REVIEW

Blood culture-based diagnosis of bacteraemia: state of the art

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Abstract

Blood culture remains the best approach to identify the incriminating microorganisms when a bloodstream infection is suspected, and to guarantee that the antimicrobial treatment is adequate. Major improvements have been made in the last years to increase the sensitivity and specificity and to reduce the time to identification of microorganisms recovered from blood cultures. Among other factors, the introduction in clinical microbiology laboratories of the matrix-assisted laser desorption ionization time-of-flight mass spectrometry technology revolutionized the identification of microorganisms whereas the introduction of nucleic-acid-based methods, such as DNA hybridization or rapid PCR-based test, significantly reduce the time to results. Together with traditional antimicrobial susceptibility testing, new rapid methods for the detection of resistance mechanisms respond to major epidemiological concerns such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase or carbapenemases. This review presents and discusses the recent developments in microbial diagnosis of bloodstream infections based on blood cultures.

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Introduction

Bloodstream infections (BSIs) are severe diseases characterized by a high morbidity and mortality, which is directly related with the delay in administration of the first adequate anti-infectious agent [1–7]. Empirical anti-infectious treatments are chosen on the basis of the clinical and epidemiological data and are started immediately after the sampling of blood bottles; however, until microbiological documentation, their adequacy cannot be guaranteed [8–10], especially in the context of an increasing rate of multidrug-resistant organisms [11]. Rapid microbiological investigations—identification of the causative agent and antimicrobial susceptibility testing (AST)–are therefore very important: 1) to adjust the anti-infectious therapy and to avoid inefficient treatment, 2) to reduce the spectrum of the anti-infectious therapy so as to limit the selection of resistant strains and 3) to limit the toxicity and negative impact on beneficial bacteria of some broad-spectrum antibiotics or combined therapy. The quantity of microbes present in the blood during BSIs ranges from 1 to 10 CFU/mL [12–15] to 1×10^3 and 1×10^4 CFU/mL [16]. Blood cultures currently represent the main method to determine the aetiology of a BSI because they are highly sensitive and easy to perform. The sensitivity of blood cultures is largely related to the volume of the sample. For adults, one blood sampling generally represents up to 20 mL of blood used to inoculate two bottles (one aerobic bottle and one anaerobic bottle). Before antibacterial treatment two to four blood cultures, i.e. 40 mL to 80 mL of blood are necessary to detect a causative agent in 80% to 96% of bacteremias [17,18]. Upon blood-culture positivity, the first step is to perform a Gram staining with a blood-culture aliquot. This is a mandatory analysis to confirm the presence of bacteria/fungi in the blood bottle. If microbes are present, the morphotype provides a first hint on the aetiology of the infection. Then microbe identification can be achieved using various approaches that are described and discussed in this

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review: 1) starting from a subculture, 2) directly from the positive blood culture using nucleic-acid-based methods such as hybridization and microarray or 3) after a bacterial enrichment and purification step to obtain a 'bacterial pellet', suitable for a variety of approaches including matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Blood-culture sampling and technologies

Standard bottles containing rich media have been designed for aerobic and anaerobic growth conditions, respectively. They are designed for up to 10 mL of blood. However, because of the difficulty of obtaining large volumes of blood, specific paediatric blood bottles have been designed for the culture of volumes <3 mL. To neutralize antibiotics given prior to sampling, charcoal or resins have been introduced in specific bottles (Table 1). Lytic agents added in some growth media promote the recovery and growth of organisms that have been endocytosed by phagocytes. Standard incubation time is 5 days, which is sufficient for the recovery of the majority of organisms including the HACEK group of fastidious bacteria (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikuenella and Kingella*) bacteria and *Brucella* spp. [19,20]. However, the incubation time should be increased for slow-growing organisms such as fungi and *Mycobacteria* spp.; the latter being grown in bottles containing Middlebrook 7H9. Modern laboratories rely on automated incubators (Table 1) including continuous monitoring for positive bottle detection, which significantly reduces the working load, the incubation time and the contamination rate. Blood-culture positivity is generally detected by following CO_2 production by growing microorganisms that trigger a pH increase visualized by colour changes, fluorescence signal or red-ox variations.

