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Narrative review

Rapid microbiological tests for bloodstream infections due to multidrug resistant Gram-negative bacteria: therapeutic implications☆

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ABSTRACT

Background: Treating severe infections due to multidrug-resistant Gram-negative bacteria (MDR-GNB) is one of the most important challenges for clinicians worldwide, partly because resistance may remain unrecognized until identification of the causative agent and/or antimicrobial susceptibility testing (AST). Recently, some novel rapid test for identification and/or AST of MDR-GNB from positive blood cultures or the blood of patients with bloodstream infections (BSIs) have become available.
Objectives: The objective of this narrative review is to discuss the advantages and limitations of different rapid tests for identification and/or AST of MDR-GNB from positive blood cultures or the blood of patients with BSI, as well as the available evidence on their possible role to improve therapeutic decisions and antimicrobial stewardship.
Sources: Inductive PubMed search for publications relevant to the topic.
Content: The present review is structured in the following way: (a) rapid tests on positive blood cultures; (b) rapid tests directly on whole blood; (c) therapeutic implications.
Implications: Novel molecular and phenotypic rapid tests for identification and AST show the potential for favourably influencing patients' outcomes and results of antimicrobial stewardship interventions by reducing both the time to effective treatment and the misuse of antibiotics, although the interpretation about their impact on actual therapeutic decisions and patients' outcomes is still complex. Factors such as feasibility and personnel availability, as well as the detailed knowledge of the local microbiological epidemiology, need to be considered very carefully when implementing novel rapid tests in laboratory workflows and algorithms. Providing high-level, comparable evidence on the clinical impact of rapid identification and AST is becoming of paramount importance for MDR-GNB infections, since in the near future rapid identification of specific resistance mechanisms could be crucial for guiding rapid, effective, and targeted therapy against specific resistance mechanisms. **D.R. Giacobbe, Clin Microbiol Infect 2020;26:713**

Introduction

Multidrug-resistant Gram-negative bacteria (MDR-GNB), now endemic in several countries, may cause severe infections that are usually associated with high mortality [1,2]. Treating severe infections due to MDR-GNB is indeed one of the most important

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challenges for clinicians worldwide, partly because resistance may remain unrecognized until identification of the causative agent and/or antimicrobial susceptibility testing (AST) [3]. This implies a non-negligible risk of delaying the initiation of an active antibacterial therapy, with potential unfavourable consequences in terms of survival and other outcomes.

In this perspective, reducing the time to response (i.e. the time elapsing from the collection of microbiological specimens to the identification of the causative agent/s) and the time to AST by means of rapid diagnostic tests is expected to improve the outcome of patients with severe MDR-GNB infections, and to help avoid misuse of last-resort antibiotics in line with antimicrobial stewardship principles.

In this review, we discuss the characteristics of different rapid tests for identification and/or AST of MDR-GNB from bloodstream infection (BSIs), as well as their possible role for guiding therapeutic decisions.

Methods

Literature search methods and a brief description of conventional methods for identification and AST are available (please see supplementary material). The different rapid tests are described in the following paragraphs and summarized in Tables 1 and 2, which also include the available evidence on their impact on therapeutic decisions and relevant outcomes.

Rapid tests on positive blood cultures

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

The basis for microbial identification through matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology is that microorganisms have unique protein profiles, which can be registered by transferring bacterial cells into a ionization chamber, to be pulsed with a laser. Species-level identification is achieved by registering the different speed of protein migration and comparing the resulting mass spectrum with a reference database. MALDI-TOF has shown >95% agreement with conventional biochemical testing systems, reflecting good accuracy for identification, and has been implemented in many laboratories [4].

MALDI-TOF identification can be carried out from isolated colonies but also from bacterial growth obtained after short incubation (2–6 hr) of positive blood cultures on a solid medium, or even more rapidly after purification of bacterial pellets from the positive culture (requiring from 30 min to 2 hr), although the latter method usually implies additional laboratory workload [5,6].

Wenzler and colleagues [7] compared a pre-intervention phase (identification through conventional methods and no antimicrobial stewardship (AMS) interventions) with an intervention phase (identification through MALDI-TOF plus an AMS intervention based on rapidly informing clinicians of MALDI-TOF results) for the treatment of *Acinetobacter baumannii* infections (BSI and pneumonia) in a setting with >60% prevalence of MDR. They observed an important reduction in the median time to adequate therapy (from 77.7 to 36.6 hr, $p < 0.001$), arguing that MALDI-TOF plus AMS were able to improve rates of adequate early treatment of severe MDR-GNB infections by anticipating identification [7]. Other experiences testify to the effect of MALDI-TOF plus AMS interventions in reducing time to adequate therapy, as well as in decreasing hospital length of stay and costs [8].

