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Narrative review

Rapid phenotypic methods to improve the diagnosis of bacterial bloodstream infections: meeting the challenge to reduce the time to result

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ABSTRACT

Background: Administration of appropriate antimicrobial therapy is one of the key factors in surviving bloodstream infections. Blood culture is currently the reference standard for diagnosis, but conventional practices have long turnaround times while diagnosis needs to be faster to improve patient care. Phenotypic methods offer an advantage over genotypic methods in that they can identify a wide range of taxa, detect the resistance currently expressed, and resist genetic variability in resistance detection.
Aims: We aimed to discuss the wide array of phenotypic methods that have recently been developed to substantially reduce the time to result from identification to antibiotic susceptibility testing.
Sources: A literature review focusing on rapid phenotypic methods for improving the diagnosis of bloodstream infection was the source.
Content: Rapid phenotypic bacterial identification corresponds to Matrix-assisted laser-desorption/ionization time of flight mass spectrometry (MALDI-TOF), and rapid antimicrobial susceptibility testing methods comprised of numerous different approaches, are considered and critically assessed. Particular attention is also paid to emerging technologies knocking at the door of routine microbiology laboratories. Finally, workflow integration of these methods is considered.
Implications: The broad panel of phenotypic methods currently available enables healthcare institutions to draw up their own individual approach to improve bloodstream infection diagnosis but requires a thorough evaluation of their workflow integration. Clinical microbiology will probably move towards faster methods while maintaining a complex multi-method approach as there is no all-in-one method.
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Introduction

Bloodstream infections (BSIs), which rank among the top seven causes of death in North America and Europe [1], are of increasing public health concern. Blood culture (BC) remains the reference standard and the first-line tool for the diagnosis of BSIs. However, conventional practices have long turnaround times (TATs), and microbiological analysis needs to be faster because BSI and sepsis have better outcomes when effective antimicrobial therapy is administered early. Thus, each hour of delay over 6 h following septic shock in antibiotic administration decreases the survival rate of more than 7% [2,3]. Reducing the time taken for identification and antimicrobial susceptibility testing (AST) contributes to improving the care of patients with BSI or sepsis, especially in the context of increasing antimicrobial resistance [4–6]. Phenotypic methods offer a huge advantage over genotypic methods in that they can identify a wide range of taxa, detect the resistance currently expressed, and resist genetic variability in resistance-associated detection [7]. Here we review the performances and workflow integration of phenotypic methods designed to speed up culture-based diagnosis of BSIs (Fig. 1).

