, 58:816–821.
15. Safdar N, Fine JP, Mak D: Meta-analysis: methods for diagnosing intravascular device-related bloodstream infection. Ann Intern Med 2005, 142:451–466.
16. Cercenado E, Ena J, Rodriguez-Creixems M, Romero J, Bouza E: A conservative procedure for the diagnosis of catheter-related infections. Arch Intern Med 1993, 150:1417–1420.
17. Fortun J, Perez-Molina JA, Asensio A, Calderon C, Casado JL, Mir N, Moreno A, Guerrero A: Semiquantitative culture of subcutaneous segment for conservative diagnosis of intravascular catheter-related infection. JPN J Parenter Enteral Nutr 2000, 24:210–214.
18. Bouza E, Munoz P, Burillo A, Lopez-Rodriguez J, Fernandez-Perez C, Perez MJ, Rincon C: The challenge of anticipating catheter tip colonization in major heart surgery patients in the intensive care unit: are surface cultures useful? Crit Care Med 2005, 33:1953–1960.
19. Blot F: Diagnosis of catheter-related infections. In Catheter-Related Infections. Edited by Seiffert H, Hansen B, Fait B. New York: Marcel Dekker; 2005:37–76.
20. Guembe M, Rodriguez-Creixems M, Sanzial-Carrilho C, Perez-Para A, Martin-Rabadan P, Bouza E: How many lumens should be cultured in the conservative diagnosis of catheter-related bloodstream infections? Clin Infect Dis 2010, 50:1575–1579.

21. Hamilton DJ, Amos D, Schwartz RW, Dent CM, Counts GW: Effect of delay in processing on lysis-centrifugation blood culture results from narrow transplant patients. J Clin Microbiol 1989, 27:1586–1593.

22. Raad I, Hanna HA, Alakbeh B, Chatzinikolaou I, Johnson MM, Tarrand J: Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. Ann Intern Med 2004, 140:18–25.

23. Sabatier C, Garcia X, Ferrer R, Duarte M, Colomina M, Alcaraz D, Fontanals D, Valles J: Blood culture differential time to positivity enables safer catheter retention in suspected catheter-related bloodstream infection: a randomized controlled trial. Med Intensiva 2014, in press.

24. Bict F, Nitenberg G, Chachaty E, Raynard B, Germann N, Antoun S, Laplanche A, Brun-Buisson C, Tancrede C: Diagnosis of catheter-related bacteremia: a prospective comparison of the time to positivity of hub/blood versus peripheral-blood cultures. Lancet 1999, 354:1071–1077.

25. Abdelkefi A, Achour W, Ben Othman T, Torjman L, Ladeb S, Lakhal A, Hsairi M, Kammoun L, Ben Hassen A, Ben Abdeladhim A: Difference in time to positivity is useful for the diagnosis of catheter-related bloodstream infection in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2005, 35:397–401.

26. Kaasch AJ, Rieg S, Hellrich M, Kern WW, Seifert H: Differential time to positivity is not predictive for central line-related Staphylococcus aureus bloodstream infection in routine clinical care. J Infect 2014, 68:S85–S91.

27. Schwartz I, Hintichs G, Reisinger EC, Kreis GJ, Olschewski H, Krause R: Delayed processing of blood samples influences time to positivity of blood cultures and results of Gram stain-acidine orange leukocyte Cytospin test. J Clin Microbiol 2007, 45:2691–2694.

28. Kaasch AJ, Rieg S, Hellrich M, Kern WW, Seifert H: Reply to Krause et al. J Clin Microbiol 2014, 52:4983–4984.

29. Bouza E, Alcalá L, Munoz P, Martin-Rabadan P, Guembe M, Rodriguez-Creixems M: Can microbiologists help to assess catheter involvement in candidemia patients before removal? Clin Microbiol Infect 2013, 19:e129–e135.

30. Leon M, Garcia M, Herranz MA, Gonzalez V, Martinez A, Castillo F, Andres E, Kaasch AJ, Rieg S, Hellmich M, Kern WV, Seifert H, Abdelkefi A, Achour W, Ben Othman T, Torjman L, Ladeb S, Lakhal A, Hsairi M, Kammoun L, Ben Hassen A, Ben Abdeladhim A: Difference in time to positivity is useful for the diagnosis of catheter-related bloodstream infection in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2005, 35:397–401.