There is also some promising evidence that MALDI-TOF could be of help for rapidly recognizing the presence of resistant organisms by detection of drug modification or spectra related to the presence of specific resistant mechanisms (e.g. extended-spectrum beta-lactamases (ESBL) or KPC enzymes in MDR-GNB), but further development and clinical studies evaluating these strategies are necessary to explore their impact on therapeutic choices and patients' outcomes [9–11].

Fluorescent in situ hybridization

AdvanDx PNA-FISH (fluorescent *in situ* hybridization) (OpGen, Gaithersburg, MD, USA) and AdvanDx Quick-FISH (OpGen, Gaithersburg, MD, USA) consist of the specific binding of fluorescent probes of nucleic acids to complementary sequences of the bacterial 16S rRNA, with binding observed through a fluorescent microscope. Specific probes are used according to Gram stain results. GNB probes are able to identify *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in 0.30–3 hr with >95% sensitivity and >90% specificity [12,13]. Identification of GNB was achieved up to >2 days more rapidly than with conventional methods in a series of 19 GNB BSI [14]. Possible reductions in antibiotic use, mortality and costs have also been reported, although in studies mostly or exclusively focused on Gram-positive bacteria [15,16]. Potential limitations are the need for skilled personnel for interpretation and the limited panel of detectable agents.

Light scattering technology

The ALFRED60 system (Alifax, Polverara, Italy) provides rapid automatic AST within 3–5 hr after positivity of blood cultures, by recording turbidity of growing bacteria in special liquid media [17]. For AST of GNB, an agreement of 91% with broth microdilution method results has been registered [18]. In a study of 115 BSI episodes (of which 51 and eight were caused by *Enterobacterales* and non-fermenting GNB, respectively), time to AST was 5 hr for the ALFRED60 system vs. 48 hr for standard microdilution method [19]. Further development (inclusion of more antibiotics active against MDR-GNB in the AST panel) and clinical studies are warranted.

FISH combined with time-lapse microscopy

The Accelerate Pheno system (Accelerate Diagnostics, Tucson, AZ, USA) is currently the only instrument able to combine identification through FISH probes with rapid phenotypic AST [20]. The GNB panel includes *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and various members of *Enterobacterales* [20]. Phenotypic AST is performed with time-lapse automated microscopy (morphokinetic cellular analysis), which evaluates growth curves at a single-colony level. Both identification and AST are fully automated, and time to identification and AST results after culture positivity might be as short as 1.5 and 7 hr, respectively [21]. Consequently, the Accelerate Pheno system might reduce time to identification and to AST by ~25 and ~40 hr, respectively, compared with conventional methods [20,21]. Considering 15 antibiotics tested against eight different GNB species and with broth microdilution or disc diffusion as reference, the overall essential agreement and categorical agreement (from 6331 AST results) were 95.4% (range 80.9–100%) and 94.3% (range 80.9–100%) respectively, with rates of very major error, major error and minor error of 0.5%, 0.9% and 4.8%, respectively [22]. Some authors have hypothesized that the Accelerate Pheno system might further anticipate time to effective therapy and time to definitive therapy even in settings where other rapid diagnostic technologies are used [23]. Nonetheless, AST might not be generated in up to 10% of cases after GNB identification, thus making the Accelerate Pheno system a valuable addition but not always a substitute for conventional methods [20].

TABLE 1
Rapid tests for identification and phenotypic/molecular antibiogram of Gram-negative bacteria (GNB) from positive blood cultures