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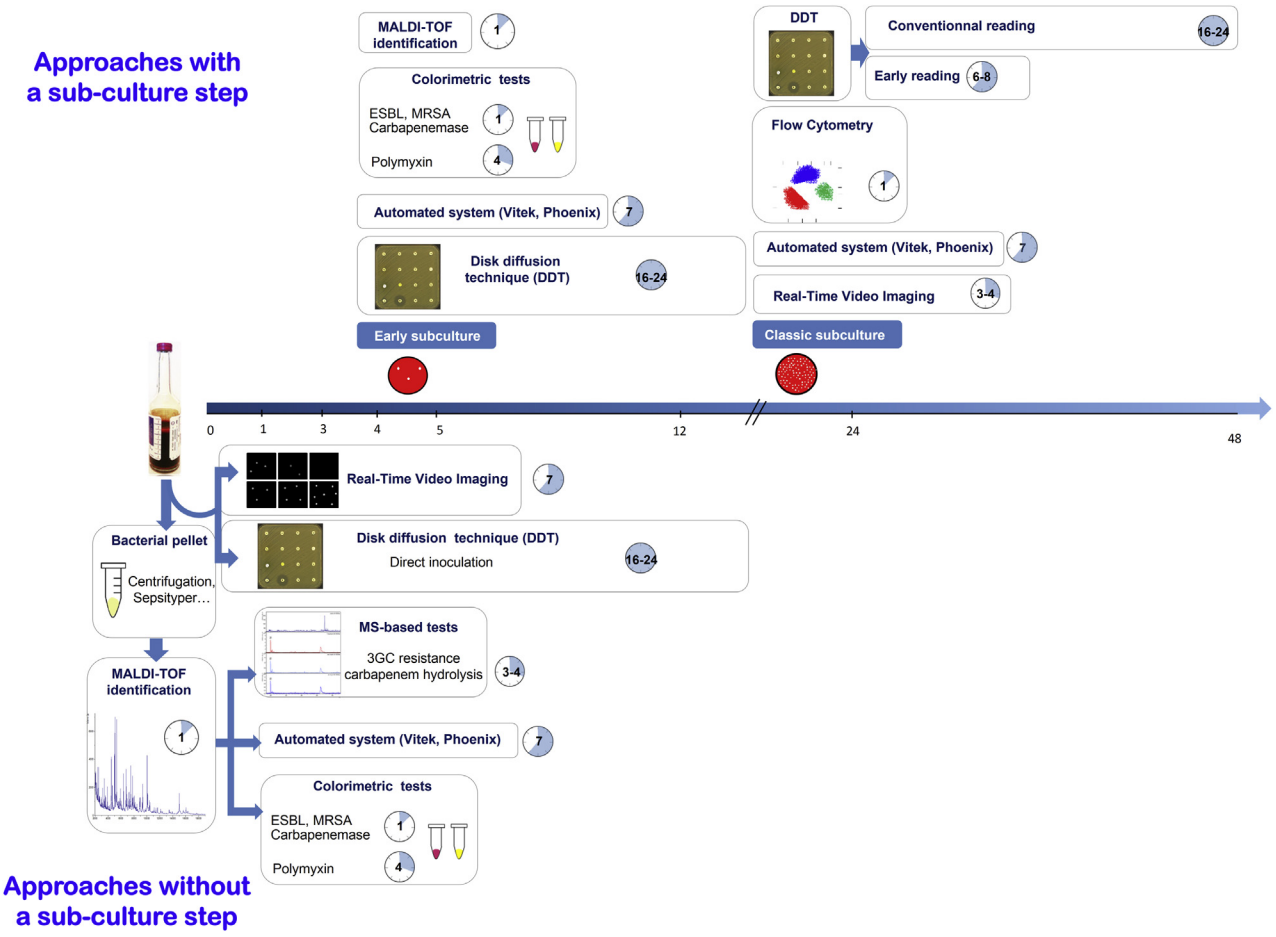

Fig. 1. Phenotypic methods implemented in microbiology laboratories to reduce time to result in reporting antimicrobial susceptibility testing (AST) from blood cultures. While accelerating conventional methods produces substantial improvements (top of the figure), obtaining a bacterial pellet from blood culture offers a dramatic reduction in the time to results with Matrix-assisted laser-desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) -based methods, colorimetric tests and direct AST (bottom of the figure).

Accelerating identification testing

Matrix-assisted laser-desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has revolutionized bacterial identification and is now widely used in clinical microbiology laboratories. Its success is due to the broad spectrum of organisms it is able to identify, the rapidity (a few minutes) in obtaining results, the method's accuracy and reproducibility, and its cost-effectiveness. Two main commercial systems are available on the market, the Vitek MS IVD (bioMérieux) and the Microflex LT Biotyper (Bruker Daltonics). These methods require at least 5×10^3 CFUs from colonies (hereafter the standard procedure) or from positive broth [8]. Because the bacterial count in positive BCs ranges from 1×10^6 CFU/mL to 2×10^8 CFU/mL for Gram-positive cocci, and from 2×10^7 CFU/mL to 1×10^9 CFU/mL for Gram-negative rods [9], MALDI-TOF MS allows for direct identification from blood vials or after short-term incubation on a solid medium provided that the positive BC is monomicrobial. Performances ranging from 58% to 86% for Gram-positive and from 84% to 98% for Gram-negative rods have been widely reported (for recent reviews see Refs [10–12]). However, these protocols require further hands-on processing time and additional costs when a commercialized kit is used.

Direct identification after purification of bacterial pellets from positive BCs

Positive BCs contain additional components, such as culture medium, blood cells and host proteins, which interfere with the bacterial proteins, so that identification by MALDI-TOF MS is ineffective [13]. However, several in-house protocols and commercial kits have been reported to remove these components and to obtain a successful identification.

In-house protocols require a volume of positive BC ranging from 1 mL to 6 mL. Some start with an additional differential centrifugation step or the use of a serum separator tube to separate bacteria from blood cells [14], especially if there is a high initial volume of positive broth. Most protocols start directly with a lysis step using a lysis buffer containing 5% saponin, ammonium chloride, trifluoroacetic acid, 10X Triton, or sterile water [15–18]. This step is followed by centrifugation and the bacterial pellet is washed with either water, ethanol or saline solution. A final centrifugation is performed to obtain a bacterial pellet, which is loaded onto a MALDI-TOF MS target plate either immediately or after applying a common extraction method for bacterial isolates, which increases the hands-on time by 15 min [19].