Interpretation of positive blood cultures: contamination versus bloodstream infection

Contamination that represents up to one-third of positive blood cultures can occur when microbes that are not present in the bloodstream are introduced into the bottle during the blood sampling [21]. Even if blood cultures are drawn under aseptic conditions, contaminations are often due to organisms that can be present in the environment and that can be part of the skin flora, such as coagulase-negative staphylococci, the third most prevalent microbe identified in blood cultures in our tertiary-care university hospital (Fig. 1), and other organisms with low or absent virulence towards humans such as *Micrococcus* spp., *Propionibacterium acnes*, most *Bacillus* spp. and most *Corynebacterium* spp. [21]. Interestingly, the rate of contamination inversely correlates with the blood volume [21,22]. A small sample volume might increase the concentration of contaminants or might be associated with difficulties in maintaining sterile conditions due to poor venous access [21,22]. Interestingly, peripheral venepuncture, arterial access or central venous accesses are associated with different contamination rates of 36%, 10% and 7%, respectively [21]. For venepuncture, a promising approach to reduce to <30% the contamination rate is named the 'initial specimen diversion technique', in which the first milliliter of blood (potentially containing bacteria not killed by skin surface antisepsis) is discarded or used for other purposes [23,24]. Nevertheless the specificity of blood culture for the diagnosis of BSI remains high as most of the top microbes recovered from positive blood cultures are primarily pathogens such as *Escherichia coli*, *Staphylococcus aureus* or *Pseudomonas aeruginosa* respectively second, fourth and fifth positions, respectively (Fig. 1).

TABLE 1. Main automated blood culture incubation systems currently commercially available

System (Manufacturer)	Main blood-culture media and characteristics	Positivity detection system
BD BACTEC (BD Diagnostics, Franklin Lakes, NJ, USA)	Standard aerobic and anaerobic broth media Media containing resin particles Media specifically designed for small blood volume inoculation Media containing a lysing agent to increase the recovery of organisms phagocytosed Media optimized for the growth of mycobacteria Specific algorithms for fastidious organisms (e.g. *Haemophilus* spp. and *Neisseria* spp.)	Fluorescent sensor of CO_2 production
BacT/ALERT 3D (bioMérieux, Durham, NC, USA)	Plastic bottles Standard aerobic and anaerobic broth media Media containing activated charcoal particles[a] or resin Media specifically designed for small blood volume inoculation Media supplemented with Middlebrook 7H9 for microbacteria growth Enriched media	Colorimetric sensor of CO_2 production
VersaTREK, (TREK Diagnostic Systems, ThermoFisher Scientific, Waltham, MA, USA)	Standard aerobic and anaerobic broth media for samples from 0.1 to 10 mL, optimized to minimize the impact of antibiotics	Monitoring of redox variations

[a]The presence of charcoal particles prevent the use of the pellet for direct identification from positive blood culture using MALDI-TOF MS.