Test	Technology Sensitivity (Se)[a] Specificity (Sp)[a]	Panel of GNB identification[b] and GNB phenotypic/molecular antibiogram	TAT from blood culture positivity[c]	Evidence from RCT and non-randomized, before–after studies on the actual[d] impact on therapeutic decisions, patients' outcomes and stewardship intervention, with focus on GNB or MDR-GNB BSI
MALDI-TOF MS	Laser desorption ionization plus mass spectrometry Se ≥75% Sp ≥95%	*Identification* • Most GNB *Molecular antibiogram* • Usually unavailable outside research laboratories, and needing further clinical evaluation	*Identification* • 0.5–2 hr if made from bacterial pellets • 2–6 hr if made after short incubation of positive cultures on a solid medium • Longer if made from isolated colonies	• In a recent RCT enrolling patients in 2 Vietnamese hospitals with invasive bacterial or fungal infection (mainly BSI, 421/628, 67%, of which 304/421,72%, caused by GNB, mostly *Enterobacterales*), MALDI-TOF MS did not improve proportion of optimal antimicrobial therapy within 24–48 hr after positivity of cultures vs. conventional methods, overall and in subgroups (including the GNB subgroup) [60]. Among *Enterobacterales*, prevalence of third-generation cephalosporin and carbapenem resistance were 48% and 5%, respectively. No subgroup analyses were performed according to antimicrobial resistance. The study was conducted in absence of AMS interventions. In another single-centre controlled trial (not randomized, allocation by weekdays) in Switzerland, no differences in duration of intravenous therapy (primary endpoint) were observed in the MALDI-TOF MS arm vs. conventional processing arm (13 vs. 14 days, p 0.7) in 242 patients with BSI [61]. Rates of admission to ICU (23 vs. 37%, 0.02) and the mean time from Gram-stain to active therapy (3.7 vs. 6.7 hr, p 0.003) were reduced in the MALDI-TOF MS arm vs. conventional processing arm. GNB were isolated in 37% of enrolled patients. The trial was conducted in a background of very rare prevalence of MDR in GNB (<5 episodes of MDR infection/year) • Before-after studies (focused or not focused only on GNB BSI) support a positive effect of MALDI-TOF plus AMS interventions in terms of increased clinical cure rates and reductions in time to optimal therapy, time to microbiological clearance, and length of stay in patients with BSI [8,16,62]
PNA-FISH Quick-FISH	FISH Se ≥95% Sp ≥90%	*Identification* • *Escherichia coli* • *Klebsiella pneumoniae* • *Pseudomonas aeruginosa* *Antibiogram*• Not available	*Identification* • 30 min to 3 hr (depending on the method)	• Clinical studies reporting reductions in antibiotic use, mortality, and costs are mostly focused/limited to Gram-positive organisms [15,63]
ALFRED60	Light scattering technology Se 97-98% Sp NA	*Identification* • Not available *Phenotypic antibiogram* • Some antibiotics used for MDR-GNB are currently not included in the AST panel	*Phenotypic antibiogram* • 3–5 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
Accelerate Pheno system	FISH/time-lapse microscopy Se 96% Sp 99–100%	*Identification (FISH)* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *Acinetobacter baumannii* • Other GNB [20] *Phenotypic antibiogram* • Some antibiotics used for MDR-GNB are currently not included in the AST panel	*Identification* • 1.5–3 hr *Phenotypic antibiogram* • 7–9 hr	• The RCT RAPIDS-GN (NCT03218397), conducted in patients with GNB BSI, and evaluating the clinical impact of the Accelerate Pheno system® plus AMS vs. standard blood culture work-up plus AMS in terms of time to first antibiotic modification (primary endpoint) and various secondary outcomes, including amongst others the development of novel infections due to MDR organisms, has been completed and results are awaited • Another RCT (NCT03745014) is expected to start in September 2019 that will compare the clinical impact of the Accelerate Pheno system® vs. standard blood culture work-up for patients with GNB BSI. The primary endpoint is a composite of patients' outcomes evaluated through the desirability of outcome ranking (DOOR) methodology • Duration of antipseudomonal therapy is one of the 2 major endpoints (the other one is duration of anti-MRSA therapy) of an ongoing RCT (NCT03744728) comparing the use of the Accelerate Pheno system® vs. standard processing plus Verigene BC-GN/GP®
Verigene BC-GN	NAAT/microarrays Se 97% Sp 100%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *Acinetobacter* spp. • Other GNB [24] *Molecular antibiogram* • CTX-M • KPC • NDM	*Identification* • <2 hr *Molecular antibiogram* • <2 hr	• In non-randomized, before-after studies, reductions in time to effective therapy, mortality, and length of stay in patients with GNB and/or MDR-GNB BSI were observed after the implementation of the Verigene BC-GN® assay [27,64,65]

(*continued on next page*)

TABLE 1 (*continued*)

Test	Technology Sensitivity (Se)[a] Specificity (Sp)[a]	Panel of GNB identification[b] and GNB phenotypic/molecular antibiogram	TAT from blood culture positivity[c]	Evidence from RCT and non-randomized, before–after studies on the actual[d] impact on therapeutic decisions, patients' outcomes and stewardship intervention, with focus on GNB or MDR-GNB BSI
		• OXA-23, -40, -48 and -58 groups • IMP • VIM		
FilmArray BCID	NAAT/microarrays Se >90% Sp 100%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [30] *Molecular antibiogram* • KPC	*Identification* • 1 hr *Molecular antibiogram* • 1 hr	• In an RCT, 617 patients with BSI (of which 33% due to GNB) were randomized in 3 arms: (a) standard processing; (b) FilmArray BCID®; (c) FilmArray BCID® plus AMS. The primary endpoint was duration of selected antimicrobial therapies, with duration of piperacillin-tazobactam therapy been lower in the FilmArray BCID® and FilmArray BCID® plus AMS arms than in the standard processing arm (44 hr and 45 hr vs. 56 hr, respectively, p 0.012) [66]. The study was conducted in a setting with low prevalence of MDR organisms. No KPC production was reported. Other RCT (NCT02743585, NCT03255759) exploring patient-level relevant outcomes are ongoing • In non-randomized, before–after studies, reductions in time to effective therapy and time to de-escalation were observed after the implementation of the FilmArray BCID® assay in samples including either only GNB BSI or both GPB and GNB BSI [33,67,68]
LFIA methods	LFIA Se >95% Sp >95%	*Identification* • Not available *Molecular antibiogram* • NDM • KPC • IMP • VIM • OXA-48-like and OXA-23	*Molecular antibiogram* • <30 min	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
Unyvero System	NAAT Se 100% Sp 99.75%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [34] *Molecular antibiogram* • KPC • NDM • IMP • VIM • OXA-48, -23, -24, -58 • CTX-M-14, CTX-M-15 • *aac* (6′)/*aph* (2″) • *aac*A4	*Identification* • 4–5 hr *Molecular antibiogram* • 4–5 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI

AMS, antimicrobial stewardship; BSI, bloodstream infections; fluorescent *in situ* hybridization; GNB Gram-negative bacteria; GPB, Gram-positive bacteria; ICU; intensive care unit; LFIA, lateral flow immunoassay; MDR, multidrug resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; NA, not available; NAAT, nucleic acid amplification tests; RCT, randomized controlled trial; TAT, turnaround time.

[a] Sensitivity and specificity are mostly referred to all the panel of identified organisms (including organisms other than GNB when detected) and are derived from pooled estimates in systematic reviews when available, and from observational, diagnostic studies when not available. For details see text.

[b] Information on identification provided for priority MDR organisms (*A. baumannii, P. aeruginosa, E. coli, K. pneumoniae*). For detailed information on identification of other GNB, please refer to references provided for each specific test.

[c] For more information and references on TAT see text.

[d] Only actual anticipation/change/optimization of therapy was considered as therapeutic decision. Studies reporting hypothetical anticipation/change/optimization of therapy/de-escalation extrapolated from turnaround times were not considered.

TABLE 2
Rapid tests for identification and molecular antibiogram of Gram-negative bacteria (GNB) directly from whole blood

Test	Technology Sensitivity (Se)[a] Specificity (Sp)[a]	Panel of GNB identification[b] and GNB molecular antibiogram	TAT from blood draw[c]	Evidence from RCT and non-randomized, before-after studies on the actual[d] impact on therapeutic decisions, patients' outcomes, and stewardship intervention, with focus on GNB or MDR-GNB BSI
LightCycler SeptiFast	NAAT Se 50-75% Sp 86-92%	*Identification* • *Escherichia coli* • *Klebsiella pneumoniae* • *Pseudomonas aeruginosa* • *Acinetobacter baumannii* • Other GNB [41]	*Identification* • 4–5 hr	• In a recent, single-centre RCT in 200 patients with sepsis comparing the use of the LightCycler SeptiFast® vs. conventional cultures, no difference was observed in antimicrobial consumption (primary endpoint). In the subgroup of patients with microbiological diagnosis in both arms (44, of which 68% were GNB) a reduction in antimicrobial consumption was observed in the SeptiFast® arm (1429 vs. 1889 DOT per 1000 patient-days). Statistically significant reductions were also observed in the time to de-escalation (8 vs. 54 hr) and in the duration of antimicrobial therapy (12 vs. 15 days), but not in mortality [69]. Antimicrobial costs were reduced for anti-GPB but not for anti-GNB agents. The trial was conducted in a setting of high prevalence of MDR (no further details provided) • In a multicentre, cluster-randomized, crossover trial, appropriate antimicrobial treatment in patients with severe infections (mostly severe sepsis) and microbiological diagnosis (n = 478) was similar in the intervention (SeptiFast®) and the control (conventional cultures) periods (92% vs. 91%) [70]. GNB were 14% of identified pathogens. Overall, SeptiFast® increased the number of septic patients with microbial diagnosis. No information on resistance prevalence in GNB was provided. • Patient-level outcomes and costs including those assigned to future resistance are among the endpoints of the Optimal Antibiotic Treatment of Moderate to Severe Bacterial Infections (CDSS) RCT, evaluating the use of SeptiFast® plus a computerized decision support system for antibiotic treatment (NCT01338116, last updated as recruiting patients in April 2016)
Magicplex Sepsis	NAAT Se 33-65% Sp 66-92%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [43]	*Identification* • 3–6 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
VYOO	NAAT Se 60% Sp 75%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [47] *Molecular antibiogram* • CTX-M • SHV	*Identification* • 7 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
SepsiTest	NAAT Se 21-85% Sp 53-100%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [51]	*Identification* • 8 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
IRIDICA BAC BSI (production discontinued)	NAAT/ESI-MS Se 81% Sp 84%	*Identification* • *E. coli* • *K. pneumoniae* • *P. aeruginosa* • *A. baumannii* • Other GNB [52] *Molecular antibiogram* • KPC	*Identification* • 6–8 hr *Molecular antibiogram* • 6–8 hr	• No RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI
T2Bacteria panel	NAAT/T2MR Se 83–90% Sp 90–98%	*Identification* • *A. baumannii* • *P. aeruginosa* • *E. coli* • *K. pneumoniae*	*Identification* • 5–6 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI

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TABLE 2 (*continued*)

Test	Technology Sensitivity (Se)[a] Specificity (Sp)[a]	Panel of GNB identification[b] and GNB molecular antibiogram	TAT from blood draw[c]	Evidence from RCT and non-randomized, before-after studies on the actual[d] impact on therapeutic decisions, patients' outcomes, and stewardship intervention, with focus on GNB or MDR-GNB BSI
T2Resistance panel	NAAT/T2MR Se NA Sp NA	*Molecular antibiogram* • KPC • OXA-48 • NDM • VIM • IMP • CTX-M-14 • CTX-M-15 • CMY • DHA	*Molecular antibiogram* • 3–5 hr	• Currently no RCT or quasi-experimental before-after studies evaluating the actual impact on therapeutic choices, patients' outcomes, and epidemiology of GNB and MDR-GNB BSI

BSI, bloodstream infections; fluorescent *in situ* hybridization; DOT, days of therapy; ESI-MS, electrospray ionization-mass spectrometry; GNB Gram-negative bacteria; MDR, multidrug resistant; MS, mass spectrometry; NA, not available; NAAT, nucleic acid amplification tests; RCT, randomized controlled trial; T2MR, T2 magnetic resonance; TAT, turnaround time.

[a] Sensitivity and specificity are mostly referred to all the panel of identified organisms (including organisms other than GNB when detected) and are derived from pooled estimates in systematic reviews when available, and from observational, diagnostic studies when not available. For details see text.

[b] Information on identification provided for priority MDR organisms (*A. baumannii, P. aeruginosa, E. coli, K. pneumoniae*). For detailed information on identification of other GNB, please refer to references provided for each specific test.

[c] For more information and references on TAT see text.

[d] Only actual anticipation/change/optimization of therapy was considered as therapeutic decision. Studies reporting hypothetical anticipation/change/optimization of therapy/de-escalation extrapolated from turnaround times were not considered.

Molecular detection systems based on nucleic acid amplification tests (NAATs) and microarrays

NAAT tests combined with microarrays consist of amplification of specific genetic targets followed by hybridization with a microarray of oligonucleotide probes for target identification. A qualitative result is obtained by amplification of the signal of hybridized probes. These systems allow for a high level of multiplexing, to cover the most important pathogens responsible of BSI and the most relevant resistance determinants with a 'syndromic panel' approach.

The Verigene BC-GN (Luminex, Northbrook, IL, USA) is able to identify four and five GNB at genus and species levels, respectively (including potential MDR-GNB such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp.) and several resistance determinants (CTX-M, KPC, NDM, OXA, IMP, VIM) with a turnaround time of <2 hr (after blood culture positivity) [24]. Sensitivity and specificity of 97.1% and 99.5% have been reported for the identification of GNB, although with possible suboptimal sensitivity for polymicrobial infections [25,26]. In a retrospective study, implementation of Verigene BC-GN reduced the time to identification of GNB from a mean of 37.9 hr when using the Vitek 2 system to a mean of 10.9 hr ($p < 0.001$) [27]. With all the limits of the retrospective design, 30-day mortality (19% (19/98) to 8% (8/97)) and mean length of stay in intensive care unit (ICU) were reduced from 16 to 12 days after implementation of the Verigene BC-GN assay ($p < 0.05$) [27]. In another retrospective study, identification of GNB with the Verigene BC-GN was obtained within 2.5 ± 1.3 hr since the Gram stain, anticipating adjustment from empirical to targeted therapy in 30% of cases within an AMS project [28]. Among 1046 GNB isolated from BSI, Verigene BC-GN showed negative predictive values (NPVs) >90% for resistance to third-generation cephalosporins in *E. coli* and *K. pneumoniae*, but lower values were registered for *P. aeruginosa*, probably because of its usually more complex array of resistance mechanisms to β-lactams in terms of porin mutations, efflux upregulation or β-lactamases that are not targeted by the probes included in the system [29]. Given these premises, the Verigene BC-GN might be of additional value to conventional methods (which remain essential for identifying species not included in the microarray panel, for testing phenotypic resistance conferred by resistance mechanisms other than the β-lactamases in the panel and for MIC measurement). Altogether, the decision whether implementing it in the laboratory workflow should consider the local microbiological epidemiology of MDR-GNB and the local type and prevalence of the different resistance determinants.