The commercial purification kits have the advantage of providing standardized protocols for laboratories that cannot easily implement published protocols. While the Sepsistyper kit protocol has a similar basis to the published ones [20–22], the Vitek MS BC kit uses a vacuum filter to purify bacteria from bloods cells [23].

The TATs of both in-house protocols and commercial kits are between 15 min and 2 h and require at least six steps, so they cannot be readily integrated into the workflow of positive BCs. Moreover, commercial tests are on average 20-times more expensive than any of the in-house protocols, which cost only a few euro cents. The additional hands-on processing time and commercial

costs limited the dissemination of these protocols, although in our experience the use of a simplified in-house protocol (15 min long) can be integrated into the routine and clearly contribute to shorter time to results.

With monomicrobial BSI episodes, the rate of identification of Gram-negative bacteria at the species level is much higher than that of Gram-positive bacteria (Table 1) [21,24–31]. In the former studies, the log (score) cut-off was that proposed by the manufacturer for identification on colonies (e.g. 2.0 for biotyper Maldi MS, Brucker Daltonics). Several authors then showed that the same performance can be obtained if the log (score) cut-off is lowered to 1.8, thereby improving the rate of correctly identified strains [26,32,33]. Finally, Schubert et al. adopted a log (score) cut-off of 1.5 and obtained acceptable performance [19] (Table 1).

Regarding polymicrobial BCs, the prevalent strain has been identified in 64% to 81.8% of specimens tested [15,20,34]. One study reported that it was possible to simultaneously identify bacteria in positive BCs by examining the top 10 matched patterns with a log-score value of >1.6 [15].

Identification after short-term incubation on a solid medium

Several authors have evaluated identification of bacteria from positive BCs by MALDI-TOF MS after short-term incubation on a solid medium for 2–6 h [35–40], which allows sufficient colonies to be obtained and subjected to MALDI-TOF MS analysis. This is feasible for rapidly growing bacteria, such as enterobacteria *or Staphylococcus aureus*. The efficiency of this method is clearly time-dependent: with Gram-negative bacteria, ≥80% of the strains were correctly identified at the species level after 2 h of preincubation, while ≥95% were correctly identified after 4 h of preincubation; with Gram-positive bacteria, preincubation of at least 4 h was required to correctly identify ≥80% of strains at the species level (Table 1). This approach is very simple, inexpensive and easy to implement in routine diagnosis, although it is slower than the purified pellet process. As with direct identification protocols, polymicrobial specimens were not successfully identified, except the predominant and rapidly growing ones.

Accelerating antibiotic susceptibility testing

Direct methods from positive BCs

Direct antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) directly from positive BCs has been widely performed in routine laboratories as it resulted in a significant time saving of approximately 24 h and the outcome of life-threatening BSIs is directly related to prompt implementation of effective therapy [41]. Many accounts of direct AST report low error rates, but these rates are frequently underestimated by the use of wrong denominators. As with any AST method assessment, the rates of very major errors (VMEs) and major errors (MEs) should be evaluated with the number of resistant and susceptible strains, rather than with the total number of tested strains, as the denominator, otherwise, the error rates will be biased by the prevalence of resistance in the strain collection being assessed [42,43]. Furthermore, error rates may vary according to the type of bacterium or antibiotic, so data should not be pooled without further analysis.

Direct AST from positive BCs has been assessed since the 1970s [44]. One of the main drawbacks of the method is the uncontrolled inoculum calibrated to 0.5 McFarland in the standard AST, which has led to substantial discrepancies [45–48] (Table 2). Nonetheless, it must be acknowledged that the method is widely used, probably because of the time saved. Inoculation in automated susceptibility testing systems yields acceptable agreement rates only when a time-consuming purification step is performed, either by filtration or with a separator tube [49,50]. Indeed, performances vary from poor to excellent depending on how efficiently the inoculum was controlled in the studies. Surprisingly, there are no readily available reports on this specific point for the disk diffusion method, although it is likely that a standardized inoculum preparation along with a culture-based check of inoculum quality yields reliable AST results. Very recently, Chandrasekaran et al. started to provide such evidence [51].