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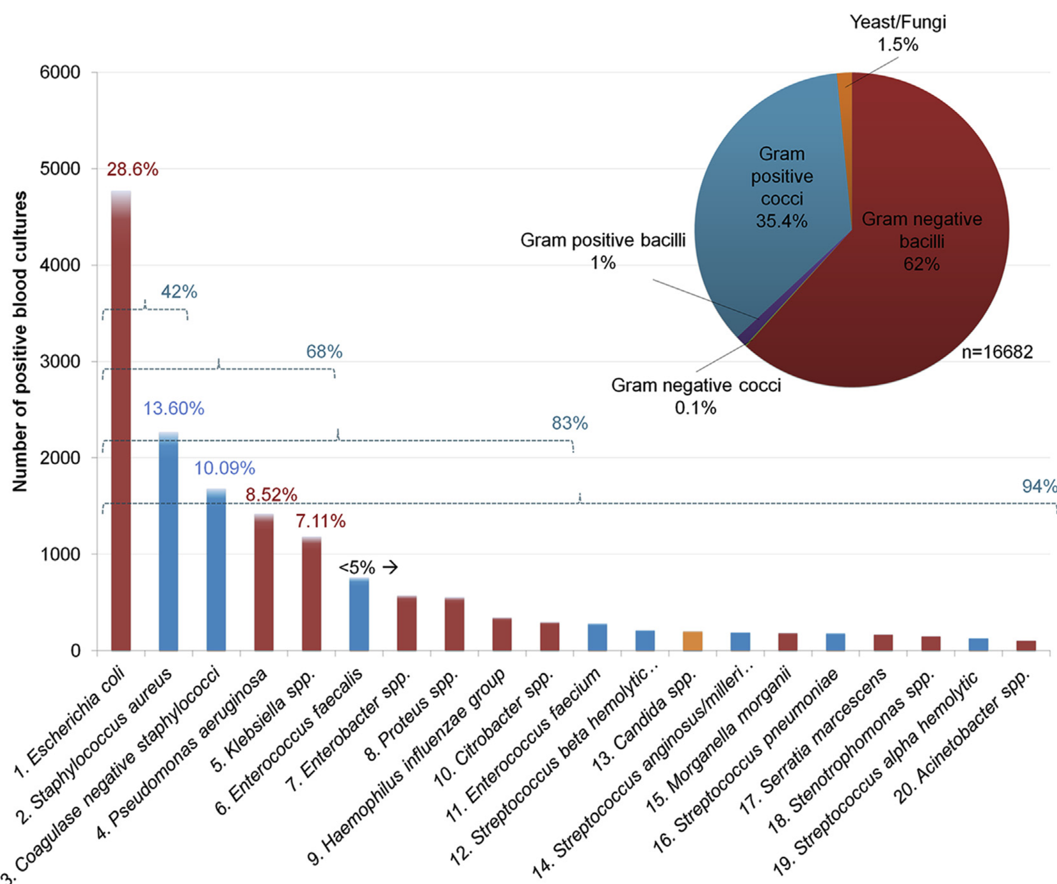

FIG. 1. Top 20 microbes identified from positive blood cultures during 1 year. Data from our 1000-bed tertiary-care university hospital during the year 2013. The pie chart represents the distribution per morphotype of all the microorganisms (total 16 682 identifications).

Together with the clinical signs and symptoms, several parameters can help to identify precisely the significance of positive blood cultures: 1) the number of positive bottles, the number of positive blood-culture pairs as well as the proportion of positive cultures [25], 2) the site of sampling—catheter versus venous puncture, and 3) the time to positivity, including the differential time to positivity between pairs collected from different sampling sites [26]. For this reason, international guidelines recommend drawing several sets of blood bottles [27]. Increasing the number of blood cultures increases the sensitivity through the increased total volume collected [28]. Blood-culture sampling should be repeated because bacteraemia often occurs intermittently. This is not the case of endocarditis or septic thrombophlebitis, where all the bottles are generally positive with pyogenic bacteria such as *S. aureus* and *Streptococcus pyogenes*.

When contamination is excluded, the organism detected and identified in the blood culture is probably present in the bloodstream at the time of sampling, which defines a bacteraemia (or fungaemia). Bacteraemia or fungaemia can be transient or sustained. Transient bacteraemia corresponds to a single episode of positive blood cultures due to the presence of microorganism during a short time-lapse in the bloodstream (less than 30 min). They are generally caused by the manipulation of contaminated mucosa or invasive respiratory, gastro-intestinal or urogenital acts [29]. Conversely, a finding of multiple positive bottles drawn at different times is a sign of sustained bacteraemia (fungaemia). Sustained positive blood cultures are encountered in the presence of endovascular infections such as endocarditis. In such cases a high number of blood cultures become positive without any difference in the time to positivity for bottles drawn simultaneously from different sites. In contrast, a catheter infection is suspected when blood cultures drawn from a catheter become positive more than 2 h before blood culture drawn from a venepuncture. This time to positivity difference exhibits an overall sensitivity of 91% and a specificity of 94% for the diagnosis of catheter infection [30]. The catheter can be removed and cultured by plating the catheter tip using the Maki roll-on semi-quantitative method [31]. Sustained bacteraemia should be