31. Schwartz I, Hintichs G, Reisinger EC, Kreis GJ, Olschewski H, Krause R: Delayed processing of blood samples influences time to positivity of blood cultures and results of Gram stain-acidine orange leukocyte Cytospin test. J Clin Microbiol 2007, 45:2691–2694.

32. Kaasch AJ, Rieg S, Hellrich M, Kern WW, Seifert H: Differential time to positivity is not predictive for central line-related Staphylococcus aureus bloodstream infection in routine clinical care. J Infect 2014, 68:S85–S91.

33. Bouza E, Alvarado N, Alcalá L, Perez MJ, Rincon C, Munoz P: A randomized and prospective study of 3 procedures for the diagnosis of catheter-related bloodstream infection without catheter withdrawal. Clin Infect Dis 2007, 44:820–826.

34. Castello-Romero A, Suberviola B, García-Astudillo LA, Holanda MS, Ortiz F, Llorca J, Delgado-Rodrigo M: Impact of the Surviving Sepsis Campaign protocols on the hospital length of stay and mortality in septic shock patients: results of a three-year follow-up quasi-experimental study. Crit Care Med 2010, 38:1036–1043.

35. Fenollar F, Raoult D: Molecular diagnosis of bloodstream infections caused by Gram-positive bacteria. Current approaches to the diagnosis of bacterial and fungal infections from bloodstream infections: from three observational trials. Crit Care 2010, 14:R186.

36. Ho YP, Reddy PM: Advances in mass spectrometry for the identification of pathogens. Mass Spectrom Rev 2011, 30:1203–1244.

37. Cherkouk A, Hibs J, Emonet S, Tangomo M, Girard M, Francios P, Schrenzel J: Comparison of two matrix-assisted laser desorption ionization-time-of-flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J Clin Microbiol 2010, 48:1159–1175.

38. Spanu T, Postoraro B, Fiori B, D’Inzzo T, Campolo S, Ruggeri A, Turnarello M, Canu G, Trecarichi EM, Parisi G, Tronci M, Sanguinetti M, Fadda G: Direct maldi-tof mass spectrometry assay of blood culture broths for rapid identification of Candida species causing bloodstream infections: an observational study in two large microbiology laboratories. J Clin Microbiol 2012, 50:176–179.

39. Juiz PM, Almela M, Melcion C, Campo I, Esteban C, Pitart C, Marco F, Vila J: A comparative study of two different methods of sample preparation for positive blood cultures for the rapid identification of bacteria using MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 2012, 31:1533–1538.

40. Leiti C, Cenci E, Cardacca A, Moretti A, D’Ago F, Pagliocchini R, Baraccca M, Ferraili S, Vioto S, Bistoni F, Menocci A: Rapid identification of bacterial and fungal pathogens from positive blood cultures by MALDI-TOF MS. Int J Med Microbiol 2013, 303:205–209.

41. Rodriguez-Sanchez B, Sanzial-Carrilho C, Ruiz A, Marin M, Cercenado E, Rodriguez-Creixems M, Bouza E: Direct identification of pathogens from positive blood cultures using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Clin Microbiol Infect 2013, 19:e1111/e1116/1245.

42. Vlek AL, Bonten MJ, Boel CH: Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One 2012, 7:e32589.

43. Reinhart K, Bauer M, Riedemann NC, Hertog CS: New approaches to sepsis: molecular diagnostics and biomarkers. Clin Microbiol Rev 2012, 25:609–634.

44. Pieraerts C, Vincent JL: Sepsis biomarkers: a review. Crit Care 2010, 14:e125.

45. Povoa P, Coelho L, Almeida E, Fernandes A, Mealha R, Moreira P, Sabino H: C-reactive protein as a marker of infection in critically ill patients. Crit Care Microbiol Infect Dis 2005, 11:101–108.

46. Povoa P, Coelho L, Almeida E, Fernandes A, Melha R, Moreira P, Sabino H: Early identification of intensive care unit-acquired infections with daily monitoring of C-reactive protein: a prospective observational study. Crit Care 2006, 10:165.

47. Lobo SM, Lobo FR, Bota DP, Lopes-Ferreira F, Soiman HM, Melot C, Vincent JL: C-reactive protein levels correlate with mortality and organ failure in critically ill patients. Chest 2003, 123:2043–2049.