MALDI-TOF MS-based approaches to detect enzymatic degradation of antibiotics

MALDI-TOF MS can reveal the disappearance of a specific peak of a native antibiotic molecule as a result of enzymatic degradation

Table 1
Standard protocols for bacterial identification from positive blood cultures

Protocols	Gram-negative bacteria tested and correctly identified (%)	Gram-positive bacteria tested and correctly identified (%)	All bacteria tested and correctly identified (%)	Identification at the species level, cut-off used	Time for sample preparation	References
On bacterial pellet from positive bood culture with	491 (84.1)	722 (57.6)	1213 (68.3)	Log (score) ≥2.0	20 min to 35 min	[15; 20; 21; 25,29; 31]
SepsisTyper (Bruker Daltonics)*	233 (87.1)	535 (68.2)	768 (74)	Log (score) ≥1.8		[26; 32; 33]
	210 (91)	303 (67.3)	513 (77)	Log (score) ≥1.7		[13; 24; 28]
	98 (89.8)	358 (86.3)	456 (87)	Log (score) ≥1.5		[19]
On bacterial pellet from positive blood culture with BioMerieux filter system	185 (90.8)	270 (82.6)	455 (85.9)	Confidence level ≥99%	<15 min	[23; 46; 27]
	84 (97.6)	237 (60.8)	321 (70.4)	≥1.5 for Gram-positive cocci; ≥1.7 bacteria for other bacteria	4 h	[39]
	229 (78.6)	286 (42)	515 (58.2)	≥1.7	2.5 h	[35]
	229 (93.4)	286 (73.4)	515 (80.6)	≥1.7	5.5 h	
	83 (95.2)	173 (77.5)	256 (83.2)	≥1.7	3 h	[30]
	323 (90.4)	527 (82.2)	850 (85.3)	≥2	5 h	[40]
Short-term incubation on agar plate (MALDI-TOF MS, Brucker Daltonics)	86 (18.6)	42 (95.2)	128 (43.8)	≥2	≤4 h	[38]
	86 (64.0)	42 (97.6)	128 (75)	≥2	≤6 h	
	86 (96.5)	42 (97.6)	128 (96.9)	≥2	≤8 h	
Short-term incubation on agar plate (Vitek MS BioMerieux)	32 (75)	121 (61.2)	153 (64)	Confidence level ≥99%	3 h	[37]
	32 (87.5)	121 (84.3)	153 (85)	Confidence level ≥99%	5 h	
	47 (91.5)	87 (87.4)	134 (89)	Confidence level ≥99%	4 h	[36]
	47 (93.6)	87 (97.7)	134 (96.3)	Confidence level ≥99%	6 h	

MALDI-TOF MS, Matrix-assisted laser-desorption/ionization time of flight mass spectrometry.
* Blood culture broth that contained two or more different species, only one of the species was identified for 23.8% (five from 21) [15], 64.3% (18 from 22) [20], and 81.8% (18 from 22) [34].

Table 2
Very major errors (VME) with reported rates >1.5% when performing direct antibiotic susceptibility testing (AST) from blood cultures

Bacterial groups/species	Sample preparation	Method for direct AST	Antimicrobial agents concerned	References
Enterobacteriaceae	Bacterial pellet, adjusted to 0.7–1 Mc Farland	Vitek 2	Amoxicillin-clavulanate, Piperacillin-tazobactam	[47]
			Cefotaxime	[45]
	Bacterial suspension from positive BC broth (dilution 1/30)	Disk-diffusion method	Ciprofloxacin	[48]
Proteus spp.	Lysis-centrifugation, adjusted to 0.5–0.63 McFarland	Vitek 2	Ceftazidime, ceftriaxone, cefazolin, ampicillin	[46]
	Bacterial pellet, adjusted to 0.7–1 McFarland	Vitek 2	Ceftazidime	[47]
Pseudomonas aeruginosa	bacterial pellet, adjusted to 0.7–1 McFarland	Vitek 2	Piperacillin-tazobactam, ticarcillin	[47]
	Lysis-centrifugation, adjusted to 0.5–0.63 McFarland	Vitek 2	Ciprofloxacin	[46]
	Bacterial suspension from positive BC broth (dilution 1/30)	Disk-diffusion method	Ceftazidime	[48]
CoNS	Lysis-centrifugation, adjusted to 0.5–0.63 McFarland	Vitek 2	Cotrimoxazole, gentamicin, vancomycin	[46]
	Bacterial pellet, adjusted to 0.5–0.63 McFarland	Vitek 2	Cotrimoxazole	[45]
	Bacterial suspension from positive BC broth (dilution 1/30)	Disk-diffusion method	Cefoxitin-screen, rifampicin	[48]
Enterococci	Bacterial suspension from positive BC broth (dilution 1/30)	Disk-diffusion method	Gentamicin high-level	[48]