differentiated from persistent bacteraemia, which is defined by the persistence of positive blood cultures despite the introduction of an anti-infectious treatment. Persistent bacteraemia (fungaemia) is generally due to organisms resistant to the prescribed antibiotic, to the presence of a second organism or to the site of infection being inaccessible to the antibiotic (e.g. septic thrombosis).

Identification and AST approaches requiring a pure subculture

After the Gram-staining examination, the positive blood culture can be plated to ensure pure culture (isolated colonies) that can be analysed using conventional biochemical methods, MALDI-TOF MS and/or nucleic-acid based methods such as PCR and gene sequencing (Fig. 2). A 12-h to 24-h incubation period is generally required to obtain enough isolated colonies for biochemical characterization, whereas relatively short incubations (<6 h) are often long enough to obtain pure subcultures. For this reason, MALDI-TOF MS, the current best method for rapid bacterial identification (see section *MALDI-TOF MS*) has been tested on colonies obtained after short incubation on solid medium. Idelevich et al. reported that a subculture shorter than 6 h yielded species identification in 97.6% of the case for Gram-negative aerobic rods and 64.0% of Gram-positive aerobic cocci using MALDI-TOF MS [32]. The mean subculture time on agar plate needed to achieve bacterial identification at the species level was 5.9 h for Gram-positive aerobic cocci and 2.0 h for Gram-negative aerobic rods [32]. Similarly Verroken et al. reported 81.1% correct identifications using MALDI-TOF MS on a 5-h brief culture of monomicrobial blood cultures. In addition a correct identification of one of the two pathogens was achieved in 82.7% of the polymicrobial blood cultures [33]. AST performed directly from a positive blood bottle is not recommended but can be achieved on a bacterial pellet preparation from the positive blood bottle (see section *Identification and antimicrobial susceptibility testing using a blood-culture microbial pellet*) or via a brief subculture. A short subculture (2.4 h for Gram-negative bacteria and 3.8 h for Gram-positive bacteria) provided reliable AST using the automated device VITEK2 (bioMérieux SA, Marcy l'Étoile, France). The total time to result from positive blood cultures was 11.2 h and 13.6 h for Gram-negative rods and Gram-positive cocci, respectively [34]. Automated systems allow the testing of a high number of antibiotics at the same time, the interpretation of the result is automated and internal expert systems are able to detect specific resistance mechanisms such as extended-