48. Gamacho-Montero J, Huici-Moreno MJ, Gutierrez-Pizarraya A, Lopez I, Marquez-Vicario JA, Machar H, Guerrero JM, Puppo-Moreno A: Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients with suspicion of sepsis. Crit Care 2014, 18:R116.

49. Garamellcols-Bourbeuils EJ, Tsangaris I, Kanni T, Mouktaroudis M, Pantelidou I, Adamis G, Atzmizidis S, Chrisofos M, Evangelopoulos V, Frantszakis F, Giannopoulos P, Giannakopoulos G, Gaitalis D, Gourgoulis GA, Kotsampasi K, Katifia K, Kofinas G, Kontopidou F, Korazis G, Kouloura V, Kouritsikou A, Kouperoti M, Kritsiki E, Leonidou L, Mega A, Mykona V, Nikolarou H, Orfanos S, Panagopoulos P, Paramythiotou E, et al: Procalcitonin as an early indicator of outcome in sepsis: a prospective observational study. J Hosp Infect 2014, 87:58–63.

50. Karlsson S, Heikkinen M, Pettita V, Alila S, Vaisanen S, Pullki K, Kolho E, Ruokonen E: Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2014, 18:R205.
59. Rello J: Bench-to-bedside review: Therapeutic options and issues in the management of ventilator-associated bacterial pneumonia. Crit Care 2005, 9:259–265.

60. Kollef MH: What is ventilator-associated pneumonia and why is it important? Respir Care 2005, 50(7):74–724.

61. Chastre J: Conference summary: ventilator-associated pneumonia. Respir Care 2005, 50:975–983.

62. Muscogere JG, Martin CM, Heyland DK: The impact of ventilator-associated pneumonia on the Canadian health care system. J Crit Care 2008, 23:10–15.

63. Hortal J, Munoz P, Cuerpo G, Litvan H, Rosseel PM, Bouza E: Ventilator-associated pneumonia in patients undergoing major heart surgery: an incidence study in Europe. Crit Care 2009, 13:R60.

64. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH: Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest 2002, 122:262–268.

65. Kuti EL, Patel AA, Coleman CM: Impact of inappropriate antibiotic therapy on mortality in patients with ventilator-associated pneumonia and blood stream infection: a meta-analysis. J Crit Care 2008, 23:81–100.

66. Bok S: Limiting the attributable mortality of nosocomial infection and multidrug resistance in intensive care units. Clin Microbiol Infect 2008, 14(15):13–23.

67. Bogaerts P, Hamels S, de Mendonça R, Huang TD, Rosin S, Remacle J, Markine-Gorsynoff N, de Longueville F, Pluster W, Denis O, Glupczynski Y: Analytical validation of a novel high multiplexing real-time PCR array for the identification of key pathogens causative of bacterial ventilator-associated pneumonia and their associated resistance genes. J Antimicrob Chemother 2014, 69:840–347.

68. Hogardt M, Trebesius K, Geiger AM, Hornef M, Rosenecker J, Heesemann J: Specific and rapid detection by fluorochrome in situ hybridization of bacteria in clinical samples obtained from cystic fibrosis patients. J Clin Microbiol 2000, 38:816–825.

69. American Thoracic Society, Infectious Diseases Society of America: Guideline for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005, 171:388–416.

70. de Jaeger A, Litalien C, Lacroix J, Guertin MC, Infante-Rivard C: Conference summary: ventilator-associated, and healthcare-associated pneumonia. Multidrug resistance in intensive care units. Clin Microbiol Infect 2000, 6(Suppl 3):198–203.

71. Torres A, El-Ebiary M: Accuracy of delayed (24 hours) processing of bronchoalveolar lavage for diagnosing bacterial pneumonia of the British Society for Antimicrobial Chemotherapy. J Clin Microbiol 2001, 39(1):409–413.

72. Berton DC, Kalil AC, Teixeira PJZ: Surrogate markers and microbiologic end points. Clin Microbiol Rev 2009, 22:2548–2560.

73. Torres A, Montecalvo MA: Bronchoscopic BAL in the diagnosis of ventilator-associated pneumonia. Chest 2000, 117(4 Suppl):2191S–2195S.

74. Berton DC, Kalil AC, Teixeira PJZ: Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia. Cochrane Database of Systematic Reviews 2014, 10(10):CD006482. DOI:10.1002/14651858.CD006482.pub4.