CoNS, coagulase-negative Staphylococci. None of the studies, except Stokkou et al.'s [48], specified the prevalence of resistance in the collection of strains studied.

following incubation with the bacterium in question. This approach was first successfully applied to colonies and was then extended to positive BCs. Given the required handling time, these tests aim to assess whether an empirical treatment is effective. Among the assays developed to detect third-generation cephalosporin resistance (3GCs) [52,53], one was highly sensitive (100%) and was able to detect both extended-spectrum beta-lactamase (ESBL)- and AmpC-producing isolates in <3 h, meaning that 3GC can reasonably be used when the test is negative [53]. Other assays have been developed with mixed results [54,55]. Accurate detection of carbapenemase-producing isolates—including non-fermenters—within 60 min has recently been reported [55]. While the 100% specificity of the test (Table 3) advises against the use of carbapenem when the test is positive, these assays cannot detect impermeability nor loss of porin. These tests are cost-effective, but the non-negligible handling time must be taken into account. In addition, the required settings of the spectrometer to determine the enzymatic degradation of antibiotics differ from those applied to identification purposes. This method requires changing the settings of MALDI-TOF, a house development method, and an interpretation of data not yet standardized. Moreover, this strategy allows testing only a limited number of molecules. Until additional development is provided to users, implementation in routine diagnostics of this method is deeply hampered by this major limitation.

Rapid tests

Colorimetric tests have recently been developed to detect enzymatic degradation—mainly of β-lactams—using a chromogenic substrate or a pH indicator for colour change in the medium. Initially designed to be performed on colonies, several of these tests have been evaluated directly on positive BC pellets with results returned within 1 h. Performances of assays of 3GC resistance in *Enterobacteriaceae* can vary [56,57], as some are unable to detect AmpC overexpressing isolates (Table 3). Similar tests exist for detecting carbapenemase-producing *Enterobacteriaceae*, or colistin-resistant *Enterobacteriaceae*. In a similar fashion, methicillin-resistant *S. aureus* can be evidenced in <30 min using an immunochromatographic assay detecting the penicillin-binding protein 2a. All these tests have been found to be reliable (high specificity) and to require minimal hands-on time, with the additional benefit of non-negligible costs [58–61]. For an extensive review, see Ref. [7].

Methods performed on early colonies

Accelerated classical methods

The early subculture approach was assessed for AST as it allows inoculum density to be controlled. The disk diffusion method performed on a 4-h to 6-h subculture with reading of inhibition zones after 16 h to 24 h of incubation saves a total of 24 h [62] with a VME rate of <0.4%. The same approach performed with Vitek dramatically reduces the time to result to <14 h with a similar performance to standard AST [63] (Table 3). Colorimetric tests and MALDI-TOF MS-based approaches for detecting antibiotic degradation can be carried out on young colonies, thereby bypassing the extraction step required when performed directly from BCs [38,60,61].

Reducing the AST reading time when the disk diffusion method is used is another strategy for shortening AST reporting. Using real-time video imaging, ESBL in *Enterobacteriaceae* could be identified in <5 h, resistance to imipenem in <3 h [64]. Early reading was therefore recently evaluated at 10 h [65] and 6 h [66] with a VME rate of <0.7%. The latter are mostly related to the penicillin+β-lactamase inhibitor association, probably because of the need to stabilize the two molecules. It should be noted that, for now, it seems early reading is not applicable to bacterial groups other than *Enterobacteriaceae* [66].

MALDI-TOF MS-based approaches for categorizing strains

Alongside enzymatic degradation assays, MALDI-TOF MS has been proposed for discriminating between strains susceptible or resistant to several antibiotic classes by analysis of the mass spectra generated. To date, this approach has only been tested on colonies using sophisticated algorithms. Several studies have been able to accurately discriminate between vancomycin-susceptible and -non-susceptible *S. aureus* isolates [67] or Van-B-positive *Enterococcus faecium* [68], while the data pertaining to methicillin resistance in *S. aureus* are conflicting [69,70]. In any case, data should be interpreted with extreme caution, as clonality between strains can greatly affect these classification methods.