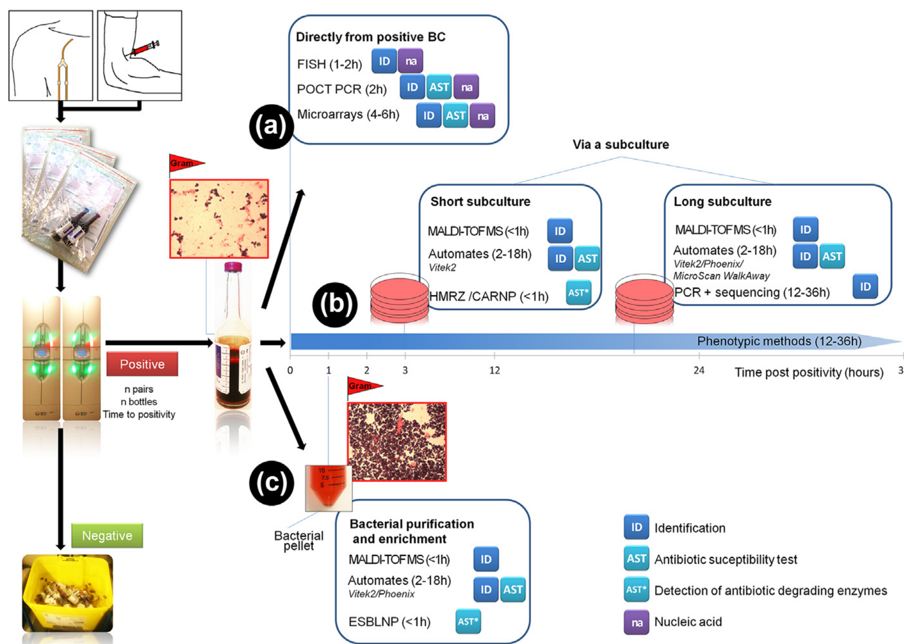

FIG. 2. Methods to identify microorganisms from positive blood cultures. When a blood culture is detected as positive, the first step is to perform a Gram staining with a sub-sample from the blood-culture bottle to confirm the presence of microbes and to determine the morphotype. The Gram staining allows disclosure of the presence of polymicrobial infections. Then the pathogen identification can be achieved: (a) Directly from positive blood culture using nucleic-acid-based methods, (b) via a subculture using phenotypic methods or via short subcultures that are suitable for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis and some antimicrobial susceptibility testing (AST) and (c) using a purified bacterial pellet also suitable for MALDI-TOF MS analysis and some AST.

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spectrum β-lactamase (ESBL) and carbapenemases. Further studies performed on a higher number of pathogen are necessary to confirm the reliability of brief culture for AST with the VITEK2 and other automated devices such as the MicroScan Walk Away (Siemens Healthcare, Sacramento, CA, USA) and the Phoenix (Becton Dickinson, Heidelberg, Germany). In addition, VITEK2 does not provide an exact MIC, in contrast to the MicroScan Walk Away and the Phoenix.

Nucleic-acid-based methods that can be used directly on positive blood cultures

Molecular methods are generally faster than phenotypic approaches because they are culture independent. However, the use of amplification-based nucleic acid methods such as PCR has to face several limitations associated with a blood sample: 1) presence of PCR inhibitors, 2) presence of a high quantity of non-microbial nucleic acids, 3) presence of contaminant DNA, and 4) persistence of DNA from dead microbes [35]. Thanks to recent advances in nucleic acid extraction and amplification, several PCR-based methods for the diagnosis of BSI directly from whole blood are now available. These methods are presented in a separate detailed review [35]. In the present review we will focus on nucleic-acid-based methods that are specifically dedicated for positive blood cultures such as probes hybridization and microarrays and some rapid PCR-based tests.

Probes hybridization and microarrays that are non-amplification-based nucleic acid methods are less influenced by inhibitors and less prone to contamination but they require a high bacterial or fungal load. Hence these methods are used on positive blood cultures in which the bacterial load can reach 1×10^6 to 2×10^8 for Gram-positive cocci and 2×10^7 to 1 × for Gram-negative bacilli (Fig. 2) [35–37].

Fluorescence *in situ* hybridization (FISH) that comprises the specific binding of fluorescent nucleic acid probes on complementary pathogen DNA sequences—16S rRNA for bacteria and 18S rRNA for fungi—can be performed directly from the positive blood cultures. The specific binding is observed using a fluorescence microscope. The choice of the probe is dependent on the Gram staining: staphylococci probes (*S. aureus*/coagulase-negative staphylococci), enterococci probes (*Enterococcus faecalis*/*Enterococcus faecium*), Gram-negative bacteria probes (*Escherichia coli*/*Pseudomonas aeruginosa*/*Klebsiella pneumoniae*), yeast probes (*Candida albicans*/*Candida glabrata*/*Candida parapsilosis*/*Candida krusei*) for instance. The commercial solutions PNA-FISH and Quick-FISH (AdvanDx, Woburn, MA, USA) display a time to result of about 1.5 to 3 h with a sensitivity and specificity of 97–100% and 90–100%, respectively (Table 2) [38–43]. The AccuProbe system (Gen-Probe, San Diego, CA, USA) is based on a DNA probe that can detect *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus* spp. and group A and B streptococci. The sensitivity and specificity are above 97%, with the exception of the *S. aureus* probes that demonstrate a sensitivity of 99.8% and a specificity of 80.8% [44,45].