The FilmArray BCID (BioFire Diagnostics, Salt Lake City, UT, USA) consists of a fully automated nested multiplex PCR that identifies *P. aeruginosa*, *A. baumannii*, *K. pneumoniae, E. coli* and other members of the *Enterobacterales* (i.e. Enterobacteriaceae, *Proteus, Enterobacter cloacae* complex, *Klebsiella oxytoca, Serratia marcescens*) [30]. It also allows for rapid identification of three resistance markers, although of them only KPC-encoding genes relate to MDR-GNB infections. The turnaround time of the test from positive culture is ~1 hr, with reported sensitivity of >90% and 100% for identification and detection of resistance, respectively, whereas possible suboptimal specificity has been reported for identifying *P. aeruginosa* [30–32]. In a quasi-experimental single-centre study, the sequential implementation of MALDI-TOF (phase 1 post intervention) and the FilmArray BCID assay (phase 2 post intervention) allowed the appropriateness of empirical antimicrobial therapy to be increased from 91% in the pre-intervention period to 95% in the phase 2 post-intervention period (p 0.02), and to reduce the median time to de-escalation from combination therapy to monotherapy (from 2.8 to 1.5 days) and from broad-spectrum agents

such as antipseudomonal β-lactams (from 4.0 to 2.5 days) and carbapenems (from 4.0 to 2.5 days) to narrower spectrum agents [33]. The decrease in the median time to de-escalation from combination therapy to monotherapy and from antipseudomonal β-lactams to narrower spectrum agents was more marked during phase 2 post intervention (after implementation of FilmArray) than during phase 1 post intervention (when only MALDI-TOF was implemented) [33]. The FilmArray BCID thus demonstrated potential usefulness in the rapid diagnosis of MDR-GNB, although the fact that detection of carbapenemases genes is limited to KPC may be an important drawback where other resistance mechanisms are prevalent.

The Unyvero System (Curetis GmbH, Holzgerlingen, Germany) is a molecular diagnostic platform for the detection of bacteria and fungi, as well as antibiotic resistance genes. This system is able to detect, among others, non-fermenters (*A. baumannii*, *P. aeruginosa*, *S. maltophilia*), *Proteus* spp., nine other members of *Enterobacterales* at species level (e.g. *K. pneumoniae, E. coli*) and various antibiotic resistance gene markers (e.g. common carbapenemase genes such as bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and $bla_{OXA\text{-}23,24/40,48,58}$) directly from positive blood culture bottles in around 5 hr, with reported 100% sensitivity and 99.75% specificity for identification of GNB and a 1.9% of invalid samples [34].

Lateral flow immunoassay for rapid detection of resistance enzymes

Lateral flow immunoassay (LFIA) methods are antibody-based methods that rely on immunological capture of epitopes specific to resistance enzymes, using colloidal gold nanoparticles bound to a nitrocellulose membrane within a lateral flow device. Different panels for resistance enzyme identification exist with the possibility to detect the five most widespread carbapenemases found in *Enterobacterales* (NDM-, KPC-, IMP- and VIM-type and OXA-48-like), the OXA-23 carbapenemase, the ESBLs of CTX-M-type and the MCR-1 enzyme (colistin resistance). These tests yield results in less than 30 min and some have already been validated and CE marketed for detection of carbapenemase producers and carbapenemase identification from bacterial culture on solid media. They are relatively inexpensive, easy to perform, and showed a high level of sensitivity and specificity when used with bacterial colonies. Recently several papers have evaluated the possible use of LFIA for the rapid detection of carbapenemase-producing *Enterobacterales* directly from positive blood cultures reporting promising levels of sensitivity and specificity (from 97.7% to 100%) [35–37]. However, a recent paper showed that some LFIA tests could miss the detection of some carbapenemase variants (e.g. KPC-31, which confers resistance to ceftazidime-avibactam) [38].

Rapid tests directly on whole blood

Nucleic acid amplification test-based methods

When molecular techniques are applied directly on whole blood, the potential diagnostic advantages are intuitive (i.e. results for both identification and AST are available just a few hours after the blood draw), and several nucleic acid amplification tests (NAATs) have thus been developed over the years for this purpose. However, they have generally shown variable diagnostic performances and possible suboptimal sensitivity (e.g. for the presence of polymerase chain reaction inhibitors in whole blood) or suboptimal specificity [39,40].

The first developed system was the LightCycler SeptiFast (Roche Molecular Diagnostics), which allowed identification in about 6 hr of a panel of 25 microorganisms, including several GNB (*E. coli*, *Klebsiella* spp., *Serratia marcescens*, *Enterobacter* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*), but not of resistance genes apart from *mec*A. Heterogenous sensitivity and specificity have been reported, with systematic reviews summarizing a global performance of 50–75% sensitivity and 86–92% specificity [41,42].