Innovative technologies

Single-cell morphological analysis is currently being widely assessed in microbiology laboratories. This method involves diffusion of the antibiotic of interest in a microfluidic channel containing bacteria in agarose [71], while visualization of the division of the

Table 3
Major features of phenotypic methods implemented in microbiology laboratories to reduce time to result in reporting antimicrobial susceptibility testing (AST) from blood cultures

Approaches of antibiotic susceptibility test	Targeted resistance mechanism	Targeted population	Protocol	Delay from positive blood culture	Performances	Comments	References
Directly on positive blood culture							
Mass spectrometric approaches	3GC resistance	*Enterobacteriaceae*	Extraction with Sepsityper kit Cefotaxime as indicator	<3 h	Se: 100%; Sp: 92.7%	ESBL and AmpC can be detected	[52]
	Carbapenemase	Gram-negative bacteria	Extraction with separator tube Ertapenem as indicator	<5 h		Poor detection of *Acinetobacter baumannii* OXA-23	[53]
	Carbapenemase	Gram-negative bacteria	Extraction using Sepsityper Kit + optimization buffer Imipenem as indicator	1 h	Se 98%; Sp 100%		[54]
Colorimetric tests	ESBL	*Enterobacteriaceae*	Treatment with saponin	15 min	Se: 95.7%; Sp: 100%	β-Lacta test	[56]
	ESBL	*Enterobacteriaceae*	Tratment with a solution of Triton 10% (vol/vol)	20 min	Se: 100%; Sp: 100%	ESBL NP test	
	Carbapenemase	*Enterobacteriaceae*	No extraction protocol	<5 h	Se: 97.9%; Sp: 100%	CarbaNP	[55]
	Methicillin resistance	*Staphylococcus aureus*	No extraction protocol	<30 min	Se:100%; Sp: 100%		[57]
	Colistin resistance	*Enterobacteriaceae*	10-fold dilution in 0.9% NaCl	4 h	Preliminary data		[58]
After short-term cultures							
Early subculture + colorimetric test	Carbapenemase	Gram-negative bacteria		9 h	Se: 75%; Sp: 95% (Se: 100%; Sp: 98% for enterobacteria)	Low sensitivity for *A. baumannii*	[60]
	Carbapenemase	*Enterobacteriaceae*		<5 h	Se: 98.1%; Sp:100%	Blue Carba test modified	[59]
Early subculture + complete AST	Complete AST	Rapid growers	Disk diffusion method	20 h to 30 h	Overall AST CA 92.3% 0.6% ME 0.4% VME		[61]
	Complete AST	Rapid growers	Automated systems	11 h to 15 h	Overall AST CA 99.2%		[62]

ESBL, extended-spectrum beta-lactamase; 3GC, third-generation cephalosporin; ME, major error; Se, sensitivity; Sp, specificity; VME, very major error.

bacteria reveals resistance to the antibiotic. This approach was integrated into a fully automated system using a 32-flow cell cassette [72] in which the 'resistance score' was calculated for each test condition. The Accelerate Pheno™ system (Accelerate Diagnostics, Inc., USA) gave promising results with colonies [72,73]. The system, evaluated directly on BC-positive broth with an additional sample clean-up step by electrofiltration, yielded exhaustive AST results with a total analysis time of <7 h [74–76]. Given the number of resistant and susceptible strains assessed, only the major error rate could be confidently evaluated (at 0% to 8.8%), showing that this promising method is still in need of improvement. Furthermore, given the non-negligible cost, routine use of this technology needs economic evaluation.

Other technologies for shortening the time required for AST reporting have been extensively researched. The use of nano-mechanical sensors to measure variations in the atomic forces of bacteria when exposed to selected antimicrobial agents yielded promising results in a preliminary study [77]. Recently, this method was applied to artificial BC pellets inoculated with *Escherichia coli* and resulted in complete AST in <3 h [78]. Flow cytometry performed on positive BC produced AST results in 2 h with a 98% agreement with the reference microdilution [79], while the Real-Time Laser Scattering Method correctly detected methicillin resistance in *S. aureus* and vancomycin resistance in enterococci in <3 h [80]. Finally, a direct-on-target microdroplet growth assay coupled with MALDI-TOF MS accurately detected antimicrobial resistance in 5 h [81]. All these methods require further extensive evaluation as only a few molecules and micro-organisms have been tested.