Microarrays allow the detection of a limited number of species which cover 90–95% of all the pathogens causing BSI. The turnaround time is about 2.5–4 h with sensitivity ranging from 10 to 10^5 CFU/mL [46]. In contrast to FISH, microarrays generally contain probes for the detection of resistance genes such as *mecA*, *vanA*/*vanB* and bla_{KPC}. For example, the Verigene system (Nanosphere, Northbrook, IL, USA) comprises two distinct kits for the detection of 12 Gram-positive and nine Gram-negative bacterial species with a sensitivity ranging from 81 to 100% and a specificity higher than 98% [47,48]. The Prove-it Sepsis assay (Mobidiag, Esbo, Finland) combines a PCR with a microarray for a turnaround time of 3.5 h, and has 95% sensitivity and 99% specificity (Table 2) [49].

Rapid PCR-based tests that allow the detection and identification of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) have been developed to blood cultures because of the clinical impact and epidemiological concern of this pathogen [50,51]. MSSA/MRSA rapid PCR-based detection systems such as GeneXpert MRSA/SA BC Assay (Cepheid, Sunnyvale, CA, USA) [52,53] and the StaphSR assay (BD GeneOhm, San Diego, CA, USA) [54,55] are based on multiplex real-time PCR to detect *S. aureus* and the presence of the *mecA* gene. The results are obtained in approximately 1.5 h. The FilmArray (Idaho Technology, Salt Lake City, UT, USA) is a multiplex PCR-based system designed to detect 25 microbes (90–95% of the pathogens involved in blood cultures) and the resistance genes *mecA*, *vanA* and *vanB* and bla_{KPC} [56].

Nucleic-acid-based methods significantly reduce the turnaround time of the microbial diagnosis of BSI, which represents a benefit for patient care. However, for the large-scale implementation of these technologies in clinical laboratories, efforts have to be made to reduce the cost and the hands-on time of some of these technologies, especially since the introduction of MALDI-TOF MS.

Subculture independent approaches: identification and antimicrobial susceptibility testing using a blood-culture microbial pellet

The use of MALDI-TOF MS technology for the identification of microorganisms is among the major revolutions in clinical microbiology laboratories during recent years. Microbial

TABLE 2. Characteristics of the commercially available systems for the identification of microbes from positive blood culture