75. Georges H, Santre C, Leroy O, Roussel-Delvallez M, Caillaux M, Beuscart C, de la Haba MC, Hartog D, Beranger A, Cribier A, Impagnatiello C, Luyt R, Wilcox M: Guidelines for the management of hospital-acquired pneumonia in the UK: report of the working party on hospital-acquired pneumonia of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 2008, 62:5–34.

76. Lutrop J, El-Ebiary M: Accuracy of delayed (24 hours) processing of bronchoalveolar lavage for diagnosing bacterial pneumonia. J Clin Microbiol 2001, 39(1):409–413.

77. Davis KA, Eckert MJ, Reed RL 2nd, Esposito TJ, Santaniello JM, Poulakidas S, Maillet JM, Fitoussi F, Penaud D, Dennewald G, Boullard D: Antimicrobial stewardship lessons: know when to say no to vancomycin. Clin Infect Dis 2013, 56(s6):616–617.

78. Torres M, Rados I, Sánchez C, Martín-Rabadán P, Muñoz P, Bouza E: Performance of Gram stain in lower respiratory tract (LRT) samples in ventilator-associated pneumonia (VAP): a prospective study. [Abstract]. In XII Congress of the Spanish Society of Infectious Diseases and Clinical Microbiology. Valencia, Spain, 2006.

79. Barsic B, Viducic L, Gonzalez S, Castro P, de Batlle J, Castro A, Bonet A: Microscopic examination of intracellular organisms in protected bronchoalveolar mini-lavage fluid for the diagnosis of ventilator-associated pneumonia. Chest 2001, 120:S13–S18.

80. Timsit JF, Chevallier C, Gachot B, Bruneel F, Wolff M, Carlet J, Regnier B: Usefulness of a strategy based on bronchoscopy with direct examination of bronchoalveolar lavage fluid in the initial antibiotic therapy of suspected ventilator-associated pneumonia. Intensive Care Med 2001, 27:560–567.

81. Mentec H, May-Michelangelo L, Rabbat A, Varon E, Le Turdu F, Bleichner G: Blind and bronchoscopy sampling methods in suspected ventilator-associated pneumonia. A multicentre prospective study. Intensive Care Med 2004, 30:1319–1326.

82. Burillo and Bouza

83. Antimicrobial stewardship lessons: know when to say no to vancomycin. Clin Infect Dis 2013, 56(s6):616–617.

84. Barsic B, Viducic L, Gonzalez S, Castro P, de Batlle J, Castro A, Bonet A: Microscopic examination of intracellular organisms in protected bronchoalveolar mini-lavage fluid for the diagnosis of ventilator-associated pneumonia. Chest 2001, 120:S13–S18.

85. Timsit JF, Chevallier C, Gachot B, Bruneel F, Wolff M, Carlet J, Regnier B: Usefulness of a strategy based on bronchoscopy with direct examination of bronchoalveolar lavage fluid in the initial antibiotic therapy of suspected ventilator-associated pneumonia. Intensive Care Med 2001, 27:560–567.

86. Mentec H, May-Michelangelo L, Rabbat A, Varon E, Le Turdu F, Bleichner G: Blind and bronchoscopy sampling methods in suspected ventilator-associated pneumonia. A multicentre prospective study. Intensive Care Med 2004, 30:1319–1326.

87. Bahrani-Mougeot FK, Paster BJ, Coleman S, Barbuto S, Brennan MT, Noll J, Bodey GP, Gorbach SL, Holland WC, Ralston SH, Provost DA, Perl TM, Samson LW, Salgado CD, Weisen RA, Wise R, Yokoe DS: Strategies to prevent ventilator-associated pneumonia in acute care hospitals. Infect Control Hosp Epidemiol 2008, 29(Suppl 1):S31–S40.

88. Barsic B, Viducic L, Gonzalez S, Castro P, de Batlle J, Castro A, Bonet A: Microscopic examination of intracellular organisms in protected bronchoalveolar mini-lavage fluid for the diagnosis of ventilator-associated pneumonia. Chest 2001, 120:S13–S18.

89. Lutrop J, El-Ebiary M: Accuracy of delayed (24 hours) processing of bronchoalveolar lavage for diagnosing bacterial pneumonia. J Clin Microbiol 2001, 39(1):409–413.