Clinical impact and workflow integration

The rationale for implementing a rapid method is the impact it has on antimicrobial prescription and patient outcome. Although the evidence is not comprehensive, it has recently been shown that identification at the species level allows the antimicrobial therapy to be modified for 20% of the bacteraemia, while this figure is only 8% with microscopy [82]. In another study, MALDI-TOF MS and rapid AST coupled with an antimicrobial stewardship programme resulted in reductions in the mean times to identification from 32 h to 6.5 h, to AST from 48 h to 30 h, and to therapy adjustment from 75 h to 30 h, while hospital costs were also lower [83]. Coupling rapid methods with an antimicrobial stewardship programme increases the impact [84–86]. Efforts should be made to improve the level of evidence.

One of the main challenges that need to be addressed in order to implement rapid phenotypic methods for BCs is workflow integration. Most of these methods, which are difficult to fully

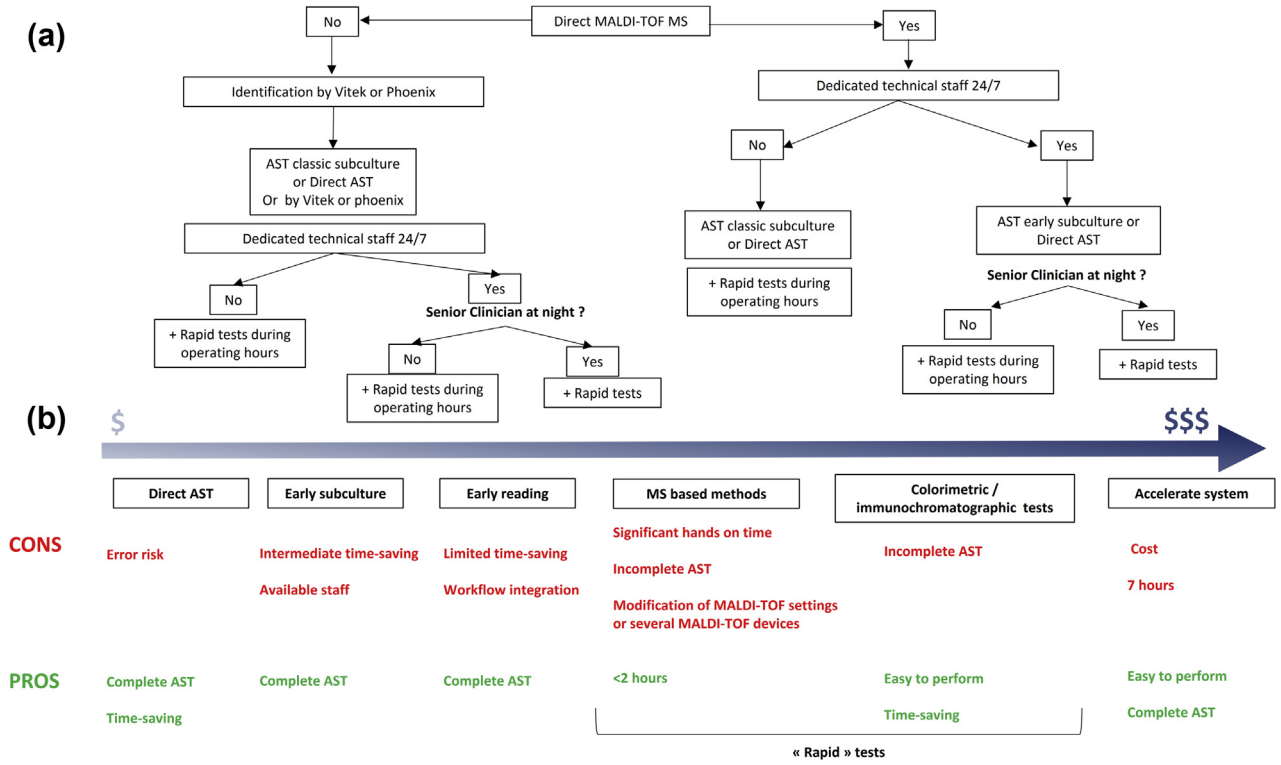

Fig. 2. Proposal of solutions for different scenarios to reduce time for identification and antimicrobial susceptibility testing (AST) from blood cultures (a). Direct Matrix-assisted laser-desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) enables a considerable time-saving for bacterial identification, especially when there are no staff who are available all day long. The implementation of rapid tests such as MALDI-TOF assays or colorimetric/immunochromatographic tests are useful when high-risk units are concerned to ensure efficacy of empirical therapy. The choice of the method to reduce AST mainly relies on the staff availability and the cost-effectiveness (b).