System (Manufacturer)	Methods	Time to result	Microorganism coverage	Resistance and virulence markers	Sensitivity Specificity Correlation with conventional methods (%)	Comments	References
PNA FISH and QuickFISH (AdvanDx, Woburn, MA, USA)	FISH	<1–3 hours	4 Gram positive 4 Gram negative 5 Fungi	0	97–100 90–100 96–99	+ : rapid, sensitive and specific – : dependent on the choice of the probes to be tested, no resistance marker	[38,41,42,75–80]
AccuProbe (Gen-Probe, San Diego, CA, USA)	FISH	<1 hour	*Staphylococcus aureus* *Enterococcus* spp. *Streptococcus pneumoniae* *Streptococcus* group A *Streptococcus* group B	0	80.8–100 98.7–100 nr	+ : high specificity – : variable sensitivity, no resistance marker, limited number of publications	[44,45]
Verigene (Nanosphere, Northbrook, IL, USA)	Microarray	2.5 hours	12 Gram positive 9 Gram negative	*mecA*, *van*A/B, KPC, NDM, CTX-M,VIM, IMP,OXA12	81–100 98–100 nr	+: detection of resistance markers, good specificity –: variable sensitivity, narrow range of pathogens detected	[47,81–89]
Prove-it Sepsis (Mobidiag, Esbo, Finland)	Microarray	3.5 hours	60 bacteria 13 fungi	*mecA*	95% 99% nr	+ : sensitive and specific – : limited number of publications	[49]
FilmArray (Idaho Technology, Salt Lake City, UT, USA)	Multiplex PCR	1 hour	8 Gram positive 11 Gram negative 5 Fungi	*mecA*, *van*A/B, KPC	97–95 91–98 nr	+ : rapid, sensitive and specific – : narrow range of pathogens detected	[56,90–92]
Xpert MRSA/SA BC (Cepheid, Sunnyvale, CA, USA)	Real-time PCR	1 hour	*S. aureus*	*mecA*	100 99–100 nr	+ : rapid, sensitive and specific – : expensive	[52,53,93–95]
StaphSR assay (BD GeneOhm, San Diego, CA, USA)	Multiplex PCR	1–2 hours	*S. aureus*	*mecA*	96–100 95–98 nr	+ : rapid, sensitive and specific – : expensive	[96,97]
StaphPlex (Genaco Biomedical Products, Huntsville, AL, USA)	Multiplex PCR + Microarray	5 hours	*S. aureus*	*mecA* (+ PVL)	100 95–100 92	– : limited number of publications	[98]
MALDI-TOF MS Brucker Daltonics (Bremen, Germany) bioMérieux (Marcy l'Etoile, France)	Mass-spectrometry	<1 hour	<1000[a]	not in routine	– – 76–99	+ : rapid, sensitive and specific – : significant hands on time for bacterial enrichment	[36,39,57,59,60,66,69]

Abbreviations: FISH, fluorescence *in situ* hybridization; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; nr, not reported.
[a]Dependent on the mass-spectrum database.

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identification using MALDI-TOF MS is based on the comparison of a protein profile obtained by mass spectrometry from a bacterial or fungal sample with a database of profiles obtained from characterized microbes [57,58]. MALDI-TOF MS is an accurate and fast approach whose performance largely depends on a microorganism's purity and quantity. Therefore, bacterial enrichment and purification procedures are required from positive blood cultures, which contain high concentrations of non-microbial material that may interfere with MALDI-TOF MS identification and AST. Several in-house and commercial methods have been developed to isolate and concentrate microorganisms from positive blood cultures including lysis centrifugation methods [59–61], saponin or equivalent mild detergent methods [62,63], serum separator method [64] and the commercial MALDI Sepsityper Kit (Bruker Daltonics Inc., Billerica, MA, USA) [65]. These pellet preparation protocols may not only be used for MALDI-TOF MS identification but also provide enough starting material for other downstream applications including Gram staining, AST [66] and rapid PCR-based tests [53].

MALDI-TOF MS on positive blood-culture pellet

The main MALDI-TOF MS systems commercially available, the Autoflex II mass spectrometer (Bruker Daltonik) and Axima Assurance system (Shimadzu Corporation, Kyoto, Japan) display similar performance [57,58,67,68]. The performances of MALDI-TOF MS identification vary according to the enrichment and purification method (Table 2). In the study performed by Prod'hom et al., 78.7% correct identification by MALDI-TOF MS was obtained from blood-culture pellets. Among samples giving no reliable identification by MALDI-TOF MS, 81% were blood-culture-positive for Gram-positive bacteria including mainly streptococci and coagulase-negative staphylococci [59]. Using a differential centrifugation protocol, March-Rossello et al. correctly identified 97.3% of Gram-negative bacteria and 98.4% of Gram-positive bacteria [69]. Poor MALDI-TOF MS identifications from blood-culture preparations are mainly observed with difficult-to-lyse bacteria (e.g. *Klebsiella pneumoniae*), closely related bacterial species (e.g *Streptococcus mitis* group) and bacteria such as anaerobes that are poorly represented in the MALDI-TOF MS database [57].