The Magicplex Sepsis real-time assay (Seegene, Seoul, South Korea) can identify more than 90 microorganisms from blood, including also potential MDR-GNB (*A. baumannii*, *P. aeruginosa* and various members of the *Enterobacterales*), with final results being available in 3–6 hr. It also allows concomitant rapid identification of some resistance genes in Gram-positive (i.e. *mec*A, *van*A, *van*B) but not Gram-negative bacteria. Variable sensitivity (33–65%) and specificity (66–92%) have been reported [43–46].

The VYOO assay (Analytik Jena, Jena, Germany) is able to detect 34 bacteria (including *P. aeruginosa, A. baumannii, K. pneumoniae, E. coli* and 14 other GNB), six fungi and five resistance genes (including *mec*A, *van*A, *van*B, but also variants of the bla_{SHV} and $bla_{CTX\text{-}M}$ genes for extended spectrum β-lactamases), with final results available in ~7 hr. Sensitivity and specificity of 60% and 75%, have been reported, respectively, with some authors also suggesting the possible use of the VYOO test coupled with serum inflammatory markers for detecting infections in patients with systemic inflammatory response syndrome and negative blood cultures [47,48].

The SepsiTest (Molzym, Bremen, Germany) allows 345 different microorganisms to be identified, including several GNB, in ~8 hr, but not resistance genes. Similar to other systems, heterogenous sensitivity and specificity have been reported in different studies (21–85% and 53–100%, respectively) [44,49–51].

Amplification by PCR can also be paired with the electrospray ionization mass spectrometry (ESI-MS), in which a high electrical charge is applied to a fluid for creating aerosols of amplicons detected by mass spectrometry. The most developed system was the IRIDICA BAC BSI platform (Abbott Molecular, Des Plaines, IL, USA), which was able to identify more than 600 microorganisms and some resistance genes (including bla_{KPC}) in 6–8 hr, with variable diagnostic performance [52,53]. However, the development of this technology has been discontinued.

A recent systematic review and meta-analysis tried to summarize the clinical effectiveness and cost-effectiveness of LightCycler SeptiFast, SepsiTest and IRIDICA BAC BSI for the rapid identification of bacteria and fungi in the blood of patients with suspected sepsis [54]. The authors found (a) the LightCycler SeptiFast test to have an estimated summary sensitivity and specificity of 0.65 (95% credible intervals 0.60–0.71) and 0.86 (95% credible interval 0.84–0.89), respectively; (b) the SepsiTest to have an estimated summary sensitivity of 0.48 (95% credible intervals 0.21–0.74) and specificity of 0.86 (95% credible intervals 0.78–0.92); (c) the IRIDICA BAC BSI to have an estimated summary sensitivity of 0.81 (95% credible intervals 0.69–0.90) and specificity of 0.84 (95% credible intervals 0.71–0.92) [54]. Overall, their conclusion was that robust data are currently unavailable to accurately assess the clinical effectiveness and cost-effectiveness of these tests, and further evidence is needed.

The T2 magnetic resonance (T2MR) nanodiagnostic system (T2 Biosystems, Lexington, MA, USA) can identify *Candida* (T2Candida panel) and bacteria (T2Bacteria panel) directly on whole blood through a fully automated method. The identification process consists of the mechanical lysis of cells, amplification of DNA by PCR and target-specific primers, and detection of amplified products by measuring agglomeration of supermagnetic particles induced by the amplicons [55]. While more data are currently available regarding the T2Candida panel, clinical experience with the T2Bacteria panel is still preliminary, but nonetheless appears promising, although the restricted number of identifiable GNB and

TABLE 3
Information to be included/considered in future studies and factors to be considered when implementing rapid tests within local diagnostic protocols for MDR-GNB

Information to be included/considered in future clinical studies evaluating the impact of rapid tests on therapeutic decisions, patients' outcomes, and stewardship intervention to allow generalization and extrapolation to local realities	Important factors to be considered when implementing rapid tests within local diagnostic protocols
• Provide baseline prevalence of MDR-GNB, stratified for different organisms, antimicrobial agents, and resistance determinants • Describe availability of laboratory personnel during the study period (e.g. results obtained employing a 24/7 laboratory service may not be extrapolated to settings with different laboratory schedules) • Consider reporting of time from identification/AST in the lab to actual therapy adjustments • Include detailed sample size calculations for the different endpoints, including development of resistance • Provide clear definitions of the study population and subgroups • Consider to possibly assess clinical outcomes following rapid test-driven therapeutic choices as a measure to explore diagnostic performances in an adequate sample of patients without conventional microbiological diagnosis • Consider direct comparison between rapid tests and of combinations of rapid tests	• Molecular rapid tests generally identify a limited spectrum of microorganisms and of resistance mechanisms • Results of molecular AST are a useful proxy but not a definite proof of resistance • Molecular AST provide qualitative but not quantitative results • Rapid identification of specific resistance mechanisms will likely be more essential in the future, because of the specific activity of some novel agents against different types of resistance mechanisms • Economic costs and personnel availability need to be necessarily taken into account when implementing one or more novel rapid tests into the laboratory workflow • Consider prioritization of specific patients' categories and wards of patients at risk to maximize cost-effectiveness • Consider feasibility of implementation of a 24/7 laboratory service