90. Cohen CM, Jacoby TA, Schuchat A, van Woon M, Ramsay G, Drent M: Influence of antibiotic therapy on the cytological diagnosis of ventilator-associated pneumonia. Intensive Care Med 2008, 34:865–872.

91. Barsic B, Viducic L, Gonzalez S, Castro P, de Batlle J, Castro A, Bonet A: Microscopic examination of intracellular organisms in protected bronchoalveolar mini-lavage fluid for the diagnosis of ventilator-associated pneumonia. Chest 2001, 120:S13–S18.

92. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

93. Coffin SE, Klompas M, Classes D, Arias KM, Podgorny K, Anderson DJ, Burstin H, Cafpe DF, Dubberke ER, Fraser V, Gerdin GN, Griffin RA, Gross P, Kaye KS, Lo E, Marschall J, Merril MA, Nicolle L, Pogue DA, Perl TM, Stainton S, Salgado CD, Weinstein RA, Wise R, Yokoe DS: Strategies to prevent ventilator-associated pneumonia in acute care hospitals. Infect Control Hosp Epidemiol 2008, 29(Suppl 1):S31–S40.

94. Barsic B, Viducic L, Gonzalez S, Castro P, de Batlle J, Castro A, Bonet A: Microscopic examination of intracellular organisms in protected bronchoalveolar mini-lavage fluid for the diagnosis of ventilator-associated pneumonia. Chest 2001, 120:S13–S18.

95. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

96. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

97. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

98. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

99. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.

100. Carson KD, Chastre J, Canagaia G, Masterton R. A European care bundle for management of ventilator-associated pneumonia. J Crit Care 2011, 26:S1–10.
98. Tenover FC: Developing molecular amplification methods for rapid diagnosis of respiratory tract infections caused by bacterial pathogens. Clin Infect Dis 2011, 52(Suppl 6):S338–S345.

99. Bolmström A, Anidson S, Ericsson M, Karlsson A: A novel technique for direct quantification of antimicrobial susceptibility of microorganisms. In 28th Interscience Conference on Antimicrobial Agents and Chemotherapy. Los Angeles, (LO), 1988.

100. Cercenado E, Rico MV, Vicente T, Bouza E: Rapid Antimicrobial Susceptibility Testing in Patients with Ventilator-Associated Pneumonia: Direct E-Test on Respiratory Samples. (Abstract: D-51), In 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy; 2002; San Diego (US), 2002.

101. Bouza E, Torres MV, Radice C, Cercenado E, de Diego R, Sanchez-Carrillo C, Munoz P: Direct E-test (AB Biodisk) of respiratory samples improves antimicrobial use in ventilator-associated pneumonia. Clin Infect Dis 2007, 44(3):382–387.

102. Muñoz P, Cercenado E, Giannella M, Bouza E: Rapid detection of microorganism resistance in patients with ventilator-associated pneumonia. Clin Pulm Med 2009, 16(6):302–308.

103. Cercenado E, Marín M, Insá R, Martin-Rabadan P, Rivera M, Bouza E: Evaluation of direct E-test on lower respiratory tract samples using a chromogenic agar medium: a rapid procedure for antimicrobial susceptibility testing. (Abstract P882), In 19th Congress of the ESCMID: 2009; Finland: Helsinki, 2009.

104. Lisboa T, Seligman R, Díaz E, Rodríguez A, Teixeira PJ, Bello J: C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. Crit Care Med 2008, 36:166–171.

105. Jung B, Embirico N, Roux F, Forel JM, Demory D, Allardet-Servent J, Jaber S, Luyt CE, Guerin V, Combes A, Trouillet JL, Ayed SB, Bernard M, Gibert C, Luyt CE, Combes A, Trouillet JL, Chastre J: Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. Crit Care 2006, 10(6):R125.

106. Siu LX, Meng K, Zhang X, Wang HJ, Yan P, Jia YH, Feng D, Xie LX: Diagnosing ventilator-associated pneumonia in critically ill patients with sepsis. Am J Respir Crit Care Med 2012, 181:e110–e119.

107. Nobre V, Harbarth S, Graf JD, Rohner P, Pugin J: Use of procalcitonin to shorten antibiotic treatment duration in septic patients: a randomized trial. Am J Respir Crit Care Med 2008, 177:496–505.