automatize, require considerable handling time due to the ongoing 'one-time occurrence' of positive BCs. The consequence of this is that the time actually saved in clinical practice is often less than expected. Integration in the laboratory workflow may be particularly challenging at night when fewer operators are available. One solution would be to reduce the TAT required to obtain the bacterial pellet. For instance, a modified protocol using a lysis buffer resulted in a TAT of 15 min [87]. However, the time saved is at the expense of performance, as only 77.8% of the microorganisms were identified, which appears to be lower than in other studies using more complex extraction protocols [88]. The batchwise use of MALDI-TOF MS at night is also a worthwhile option, as it is not constrained by staff availability and it slightly reduces the TAT (i.e. to 4.9 h) compared with continuous MALDI-TOF MS identification [89]. Similarly, identification and AST can be performed twice a day on early subcultures, except for those flagged as positive at night, allowing same-day reporting of results. Combining this workflow with rapid tests on young colonies could reduce the mean time to optimal antimicrobial treatment by at least 16 h [90].

To better integrate rapid methods into the workflow, modifications have to take into account TAT, easy implementation, cost-effectiveness and staff availability, but also clinical needs, antibiotic stewardship, presence of a senior clinician, and local antibiotic resistance epidemiology [91]. Among all the available methods and strategies, we summarize the drivers, pros and cons in Fig. 2. The broad panel of phenotypic methods currently available to improve BSI diagnosis enables healthcare institutions to draw up their own individual approach [92], and as there is no all-in-one method, clinical microbiology will likely move towards faster methods while maintaining a complex multi-method approach.

Accreditation of rapid methods according to international standard 15189

Rapid methods described here include either adapted methods (e.g. direct identification by MALDI-TOF, direct AST) or commercialized rapid methods (e.g. immunochromatographic assay). In both cases, accreditation should rely on (a) a critical analysis to identify the critical steps of the test method and define ways to control these steps, and (b) an evaluation of the test method's performance. The latter should be primarily assessed using literature and supplier data to establish sensitivity, specificity, ruggedness and stability [93] Importantly, in-house testing methods require method validation and not only method verification until there is availability of enough level of evidence in the literature to ensure enough confidence. To date, enough data is available for most protocols dedicated to the direct identification by MALDI-TOF while preliminary data concerning direct AST currently does not allow a full validation of the method.

Conclusion

MALDI-TOF MS, performed either on BCs or on young colonies, has substantially shortened the time for bacterial identification of BSIs. Despite the wide spectrum of tools that can be currently used to reduce time to detect bacterial resistance, the direct AST from BCs is very convenient as it allows a considerable time-saving, is easy to implement in the laboratory workflow and does not generate additional costs. In that respect, intense efforts are currently dedicated to including direct AST in expert guidelines recommendations (e.g. 2018 French recommendations) in agreement with international standard ISO 15189. Besides, rapid tests

including colorimetric/immunochromatographic/MALDI-TOF MS assays offer the opportunity to ensure efficacy of empirical therapy in a record time. Microbiologists should also consider the syndromic approach [94], but we believe that phenotypic methods currently represent the reference standard for identifying strains recovered from positive BCs and evaluating their antimicrobial resistance. Indeed, they combine the highest value for accuracy, range of identification and resistance detection, cost-effectiveness, timely diagnosis, and ease of integration for most laboratories. Strategies to reduce time to results should also promote reduced time of sample transportation, 24/7 laboratories for patients of poor condition at high risk of sepsis, and antimicrobial stewardship.

Transparency declaration

The authors declare no conflicts of interest. This work received no funding.

Authors' contributions

G.D., B.L. and R.R. designed the review; R.R. reviewed fast identification; G.D. reviewed fast AST; D.G., B.L. and R.R. reviewed method assessment and workflow integration; G.D., B.L. and R.R. drafted the paper; G.D., B.L. and RR critically revised the manuscript; all authors read and approved the final manuscript.

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