AST, antimicrobial susceptibility testing; GNB, Gram-negative bacteria; MDR, multidrug resistant.

the absence in the panel of resistance genes are among its limitations. In a recent single-centre observational study, 140 samples from 129 patients with BSI were studied [56]. Sensitivity and specificity for the detection of ESKAPEc bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *E. coli*) were 83.3% and 97.6%, respectively. The mean times to identification and negative results were 5.5 ± 1.4 hr and 6.1 ± 1.5 hr, respectively [56]. In a cohort of 1427 patients, the mean time to species identification was 3.6 ± 0.2 hr and 7.7 ± 1.4 hr, depending of the number of tested samples [57]. The per patient sensitivity and specificity (for proven BSI) were both 90%, with 99.7% NPV [57]. A T2Resistance™ panel is under development that can identify various carbapenemases (KPC, OXA-48, NDM, VIM, IMP) and other resistance genes.

Therapeutic implications

Evidence regarding the impact of rapid tests on therapeutic decisions (i.e. on actual rapid test-driven therapeutic choices and not only on turnaround time) and on relevant outcomes in patients with MDR-GNB infections is still fragmentary and difficult to interpret. Indeed, besides the absence of comparable levels of evidence across tests (with only a few randomized controlled trials (RCTs) available and rarely involving direct comparisons) there are relevant questions that we need to ask ourselves when interpreting the results of RCT and observational studies and extrapolating them to local diagnosis/treatment protocols. Two major questions are: (a) Is the epidemiology of MDR-GNB in the study population similar to that of my hospital/wards? (b) Is the epidemiology of the specific resistance determinants detected by the investigated rapid test/s similar to that of my hospital/wards? Unfortunately, as already reported in the Tables 1 and 2, only a few high-quality studies comprehensively address the baseline epidemiology of MDR, a fact fuelling uncertainty about the best test/s to adopt in different local realities. Furthermore, the absence of other relevant baseline data (e.g. staff availability during night and weekend days) may further complicate the extrapolation of study results to local settings (Table 3).

Together with some important initiatives taken to improve the design and the comparability of diagnostic studies [58], providing comparable high-level evidence on the clinical impact of rapid tests is therefore of paramount importance to firmly inform future decisions about which rapid test/s to include in laboratory workflows. However, it is also true that MDR-GNB are already endemic in several areas, thus implementation of rapid tests for guiding therapeutic decisions based solely on available diagnostic studies and the tests characteristics (e.g. panel of identified organisms and turnaround time) may be already considered reasonable pending further clinical evidence to maximize cost-effectiveness. For example, the combined use of MALDI-TOF plus a rapid molecular or LFIA AST to identify the locally prevalent carbapenemase genes could be useful in settings where carbapenemases are the major driver of carbapenem resistance, while rapid phenotypic AST may be a more expensive but more reasonable choice where they are not. In this regard, some important factors (also summarized in Table 3) should always be carefully balanced in the decisions about the implementation of rapid tests in local laboratory workflows: (a) when molecular rapid tests are used, they generally identify a limited spectrum of microorganisms and of resistance mechanisms, thus they need to be paired with old (or novel) phenotypic tests for identification and AST (e.g. molecular tests are inherently unable to detect still unknown resistance mechanisms); (b) results of molecular AST are a useful proxy but not a definite proof of resistance (e.g. identification of inactivated or unexpressed genes in presence of phenotypic susceptibility); (c) molecular AST provide qualitative but not quantitative results (e.g. MIC) that nonetheless might be useful when deciding whether to increase dosages of a specific drug or to use alternative agents; (d) rapid phenotypic tests might show some advantages over molecular methods, but it is also true that rapid identification of specific resistance mechanisms are likely to be more essential in the future, because of the specific activity of some novel agents against different types of resistance mechanisms. Costs and personnel availability are other important factor to be necessarily taken into account when implementing one or more novel rapid tests into the laboratory workflow [16]. Finally, it may be critical to prioritize specific patient categories and wards according to the local microbiological epidemiology and the peculiar antimicrobial stewardship needs of any given hospital [59].

Conclusions

Novel molecular and phenotypic rapid tests for identification and AST show the potential for favourably influencing patients' outcomes and antimicrobial stewardship interventions, by reducing both the time to effective treatment and the misuse of novel antibiotics, although high-quality evidence to define their actual impact on therapeutic decisions and outcomes of MDR-GNB BSI is still fragmentary. High-level evidence on patients' outcomes

will also be essential for other emerging methodologies applied to the early diagnosis of BSI, such as next generation sequencing and other innovative approaches.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2019.09.023.

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