108. Linser CR, Farr ER, Drent M, Jacobs JA: C-reactive protein and procalcitonin concentrations in bronchoalveolar lavage fluid as a predictor of ventilator-associated pneumonia. Ann Clin Biochem 2008, 45:293–298.

109. Hillas G, Vassilakopoulos T, Planta P, Rasidakis A, Bakakos P: C-reactive protein and procalcitonin as predictors of survival and septicaemic shock in ventilator-associated pneumonia. Eur Respir J 2010, 35:805–811.

110. Chastre J, Luyt CE, Trouillet JL, Combes A: New diagnostic and prognostic markers of ventilator-associated pneumonia. Curr Opin Crit Care 2006, 12:446–451.

111. Conway Morris A, Kefala K, Wilkinson TS, Moncayo-Nieto OL, Dhaliwal K, Farrell L, Walsh TS, Mackenzie SJ, Swann DG, Andrews PJ, Anderson N, Govan JR, Lauresson F, Reid H, Davidson DJ, Haslett C, Sallanen JM, Simpson AJ: Diagnostic importance of pulmonary interleukin-1beta and interleukin-8 in ventilator-associated pneumonia. Thorax 2010, 65:201–207.

112. Luyt CE, Combes A, Trouillet JL, Chastre J: Value of the serum procalcitonin level to guide antimicrobial therapy for patients with ventilator-associated pneumonia. Semin Respir Crit Care Med 2011, 32:181–187.

113. Pugh R, Grant C, Cooke RP, Dempsey G: Short-course versus prolonged-course antibiotic therapy for hospital-acquired pneumonia in critically ill adults. Cochrane Database Syst Rev 2011, CD007577.

114. Goulden BE: Assessment of the usefulness of the examination of a gram smear of fresh uncentrifuged urine in the determination of significant bacteriuria in dogs. N Z Vet J 1968, 16:1–2.

115. Jorgensen JH, Jones PW: Comparative evaluation of the Limulus am arginine esterase test and the direct Gram stain for detection of significant bacteriuria. Am J Clin Pathol 1975, 63:142–146.

116. Robins DG, Rogers KB, White RH, Osman MS: Urine microscopy as an aid to detection of bacteriuria. Lancet 1975, 1476–1478.

117. Crou T, Ilton RC: Rapid screening of urine for significant bacteriuria by Gram stain, acidine orange stain, and the Autobac MTS system. Diagn Microbiol Infect Dis 1984, 2179–186.

118. Ilton RE, Ilton RC: Automated direct antimicrobial susceptibility testing of microscopically screened urine cultures. J Clin Microbiol 1990, 1175–1161.

119. Jorgensen JH, Barlow SF, Turner RM, Hodgson E, Craig JC: Absolute and relative accuracy of rapid urine tests for urinary tract infection in children: a meta-analysis. Lancet Infect Dis 2010, 10:240–250.

120. Lewis JF, Alexander M: Microscopy of stained urine smears to determine the need for quantitative culture. J Clin Microbiol 1976, 4372–374.

121. La Scola B, Rouault D: Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. PLoS One 2009, 4:e8804.

122. Ferroni A, Suarez S, Beretti JL, Dauphin B, Bille E, Meyer J, Bougnoux ME, Alario A, Berche P, Nassif X: Real-time identification of bacteria and Candida species in positive blood culture broths by matrix-assisted laser desorption ionization-time of-flight mass spectrometry. J Clin Microbiol 2010, 48:1542–1548.

123. Ferreira L, Sanchez-Juanes F, Gonzalez-Avila M, Cembrero-Fucinos D, Herrera-Hernandez A, Gonzalez-Buitrago JM, Munoz-Bellido JL: Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of-flight mass spectrometry. Clin Microbiol 2010, 48:2110–2115.

124. Jorgensen JH, Barlow SF, Turner RM, Hodgson E, Craig JC: Rapid detection of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of-flight mass spectrometry. J Clin Microbiol 2010, 48:2110–2115.

125. Wang YH, Zhang G, Fan YY, Yang X, Xiu WJ, Lu XW: Direct identification of bacteria causing urinary tract infections by combining matrix-assisted laser desorption ionization-time of-flight mass spectrometry with a rapid diagnosis of urinary tract infection. PLoS One 2014, 9:e86915.