Antimicrobial susceptibility testing on positive blood-culture pellet

Automated microbial system cards and manual disc diffusion assays were directly used to perform AST from purified and/or enriched microbial samples obtained from positive blood cultures. Machen et al. performed a same-day AST by directly inoculating the AST automated microbial system (VITEK 2) with filtered microorganisms from positive blood cultures. A 93.5% category agreement with tested antibiotics was obtained with only 1.7% major errors (ME) and 1.3% very major errors (VME) according to definitions given by the US Food and Drug Administration for interpretive agreement results [70]. Similar results were observed when the VITEK 2 was inoculated with bacterial pellets obtained by ammonium chloride lysis centrifugation [66] or when the Phoenix system was inoculated with bacteria harvested using serum separator tubes [71]. However, some antibiotics known to present frequent discrepancies compared with conventional approaches need to be confirmed by disc diffusion assays and/or gradient diffusion methods directly performed from the same blood culture bacterial preparations [66].

The emergence of broad-spectrum antibiotic resistance mechanisms triggered the development of methods allowing rapid detection of ESBL and carbapenemase activities on blood cultures positive for Gram-negative bacteria. Using a Triton lysis–centrifugation method, ESBL activities can be directly and rapidly (less than 1 h) detected from spiked blood cultures using the ESBL Nordmann/Dortet/Poirel (ESBL NDP) test with 100% sensitivity and specificity [72]. Interestingly, the ESBL NDP test applied to blood-culture pellets showed higher performance compared with the same test performed on bacteria grown on agar plates, which exhibited a 100% specificity; the decreased sensitivity (92.6%) is due to the poor performance of the test (25% sensitivity) for the detection of non-CTX-M ESBL producers. The higher performance observed with this test applied on blood culture samples is probably explained by the very high bacterial inoculum recovered from positive blood cultures. Another approach to detect ESBLs from positive blood cultures is the chromogenic cephalosporin HMRZ-86 βLACTA test (Bio-Rad, Marnes-la-Coquette, France) exhibiting a 100% sensitivity and specificity following a 2-h subculture in tryptone soya broth to prevent inhibition of the test by lysed blood [73]. Hence in Lausanne, we applied it on purified bacterial pellets with accurate results (Prod'hom et al. submitted). The detection of carbapenemase activity from positive blood cultures can be performed with the Carba NDP with 97.9% sensitivity and 100% specificity test following a 3-h selective enrichment in brain–heart infusion containing imipenem [74]. A lower sensitivity (91.3%) was observed with OXA-48 producers whereas 100% of sensitivity was obtained with other classes of carbapenemases.

Rapid PCR-based tests on positive blood-culture pellet

Rapid PCR-based tests, such as the GeneXpert MRSA/SA BC Assay, can be applied on both native and microbial purified and enriched fractions of positive blood cultures (Fig. 2). The GeneXpert MRSA/SA test was applied to *S. aureus* blood culture bacterial pellets identified by MALDI-TOF MS and showed 99% sensitivity and 100% specificity, allowing a significant reduction of anti-MRSA antibiotics misuse from 26.1% to 8.1% [53].

Conclusions

New technologies and new methods for the diagnosis of positive blood cultures allow a significant reduction of the turn-around time for both identification and AST, with a positive impact on the management of patients suffering from BSIs.

Some of these approaches such as microbial enrichment via centrifugation require significant hands on time, which may hinder their implementation in laboratories with limited human resources. Hence, the emergence of new laboratory methodologies and new laboratory automated technologies, should help the implementation of these new diagnostic approaches. This should also be accompanied by significant efforts in increasing human resources.

The example of recent developments in the diagnosis of BSIs highlights the importance of a dynamic research and development process in diagnostic laboratories that promote innovation and implementation of the most recent technologies for the benefit of patients' care, while keeping an acceptable cost per test ratio.

Transparency declaration

No conflict of interest declared.

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