126. Oakes AR, Badger R, Grove DL: Comparison of direct and standardized testing of infected urine for antimicrobial susceptibilities by disk diffusion. J Clin Microbiol 1994, 32:40–45.

127. Allen JS, Check D, Kujawa R, Sheehan A, Kim J, Slade M, Medalia O, Delage B, Cukierman A, Griffiths R, Woodford N, Breiman RF, Patterson D, Neu HC, Karchmer AW, Martin P, Waldvogel FA, Cassel JB, Perlin DS, Telledou B, Dellinger EP: Accuracy of urine antigen testing for the diagnosis of invasive bacterial infections: a meta-analysis. JAMA 2011, 306:1310–1320.

128. Johnson JR, Tiu FS, Stamm WE: Direct antimicrobial susceptibility testing for acute urinary tract infections in women. J Clin Microbiol 1995, 33:2316–2323.

129. Gillessen-Iv J, Clark WH: Tentative direct antimicrobial susceptibility testing in urine. J Urol 1996, 156:149–153.

130. Brennemann T: Direct antimicrobial susceptibility testing in bacteriuria. APMA 1999, 107(4):437–444.

131. Breterer KB, Rentenaar RW, Verkaart G, Sturm PD: Performance and clinical signification of direct antimicrobial susceptibility testing on urine from hospitalized patients. Scand J Infect Dis 2011, 43:771–776.

132. Tilton RE, Miller JM, Weinstein MP, Richter SS, Gligan PH, Thomson RB Jr, Bourbeau P, Carroll KC, Kehl SC, Durne WM, Robinson-Dunn B, Schwartzman JD, Chapman KC, Snyder JM, Forbes BA, Patel R, Rosenblatt JE, Pitts BS: A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 2013, 57(2):e11–e121.

133. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya XS, Wade JC: Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin Infect Dis 2005, 41:1373–1406.
Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays, J Clin Microbiol 2009, 47:823–826.

140. Dubouix-Bour Andy A, de Ladoucette A, Pietri V, Méhdi N, Benzaquen D, Guinand R, Gandois JM: Direct detection of Staphylococcus osteoarticular infections by use of Xpert MRSA/SA SSTI real-time PCR. J Clin Microbiol 2011, 49:4225–4230.

141. Titecat M, Loiez C, Senneville E, Wallet F, Dezeque H, Legout L, Migaud H, Courcol RJ: Evaluation of rapid mecA gene detection versus standard culture in staphylococcal chronic prosthetic joint infections. Diagn Microbiol Infect Dis 2012, 73:318–321.

142. Petts DN, Lane A, Kennedy P, Hadfield SG, McIlmurray MB: Direct detection of groups A, C and G streptococci in clinical specimens by a trivalent colour test. Eur J Clin Microbiol Infect Dis 1988, 7:34–39.

143. de Quiros JCL B, Moreno S, Muñoz P, Rodríguez-Créixems M, Catalán P, Bouza E: Antigen detection in the rapid diagnosis of severe extrapharyngeal infections caused by group A streptococcus. Infect Dis Clin Pract 1992, 1:372–376.

144. Garcia-Lechuz Moya JM: Clinical microbiological case: a Nicaraguan woman with skin lesions on the left elbow and foot. Clin Microbiol Infect 2001, 7:84–87.

145. Sinclair A, Xie X, Telfscher M, Dendukuri N: Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by Streptococcus pneumoniae. J Clin Microbiol 2013, 51(7):2303–2310.

146. de Ory F, Minguito T: Comparison of five commercial assays for the detection of Legionella pneumophila antigens in urine. Enferm Infect Microbiol Clin 2009, 27(2):81–84.

147. Emmadi R, Boonyaratarkomkit J, Selvarangan R, Shyamala V, Zimmer BL, Williams L, Bryant B, Schutzbank T, Schoonmaker MM, Amos-William JA, Hall L, Pancholi P, Bernard K: Molecular methods and platforms for infectious diseases testing: a review of FDA-approved and cleared assays. J Mol Diagn 2011, 13(6):583–604.

148. Tenover FC: Potential impact of rapid diagnostic tests on improving antimicrobial use. Ann N Y Acad Sci 2010, 1213:70–80.

doi:10.1186/s12879-014-0593-1
Cite this article as: Burillo and Bouza: Use of rapid diagnostic techniques in ICU patients with infections. BMC Infectious Diseases 2014 14:593.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit