Advances in rapid identification and susceptibility testing of bacteria in the clinical microbiology laboratory: implications for patient care and antimicrobial stewardship programs

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Abstract

Early availability of information on bacterial pathogens and their antimicrobial susceptibility is of key importance for the management of infectious diseases patients. Currently, using traditional approaches, it usually takes at least 48 hours for identification and susceptibility testing of bacterial pathogens. Therefore, the slowness of diagnostic procedures drives prolongation of empiric, potentially inappropriate, antibacterial therapies. Over the last couple of years, the improvement of available techniques (*e.g.* for susceptibility testing, DNA amplification assays), and introduction of novel technologies (*e.g.* MALDI-TOF) has fundamentally changed approaches towards pathogen identification and characterization. Importantly, these techniques offer increased diagnostic resolution while at the same time shorten the time-to-result, and are thus of obvious importance for antimicrobial stewardship. In this review, we will discuss recent advances in medical microbiology with special emphasis on the impact of novel techniques on antimicrobial stewardship programs.

Introduction

The pace of diagnostic processes in clinical microbiology laboratories has largely been unchanged for almost 100 years, as availability of diagnostic results essentially depended on the growth of bacteria. Using traditional approaches, it takes at least 24 hours for obtaining growth from clinical specimens, and an additional 24 hours for down-stream isolate characterization (*i.e.* biochemical identification and phenotypic susceptibility testing). As a consequence, therapeutic decisions are commonly made empirically until the availability of species identification and resistance patterns. The emergence of pathogens carrying acquired resistance determinants, *e.g.* methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase- (ESBL-) producing *Enterobacteriaceae*, or carbapenem-resistant Gram-negative rods, has resulted in increasingly broad empiric treatment regimens, often including glycopeptides and broad-spectrum beta-lactams such as piperacillin-tazobactam or carbapenems. The resulting overuse of these reserved agents itself drives the emergence and spread of multi-resistant organisms. The situation is aggravated by the often unsuccessful recovery of pathogens from patients receiving prior broad-spectrum antibiotics and, in consequence, unavailability of subsequent drug susceptibility data. Moreover, it is a common problem that (successful) empiric broad-spectrum therapy remains in place although microbiological test results justify de-escalation.[1] Therefore, it is evident that overtreatment is, at least partially, linked to the discrepancy between traditional microbiological procedures and the clinical need for more rapid results. Over the past couple of years, several new technologies have entered clinical microbiology laboratories. Accelerated phenotypic methods, molecular techniques, MALDI-ToF and next generation sequencing (NGS) all hold promise or have already proven to not only optimize workflows within the lab, but also to offer increased diagnostic resolution and decreased time-to-result. In this article, we will discuss recent advances in medical microbiology with special emphasis on the impact of novel techniques on antimicrobial stewardship programs.

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Rapid phenotypic susceptibility testing

Antimicrobial susceptibility testing (AST) of bacterial pathogens is one of the principal tasks of the clinical microbiology laboratory and phenotypic AST is still considered the gold standard for the determination of antimicrobial susceptibility. Phenotypic AST offers two advantages as compared to genotypic testing methods: i) it predicts not only drug resistance but also drug susceptibility; ii) it permits to quantify the level of susceptibility of a bacterial isolate to individual antimicrobials (quantitative AST). Quantitative AST is of major importance as a clear correlation between the presence of a genetic resistance marker and the resulting drug susceptibility phenotype is not always possible, *e.g.* due to variable expression levels or sequence variations causing unknown substrate specificities.

Delays in phenotypic AST lead to prolonged hospitalization, increased cost and patient mortality.[2-7] Therefore, efforts to reduce the time-to-result for phenotypic AST are crucial to facilitate timely administration of appropriate antimicrobials and to improve patient outcome and cost-effectiveness of anti-infective therapies. In principle, two strategies exist to meet this challenge: i) acceleration of classical phenotypic AST techniques and ii) introduction of novel, more rapid methods for phenotypic AST. Using these strategies, significant progress towards accelerated reporting of antimicrobial susceptibility data was made in recent years. However, some major issues need to be addressed before rapid phenotypic AST can be provided on a large scale.

Acceleration of classical techniques

Classical AST techniques such as broth microdilution, disk diffusion, gradient tests, agar dilution and breakpoint tests (testing bacterial growth at breakpoint concentrations only) are based on continuous exposure of a bacterial isolate to a set of antimicrobials followed by visual detection of growth (Table 1). Classical AST techniques involve a defined inoculum of a bacterial pure culture and are standardized to 16 to 20

hours of incubation with some species requiring longer incubation periods of 72 hours and more. Accordingly, breakpoints by AST committees such as EUCAST and CLSI were calibrated to these incubation periods. For positive blood cultures, this leads to a time-to-result of at least 2 working days, as both primary growth from a positive blood culture bottle and subsequent AST from the isolated bacteria require overnight incubation. In order to shorten this period, three possibilities exist: i) replacement of standardized inocula prepared from pure bacterial cultures by directly using sample material such as positive blood cultures or urine as a starting point for AST (direct AST); ii) acceleration of bacterial growth; iii) more sensitive detection.

With respect to bacterial growth, both intrinsic properties of the pathogen and experimental conditions influence the behavior of the AST system. The lag phase, in which cells are adjusting to a new environment through synthesis of RNA, enzymes and other molecules, presents a lower boundary for accelerated AST using classical techniques, as cell division is greatly down-regulated.[8] Furthermore, basic test parameters of classical AST systems (temperature, nutrient supply) are already set in order not to limit bacterial growth during the subsequent log phase. Therefore, novel strategies for rapid classical AST focus on more sensitive growth detection. Semi automated devices (*e.g.* Biomerieux Vitek, BD Phoenix, Beckman Coulter MicroScan), which use optical systems to measure subtle changes in bacterial growth, can produce susceptibility test results in a shorter period than manual readings (6 to 12 hours). Recently, Kahlmeter *et al.* reported results of disk diffusion AST read after 6, 8 and 12 hours of incubation (ESCMID eLibrary 2016 Tentative breakpoints for early reading of disk diffusion tests for *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*). The data show that separation between wild type and non-wild type populations was poorer with short compared to standard incubation. Notably, application of the standard EUCAST meropenem screening diameter for detection of carbapenemase production would have resulted in a significantly larger number of false-positive screening results after 6 hours as compared to the standard time point (18 hours). Similarly, MRSA screening using the EUCAST recommended cefoxitin 30 ug disk with a threshold zone diameter of 22 mm would have led to significantly more methicillin-susceptibly strains being classified as methicillin-resistant after 8 hours as compared to the regular

Table 1. Advantages and disadvantages of different approaches to accelerated antimicrobial susceptibility testing using blood cultures as an example.

Method	Time-to-result[a]	Advantages	Disadvantages
Conventional AST (disk diffusion, manual broth microdilution, gradient tests)	38-48 hours	Reference method; Established breakpoints; MIC distributions and epidemiological cut-off values available[b]; High-throughput testing possible; Widely used methods, large amount of validation data published; Full flexibility regarding choice of tested antimicrobials; Detection of inducible resistance, *e.g. D test* for clindamycin or *Keyhole effect* for ESBL in disk diffusion testing; Relatively cheap	Relatively slow; Expert knowledge on data interpretation required at all times; Laboratory is fully responsibility for quality control
Automated AST	22-48 hours	Widely used method, large amount of validation data published; Pre-defined set of rules to support data interpretation (expert system); Detection of inducible resistance; Manufacturers responsible for product quality	Higher cost; No direct visual feedback, e.g. on incremental development of drug resistance in follow-up samples
Direct AST	14-24 hours	High-throughput testing possible; Free choice of compounds (unless an automated system is used); No additional equipment required	No systematically evaluated breakpoints; No standardized inoculum; Not controlled for polymicrobial infections; Not controlled for substances in the specimens that affect results
Rapid conventional AST	18-32 hours	High-throughput testing possible; Free choice of compounds (unless an automated system is used)	No valid breakpoints; Inducible resistance may be missed; Additional equipment may be required (e.g. high-resolution cameras)
Novel approaches to phenotypic AST	3-8 hours	Very short time-to-result (same working day); Some systems offer simultaneous species identification	Few commercial products available, additional equipment may be required; High throughput testing may be limited; Flexibility regarding choice of tested compounds may be limited; Breakpoint validation required
Genotypic AST	1-5 hours[c]	Very short time-to-result (same working day); Full flexibility and relatively low cost for in-house systems	Unambiguous genotype-phenotype relation required; No detection of novel resistance mechanisms; Little flexibility and relatively high cost for commercial systems; Laboratory is fully responsible for validation and quality control of in-house systems

[a] Time-to-result was defined as the time required from blood culture positivity until the generation of an AST report. For estimates of the time-to-result, standard weekday working hours of 8 a.m. until 6 p.m. were taken as a basis. [b] Breakpoint distributions for both minimal inhibitory concentrations and disc diffusion zone diameters available through the EUCAST website (http://www.eucast.org/mic_distributions_and_ecoffs/). [c] Depending on the assay format.

reading after 18 hours of incubation. In addition, inducible expression of chromosomal or plasmid-borne resistance genes such as *amp*C (resistance to betalactams) in many *Enterobacteriaceae* or *erm* (resistance to macrolides, streptogramins and lincosamids) *e.g.* in streptococci, presents another major challenge for rapid phenotypic AST.[9-11] Little is known regarding the resistance phenotype of strains carrying such genes as a function of incubation time in classical AST systems and more research is needed to clarify these important issues. In conclusion, new breakpoints will have to be established and minimal incubation periods for reliable detection of inducible resistance markers will have to be evaluated before rapid AST data based on conventional AST systems can be delivered.

Novel approaches to rapid phenotypic antimicrobial susceptibility testing

Novel approaches to rapid phenotypic AST typically require shorter exposure to antimicrobials and are either designed for early reporting of a full surrogate of the conventional antibiogram or focused on early detection of resistance to particularly critical compounds. For example, Entenza *et al.* demonstrated that reduced susceptibility to vancomycin in *S. aureus* can be detected in under 8 hours by microcalorimetry, *i.e.* by measuring reduced bacterial heat production in the presence of vancomycin.[12] Similarly, novel techniques for phenotypic carbapenemase detection in *Enterobacteriaceae* and nonfermenters have been reported. These tests, such as the Rapid Carb Blue Kit (Rosco Diagnostica, Taastrup, Denmark) and the Rapidec Carba NP test (bioMérieux, Marcy L'Etoile, France), can be performed directly from colonies grown on selective or non-selective agar plates.[13,14] Imipenem hydrolysis due to carbapenemases is detected using a colorimetric pH indicator in as little as 30 minutes. Of note, known limitations of the Carba NP test such as relatively low sensitivity for detection of Oxa-48 carbapenemases and false negative results with *Providencia rettgeri, Providencia stuartii* or *Proteus mirabilis* can be overcome by running the test for 120 minutes and doubling the inoculum recommended by the manufacturer.[15,16] With a sensitivity of >90% and a specificity of 100% these tests can be considered useful tools for rapid confirmation of carbapenemase-producing *Enterobacteriaceae* not least because the continual discovery of novel, genetically distinct carbapenemases presents a technical challenge for PCR-based detection.

Commercially available systems to shorten the time-to-result for the entire antibiogram include the Accelerate ID/AST platform (Accelerate Diagnostics, Tucson, USA) which, after a gel electro-filtration step, uses fluorescence in situ hybridization for species identification (1 hour) and automated time-lapse microscopy on individual bacterial cells for AST (5 hours) directly from positive blood cultures. MICs are determined by matching growth patterns to reference profiles, for which correlations to conventional MICs have been established. An agreement of >92% as compared to the reference method (broth microdilution) could be demonstrated for common compound/species combinations.[17] Other novel developments such as two-photon excitation assays (ArcDia, Turku, Finland), ultra-high-resolution bacterial mass measurement (LifeScale, Santa Barbara, USA) or pathogen-specific bioparticles that bind to specific bacterial targets and deliver custom-designed DNA molecules causing viable bacteria to express luciferase (Roche, Basel, Switzerland) are under commercial development and show promising potential. However, more peer-reviewed studies will be required to assess their usefulness in the routine clinical microbiology laboratory.

In addition to these commercial or pre-commercial developments, various studies describe novel approaches to rapid phenotypic AST, which warrant further exploration. For instance, Huang *et al.* reported a novel method utilizing flow cytometry and adaptive multidimensional statistical metrics to analyze the data.[18] Matsumoto and co-workers described a microfluidic channel method for rapid AST (3 hours) of *Pseudomonas aeruginosa* by automated microscopic detection of cell growth and morphology of single bacterial cells following incubation in antimicrobial-coated microfluidic channels and good correlation with the reference (broth microdilution) method was demonstrated.[19] Weibull *et al.* developed a high-throughput nanowell AST device allowing heat map representation of MIC data within 4 hours.[20] Finally, Metzger *et al.* reported a general method for rapid species identification and AST involving a short initial cultivation step in the absence or presence of different antimicrobials followed by padlock probe detection of bacterial target DNA as a surrogate for bacterial growth. In a small clinical validation study, antibiotic susceptibility profiles of *E. coli* for ciprofloxacin and trimethoprim were determined with 100% accuracy in 3.5 hours.[21]

In conclusion, it is clearly established that rapid phenotypic susceptibility testing lowers the rate of incorrect empiric treatment choices, shortens the length of hospital stay and reduces patient mortality. Many novel options for rapid phenotypic AST will be available in the near future. Before adopting one or more of these systems, clinical microbiologists will need to evaluate their benefit in the context of local requirements: Is there a need to bridge a particular diagnostic gap such as rapid AST in sepsis? What is the capacity of the system (parallel processing)? Is it cost-efficient under local circumstances? Is there sufficient peer-reviewed validation data? How is the flexibility of in-house solutions weighted against the ease-of-use of proprietary systems (*black box*)? Finally, the benefits of rapid phenotypic AST will not translate into improved patient care unless extended staffing schedules and more rapid transmission of verified results can be provided.

Usefulness of MALDI-TOF to optimize anti-infective therapies

MALDI-TOF mass spectrometry fingerprinting has now been widely adopted by clinical microbiology laboratories for rapid identification of cultured microorganisms.[22-25] Compared to other conventional (*e.g.* biochemical) identification workflows, turnaround times are typically reduced by at least one working day up to several days for slower growing species or isolates that require complex tests for definite identification.[26,27]

Precise speciation can inform treatment decisions by facilitating better judgment of clinical relevance of microbial isolates (*e.g. S.aureus vs*. coagulase-negative staphylococci) or directly guide selection of antimicrobials based on known patterns of intrinsic resistance (*e.g*. according to EUCAST expert rules) and local susceptibility data. Targeted modification of antimicrobial treatment can often be suggested upon identification of non-fermenting Gram-negative bacilli (*Acinetobacter spp*., *Pseudomonas spp*., *Stenotrophomonas maltophilia*), the CESP group of *Enterobacteriaceae* (*Citrobacter spp., Enterobacter spp., Serratia spp., Providencia spp*., *Hafnia spp.,*) or enterococci. Given the usually low rates of acquired resistance, species identification is exceptionally useful for the treatment of fungal infections. In observational studies, introduction of MALDI-TOF with antimicrobial stewardship intervention significantly reduced time to effective antimicrobial treatment in patients with bloodstream infection[26-28] and *Acinetobacter baumannii* pneumonia[29] and shortened inappropriate use of vancomycin in patients with CoNS-contaminated blood cultures by more than 60 hours.[30]

Highest impact on turnaround times and prescription policies is expected for rapid identification from positive blood culture bottles.[27,31] While MALDI-TOF fingerprinting had originally been introduced and approved for the identification of solid media cultures, it has readily been adopted for liquid enrichment cultures.[32] Currently, sample preparation kits for blood cultures are offered by both major suppliers of MALDI-TOF fingerprinting systems.

Compared to conventional processing, direct identification of organisms from positive blood-cultures by mass spectrometry reduced turnaround times by at least one working day and provided species level identification results the day after sample collection in more than three fourths of cases.[32] The technique is thus suitable to inform clinicians within the critical phase of septic illness when laboratory reports are known to have highest impact on treatment decisions.[33] In observational studies, identification by MALDI-TOF added significantly to Gram stain reports, leading to additional treatment modifications in more than 10% of cases.[27,31,34] Combined with selected molecular resistance marker tests or modified phenotypic susceptibility tests, MALDI-TOF based workflows can provide sufficient information for definite treatment within 12 hours of blood culture positivity.[35,36] Two recent studies found a reduction in time to optimal antimicrobial therapy, a reduction in hospital length of stay and a reduction of hospital costs upon introducing direct pathogen identification from positive blood cultures by MALDI-TOF.[37,38] The latter study could even show a reduction in 30-day mortality, the most meaningful clinical parameter. Yet, in both studies, direct MALDI-TOF identification was only one aspect of an intervention bundle, which also comprised rapid susceptibility testing from positive blood cultures and intensified antimicrobial stewardship measurements. Thus, the exact contribution of rapid pathogen identification by MALDI-TOF remains difficult to assess. However, MALDI-TOF-based identification of bacterial pathogens directly from positive blood-culture bottles is comparably labor-intensive and currently few laboratories offer the service as part of their routine blood culture workup.

Beyond species identification, mass spectrometry has also been utilized for rapid susceptibility testing. The technique can be used to detect products of beta-lactam hydrolysis in bacterial cultures with unprecedented sensitivity and specificity. It has successfully been used to detect ESBL and carbapenemase production within 30 to 150 minutes.[39-41] Other approaches rely on the detection of changes in the proteomic profile of cells exposed to antimicrobial agents and should be applicable to a broader range of substances.[42,43] When made available for routine testing, these assays could add to the armamentarium of rapid susceptibility tests needed to reduce time to optimal antimicrobial therapy. Another promising approach involves direct identification of resistance determinants or biomarkers expressed by resistant bacteria by MALDI-ToF. A recent study could prove high sensitivity and specificity by detecting a protein specifically present in a subset of MRSA strains in the mass spectra generated by MALDI-TOF.[44]

In selected cases, MALDI-TOF mass spectrometry might also provide treatment relevant information via sub-species level differentiation of microbial pathogens. Certain lineages with known susceptibility traits might be identified by characteristic marker peaks in their MALDI-TOF mass spectrum. While the achievable phylogenetic resolution varies considerably between species and is generally lower than with established typing tools,[45] the technique is much faster and cheaper than MLST or PFGE. If MALDI-TOF markers can be established for the trait of interest, clinical isolates could be monitored with little additional effort. So far, MALDI-TOF typing has successfully been used during a large outbreak of ESBL-EHEC[46] and to classify methicillin-resistant *Staphylococcus aureus*.[44,47,48] However, neither standardized workflows nor databases or software tools are currently available for routine application.

The introduction of MALDI-TOF mass spectrometry into the clinical microbiology laboratory has considerably reduced time-to-result for species identification in culture based diagnostics. However, its impact on the rational use of antimicrobials critically depends on the timely translation of test results into clinical decision making via policies for empirical treatment based on local susceptibility data. Application of MALDI-ToF mass spectrometry for rapid susceptibility testing or epidemiological problems is currently hampered by the lack of standardized protocols, test kits and software tools. While the analytical sensitivity of MALDI-ToF is insufficient for direct application to clinical samples, the low cost-per-sample and broad applicability make it an attractive bridging technology, which can be well complemented with nucleic acid based tests and conventional assays.

Clinical impact of amplification-based diagnostics

Approaches towards direct pathogen identification using nucleic acid amplification techniques

During the last two decades, amplification-based approaches towards pathogen detection have become irreplaceable in the clinical microbiology laboratory. More recently, the introduction of commercial multiplex PCR assays made rapid, sensitive and specific detection of both bacterial and viral pathogens from a single specimen broadly available. These assays can help to avoid unnecessary antibacterial treatment if viral pathogens are detected, which is of particular importance in infections of the respiratory system.[49] Although it is well known that the majority of respiratory infections are caused by viral pathogens, prescription of antibiotics is frequent, promoting the development of antimicrobial resistance and the occurrence of complications such as *Clostridium difficile* infection.[50] Thus, an obvious strategy to reduce antimicrobial consumption in these infections is to broadly screen by PCR for both bacterial and viral pathogens and to discontinue antimicrobial therapy once evidence for a viral infection is generated.[51] Molecular techniques can identify multiple different viral pathogens in one analysis with test results being available on the same working day. Some commercially available assays additionally detect a number of bacterial pathogens implicated in lower respiratory tract infections, *e.g.* pneumococci, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Chlamydophila pneumonia*, *Mycoplasma pneumoniae* and sometimes also *Staphylococcus aureus* thereby facilitating empiric treatment choices in case of a bacterial infection.[52] However, surprisingly few studies are published which analyze whether this workflow leads to a decrease in unnecessary antibacterial therapies. Nevertheless, the published studies highlight some important problems. Most importantly, while one would assume that identification of a single viral pathogen in respiratory samples results in immediate discontinuation of antimicrobial treatment, several studies found that this is not generally the case.[53-57] This phenomenon may in part be explained by delayed communication of the test results to the clinician or by clinical improvement of the patient upon other therapeutic interventions, *e.g.* antipyretics and administration of oxygen, being erroneously related to the empiric antimicrobial therapy.

Interpretation of rapid molecular screening results becomes more complicat-

ed when bacterial pathogens are targeted by multiplex PCR. In case of respiratory infections, typical patient samples include sputum and nasopharyngeal swabs.[56] Yet, many bacterial pathogens causing respiratory tract infections, particularly pneumococci, *H. influenzae*, *M. catarrhalis* and in part also *S. aureus*, all colonize the upper respiratory tract as commensals of the physiological flora. Not surprisingly, a study by Gilbert and co-workers using a combination of culture-based diagnostics and molecular tests to screen for viral or bacterial pathogens in community-acquired pneumonia, found bacteria as causative agents for respiratory infections at rates close to the reported colonization frequencies and often in conjunction with viral pathogens.[56] Therefore, while positive results in these assays may reflect true bacterial or bacterial/viral co-infection, they may also represent mere contamination of the sample. In consequence, false-positive rapid molecular test results may even trigger antimicrobial therapy when none is required and thus have a detrimental effect on antimicrobial stewardship initiatives.

Bloodstream infections present another category of infections where rapid molecular diagnostics hold great promise to rationalize empiric antimicrobial therapy.[58] Molecular assays could not only accelerate pathogen detection, but may also be of value in patients in which blood cultures remain negative.[59] This is the case in up to 50% of bacteremic patients and relates to low numbers of circulating bacteria, presence of fastidious organisms, delayed transportation and incubation of blood culture bottles with resultant decreased viability of bacteria or growth inhibition due to antibiotic pre-treatment.[60,61] Most available commercial systems are reported to provide species identification within 3-6 hours and have a lower limit of detection between 10 and 100 CFU/mL.[59] The optimism created by these excellent technical outlines was thwarted by ambiguous results when molecular assays were validated in comparison to standard blood cultures. In fact, various commercial PCR assays from whole blood specimens remained negative while bacteria were recovered using conventional blood culture bottles, indicating a potential sensitivity issue with molecular sepsis assays.[62] These apparently conflicting results may in part be explained by relatively low blood volume from which bacterial DNA was isolated (ranging from 1-6 mL), or the presence of PCR-inhibitors (*e.g.* iron, heparin, immunoglobulins) hampering DNA amplification.[59] On the other hand, in some cases PCR-based methods detected microorganisms that could not be grown using conventional blood cultures, putting forward the question which gold standard is best for validating test accuracy. The interpretation of these results and the (necessary) differentiation from probable contaminations during sampling remains open. Most importantly, at present no data are available demonstrating the clinical impact of (cost intensive) PCR assays for direct pathogen identification in whole blood. Although a recent study found a change in clinical management (*e.g.* change in antimicrobial therapy) in about a third of the study population as a consequence of PCR results from directly drawn blood samples from newborns, differentiating false- from true-positive PCR results was regarded as difficult.[63] Nearly two thirds of PCR-positive samples, often with CoNS, remained culture-negative. Thus, at present PCR assays are still waiting to find their place in sepsis diagnostics.

Usefulness of nucleic acid amplification-based methods for rapid pathogen characterization from positive blood cultures

Rapid, amplification-based methods could help to avoid unjustified broad-spectrum pathogen coverage and fast de-escalation of empiric antimicrobial therapy by immediate identification of molecular resistance mechanisms as soon as enough bacterial material becomes available during culture. Obvious clinical need and the availability of abundant organisms have made positive blood cultures a primary target of tailored commercial assays.[58,64,65] A common feature of these systems [*e.g.* Verigene BC-GP/-BN (Luminex), Xpert MRSA/SA BC assay (Cepheid), FilmArray BCID (Biomerieux/BioFire)] is the possibility to differentiate bacteria to the species level. This already could have important stewardship implications, as knowledge on naturally occurring resistance phenotypes and availability of specific local resistance epidemiology could help to optimize antimicrobial therapies at an early stage of the diagnostic work up. Moreover, in certain scenarios a confirmed species identification could already help to discard bacteremia as a diagnosis and thus cease an antibacterial therapy (*e.g.* if coagulase-negative staphylococci are encountered).[66,67] Today, certainly MALDI-ToF-based direct identification of bacteria from positive blood culture bottles offers a broader diagnostic precision at a lower cost as compared to amplification-based, commercial systems (see above).

However, a major drawback of every approach that is restricted to rapid identification of bacterial pathogens is the lack of information on possible acquired resistance markers. Therefore, inclusion of primer sets for detection of specific resistance determinants is an obvious extension of PCR-based assays as long as there is an unambiguous association with a specific drug-susceptibility phenotype. Here, due to the tremendous variability of resistance mechanisms, PCR-based methods as a basis to extrapolate a dedicated resistance phenotype are obviously of limited value in Gram-negative organisms.[68] Conversely, in staphylococci deduction of beta-lactam susceptibility from genetic information is feasible through detection of *mecA*. The almost monocausal reason for beta-lactam resistance in *S. aureus* depending on the expression of PBP2a has driven the development of various in-house as well as commercial systems for *mecA* detection.[68,69]

Most available in house as well as commercial assays target positive blood cultures yielding growth of cluster forming Gram-positive cocci. They allow to differentiate between coagulase-negative staphylococci and *S. aureus* [*e.g.* Xpert MRSA/SA BC assays (Cepheid), GeneOHM StaphSR assay (BD)],[70,71] and are able to detect *mecA* (and at least in some assays also for *mecC*).

Turn-around times for PCR-based assays are between one to three hours and can thus significantly accelerate time to optimal targeted antimicrobial therapies or discontinuation of a running therapy, *e.g.* if evidence for coagulase-negative staphylococci is provided and contamination is likely.[72,73] Interestingly, statistical modeling of the impact of a rapid (PCR) assay detecting MRSA in blood cultures indicated that such a strategy has the potential to reduce mortality in hospital-acquired bacteremia over a range of MRSA prevalences from 2-80%. Moreover, data from the same study indicate that rapid MRSA detection is cost effective, *e.g.* by lowering cost for broad range empiric antimicrobial therapy.[74] These conclusions were indeed confirmed in clinical studies.

In a single center study from the USA (local MRSA prevalence of 65% in *S.aureus* bacteremia) the effect of PCR-based differentiation of cluster forming Gram-positive cocci by using the Xpert MRSA assay in combination with intervention of an infectious disease pharmacist aiding to optimize antimicrobial therapy was assessed. If possible, the time to switch from empiric vancomycin therapy to a beta-lactam was 1.7 days shorter as compared to the control (no PCR, no ID intervention). Moreover, the mean length of hospital stay was 6.3 days shorter and the mean treatment costs were on average $ 21,387 less in the intervention group.[75]

The diagnostic strategy of PCR-based differentiation of Gram-positive cocci directly from positive blood cultures includ-

ing detection of *mecA* was reinforced by a later study from Australia investigating 151 *S. aureus* bacteremia episodes (local MRSA prevalence of 20% in *S. aureus* bacteremias), Implementation of the Xpert MRSA/SA BC assay allowed for earlier appropriate prescription of vancomycin in 54% of patients with MRSA infections. In 25% of all patients, unnecessary vancomycin was avoided, and in 16% of all patients, therapy was ceased because no *S. aureus* was detected.[76]

Emonet and co-workers recently analyzed the effect of an in-house multiplex real time PCR including specific primers for *S. aureus*, *S. epidermidis*, and *mecA* on management of patients with bacteremia caused by Gram-positive cocci. PCR was used to differentiate and preliminary deduce susceptibility of *S. aureus* and *S. epidermidis*. Introduction of the PCR assay significantly shortened the time-to-result to detect methicillin-susceptibility as compared to the standard workflow from 25.4 hours to 3.9 hours after availability of a Gram stain. More rapid availability of presumable beta-lactam susceptibility allowed for a quicker switch to an appropriate therapy in *S. aureus* bacteremia cases (5 hours *vs.* 25.5 hours). Switching most often occurred in MSSA bacteremia, in which empiric glycopeptide usage was stopped and patients were treated with a beta-lactam instead.[77]

A drawback of these studies is that PCR was performed on all blood cultures yielding growth of Gram-positive cocci, resulting in significant costs especially when commercial systems are in use. A way to lower these costs is to differentiate between coagulase-negative staphylococci and *S. aureus*, *e.g.* by direct identification of cultured bacteria using MALDI ToF,[78] and to restrict the use of (commercial) *mecA* PCRs to those samples showing growth of *S. aureus.*[79] In a study from Switzerland this approach was prospectively analyzed during a one year period. In total, MALDI-ToF identified growth of *S. aureus* in 197 blood cultures. 106 samples included in the intervention group in which cultures yielding growth of Gram-positive cocci were processed including MALDI-ToF identification directly from blood cultures and subsequent Xpert MRSA/SA assay. Ninety-six samples were assigned to the control group. Here, direct identification was followed by conventional susceptibility testing. Intriguingly, there was less unnecessary glycopeptide usage in patients with MSSA bacteremia in the intervention group (8.1% *vs*. 26.1%; P<0.01).[80]

Yet, despite the seemingly straightforward genotype-phenotype correlation for beta-lactam susceptibility in *S. aureus*, limitations of currently used PCRs must be kept in mind. Rates of false-positive MRSA-PCR results, for example due to *mecA*-negative SCCmec-elements, can reach significant levels.[81] Thus, particularly in low prevalence regions, care must be taken, that false-positive PCR-results do not exaggerate glycopeptide use instead of lowering it. Nevertheless, the studies related to staphylococcal bacteremia highlighted above demonstrate the significant impact of direct bacterial species identification and detection of genetic resistance markers can have on the clinical management of septic patients. A similar strategy may also be applicable to other species, given that a reliable association between genotype and phenotype exists and that the respective genetic markers are of low variability. For instance, this applies for vancomycin resistance in enterococci carrying *vanA* or *vanB*. In the past, optimal treatment (ampicillin versus vancomycin) of enterococcal bacteremia could be readily deduced from species identification, as resistance to aminopenicillins is low in *E. faecalis* and high in *E. faecium*. However, due to the emergence of vancomycin-resistant enterococci (VRE) in Europe[82] and the high VRE prevalence in specific risk groups,[83] empiric administration of vancomycin may today be inappropriate even in *E. faecium* depending on local epidemiology. At least one report found that implementation of a commercial assay to detect *vanA/B* in enterococcal isolates from positive blood cultures (Verigene BC-GP, Luminex) significantly shortened the time to appropriate therapy in patients with VRE bacteremia (reduction by 31.1 hours, P<0.0001). In parallel, introduction of a molecular assay to detect *vanA/B* was associated with shorter mean length of stay and lower mean hospital costs.[84] Of note, a recent study in which the impact of the FilmArray BCID assay was tested in comparison to standard procedures did not find a clinical impact on patient outcome by using fast VRE detection in blood cultures.[85] As mentioned above, molecular detection of resistance determinants and reliable deduction of resistance phenotypes is much more challenging in Gram-negative bacteria as compared to Gram-positive species.[86] However, given the raise and rapid spread of multidrug-resistant Gram-negative species, availability of rapid molecular test would be highly desirable, especially in blood stream infections. Over the past couple of years several in-house as well as commercial systems have been developed, partially in integrated solutions in which Gram-negative and Gram-positive bacteria and some of their key resistance determinants are detected simultaneously from positive blood culture bottles [*e.g.* FilmArray BCID (Biomerieux/Biofire), Unyvero BCU (Curetis), Verigene BC-GN assay (Luminex)]. These assays essentially focus on the detection of *mecA* and *vanA/B* in staphylococci and enterococci, respectively, and various beta-lactamases in gram-negative rods. Frequently, $bla_{\text{CTX-M}}$ as a marker for an ESBL-phenoptype is targeted (Verigene BC-GN/-GP, Unyvero BCU), in combination with common carbapenemases (*e.g.* bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}; FilmArray BCID, Verigene BC-GN/-GP, Unyvero BCU).[87-91] The list of resistance determinants is far from comprehensive, and completely neglects ESBL enzymes other than CTX-M and genes that confer resistance against fluoroquinolones or aminoglycosides. In addition, resistance phenotypes involving changes in gene expression levels or combined effects (*e.g.* ESBL-/AmpC-overexpression and porin loss leading to elevated carbapenem MICs) are currently undetectable using commercial amplification techniques. As a consequence, rapid reporting of molecular resistance results could potentially lead to wrong empiric treatment decisions by suggesting an *all-clear* to the clinician. Those reports should therefore generally include a comment on the limitation of the tests, and advise on considering the clinical context of the patient (for example results from recent colonization screenings, epidemiological background, effectiveness of current antimicrobial treatment). As previously noted, rapid phenotypic methods will thus continue to be of significant importance in this context.[86] Nevertheless, given the importance of anticipated susceptibilities based on species identification and the possibility to exclude the presence of organisms for which multi-resistance phenotypes are more common (*e.g. P. aeruginosa*, *A. baumannii*), rapid PCR-based analysis of positive blood cultures growing Gram-negative rods could be of clinical value.[92]

Although available commercial assays have been thoroughly validated in technical terms,[93-95] the impact of using rapid amplification based methods on clinical decision-making and patient outcome is less well studied. However, such studies would be of significant importance in order to justify the increased cost and complexity of the diagnostic workflow.

The clinical impact of performing rapid identification and detection of resistance determinants in Gram-negative rods was tested in a retrospective study by Walker and co-workers.[96] The authors compared two periods, in which standard procedures for species identification and susceptibility testing were in place alone or in combina-

tion with the Verigene BC-GN assay. The amplification assay was performed immediately after the blood culture bottles were flagged positive, and results were directly reported. While the implementation had no effect on earlier appropriate antimicrobial coverage or de-escalation, length of ICU stay, 30-day mortality and mortality associated with multidrug resistant organisms (*e.g.* ESBL-producing *Enterobacteriaceae*) were lower in the group in which rapid molecular testing was applied. It should be noted that in this study, no additional stewardship measures were initiated to flank implementation of rapid diagnostics.

A general drawback of most studies investigating the clinical impact of rapid pathogen identification in positive blood cultures is their observational study design and the use of historic controls. In that respect, a recent publication by Banaerjee and co-workers is of special importance.[97] In a randomized clinical trial, the authors compared three approaches to differentiate organisms in positive blood cultures and communication of results. While in one group standard work up was in place, in a second group the FilmArray BCID assay was used to differentiate organism immediately after blood culture bottles were flagged positive. Results in this group were communicated by a laboratory technician during 24 hours every day, accompanied by templated comments in the electronic medical record to guide antimicrobial therapy. In a third group, FilmArray BCID results were communicated 24 hours every day by a member of the antimicrobial stewardship team. The authors found that in groups 2 and 3 clinicians were enabled to quickly initiate pathogen-directed antimicrobial therapy. In both groups an increased use of narrow spectrum antibiotics was observed, as was less usage of unnecessary vancomycin, decreased treatment of blood culture contaminants and more timely escalation if appropriate. The implementation of rapid molecular testing, however, had no effect on mortality, length of stay or costs. The study not only provides evidence that implementation of rapid PCR testing of positive blood cultures can optimize patient treatment, but demonstrates that flanking stewardship measures are important clues to translate speed in diagnostic procedures into clinical action. The importance of structured communication and stewardship decision support, especially in Gram-negative bacteremia, was also reported by others.[26,92,98] A rather simple but obviously effective way apparently is to provide real-time decision support using templated comments in medical records.[97] This solution appears to be especially interesting in settings where antimicrobial stewardship teams are not available.

In conclusion, PCR-based assays have a clear place in specific and fast, culture independent pathogen detection. The specific value in infections of the respiratory tract, especially hospital-acquired pneumonia, and the bloodstream is currently unclear. Certainly, PCR is of great value in rapid pathogen identification and resistance determinant detection in cultured bacteria. This is especially true in the work-up of positive blood cultures – however, the investment in expensive diagnostic assays is only justified if results are communicated to the clinician in a way allowing for immediate clinical action, *i.e.* adjustment of antimicrobial therapies.

Conclusions

Techniques providing rapid information on bacterial pathogens and their antimicrobial susceptibility are of key importance for the management of infectious diseases patients. The introduction of MALDI-ToF into routine diagnostics led to a significant acceleration of highly specific species identification and must be regarded as a major advance in the field of clinical microbiology. In addition, rapid molecular tests offer significant opportunities to further reduce the time-to-result for pathogen identification and information on key resistance determinants. Moreover, novel approaches in phenotypic susceptibility testing herald an era in which medical microbiology can substantially support also the early stages of clinical decision making. Information will be especially useful to limit usage of last resort antimicrobials to those cases in which narrow-spectrum antimicrobials are not appropriate. With next generation sequencing becoming implemented into routine diagnostic procedures, additional improvements are on the horizon.

Most importantly, it has already become evident that technical improvements resulting in a shorter time-to-result only translate into benefit for the patient if rapid, structured communication and interpretation of clinical microbiology results are available for the responsible clinician. In this regard, the importance of a close cooperation between the clinical microbiology laboratory and antimicrobial stewardship teams cannot be overestimated.

References

1. Soo Hoo GW, Wen YE, Nguyen TV, et al. Impact of clinical guidelines in the management of severe hospital-acquired pneumonia. Chest 2005;128:2778-87.
2. Doern GV, Vautour R, Gaudet M, et al. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J Clin Microbiol 1994;32:1757-62.
3. Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J Clin Microbiol 1999;37:1415-8.
4. Shorr AF, Micek ST, Welch EC, et al. Inappropriate antibiotic therapy in Gram-negative sepsis increases hospital length of stay. Crit Care Med 2011;39:46-51.
5. Garcia-Vazquez E, Moral-Escudero E, Hernandez-Torres A, et al. What is the impact of a rapid diagnostic E-test in the treatment of patients with Gram-negative bacteraemia? Scand J Infect Dis 2013;45:623-8.
6. Kumar A, Ellis P, Arabi Y, et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 2009;136:1237-48.
7. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
8. WADE HE. Observations on the growth phases of Escherichia coli, American type B. J Gen Microbiol 1952;7:18-23.
9. Harris PN, Ferguson JK. Antibiotic therapy for inducible AmpC beta-lactamase-producing Gram-negative bacilli: what are the alternatives to carbapenems, quinolones and aminoglycosides? Int J Antimicrob Agents 2012;40:297-305.
10. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev 2009;22:161-82.
11. Leclercq R, Courvalin P. Resistance to macrolides and related antibiotics in Streptococcus pneumoniae. Antimicrob Agents Chemother 2002;46:2727-34.
12. Entenza JM, Betrisey B, Manuel O, et al. Rapid detection of Staphylococcus aureus strains with reduced susceptibility to vancomycin by isothermal microcalorimetry. J Clin Microbiol 2014;52:180-6.
13. Novais A, Brilhante M, Pires J, et al. Evaluation of the recently launched rapid carb blue kit for detection of carbapenemase-producing Gram-negative bacteria. J Clin Microbiol 2015;53:3105-7.
14. Poirel L, Nordmann P. Rapidec carba

NP test for rapid detection of carbapenemase producers. J Clin Microbiol 2015;53:3003-8.
15. Maurer FP, Castelberg C, Quiblier C, et al. Evaluation of carbapenemase screening and confirmation tests in Enterobacteriaceae and development of a practical diagnostic algorithm. J Clin Microbiol 2014 29;JCM-14.
16. Hombach M, von Gunten B, Castelberg C, et al. Evaluation of the rapidec carba NP test for detection of carbapenemases in enterobacteriaceae. J Clin Microbiol 2015;53:3828-33.
17. Parcina M, Bartonickova L, Vojvoda V, et al. Performance characteristics of the new accelerate ID/AST system for antibiotic susceptibility testing of enterobacteriaceae clinical isolates, compared to IVD routine laboratory AST systems. San Diego: ICAAC; 2015.
18. Huang TH, Ning X, Wang X, et al. Rapid cytometric antibiotic susceptibility testing utilizing adaptive multidimensional statistical metrics. Anal Chem 2015;87:1941-9.
19. Matsumoto Y, Sakakihara S, Grushnikov A, et al. A microfluidic channel method for rapid drug-susceptibility testing of Pseudomonas aeruginosa. PLoS One 2016;11:e0148797.
20. Weibull E, Antypas H, Kjall P, et al. Bacterial nanoscale cultures for phenotypic multiplexed antibiotic susceptibility testing. J Clin Microbiol 2014;52:3310-7.
21. Mezger A, Gullberg E, Goransson J, et al. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. J Clin Microbiol 2015;53:425-32.
22. Cassagne C, Normand AC, L'Ollivier C, et al. Performance of MALDI-TOF MS platforms for fungal identification. Mycoses 2016;10.
23. Morgenthaler NG, Kostrzewa M. Rapid identification of pathogens in positive blood culture of patients with sepsis: review and meta-analysis of the performance of the sepsityper kit. Int J Microbiol 2015;2015:827416.
24. Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. Clin Microbiol Rev 2014;27:783-822.
25. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem 2015;61:100-11.
26. Huang AM, Newton D, Kunapuli A, et al. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis 2013;57:1237-45.
27. Martiny D, Debaugnies F, Gateff D, et al. Impact of rapid microbial identification directly from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on patient management. Clin Microbiol Infect 2013;19:E568-81.
28. Malcolmson C, Ng K, Hughes S, et al. Impact of matrix-assisted laser desorption and ionization time-of-flight and antimicrobial stewardship intervention on treatment of bloodstream infections in hospitalized children. J Pediatric Infect Dis Soc 2016;iw033.
29. Wenzler E, Goff DA, Mangino JE, et al. Impact of rapid identification of Acinetobacter baumannii via matrix-assisted laser desorption ionization time-of-flight mass spectrometry combined with antimicrobial stewardship in patients with pneumonia and/or bacteremia. Diagn Microbiol Infect Dis 2016;84:63-8.
30. Nagel JL, Huang AM, Kunapuli A, et al. Impact of antimicrobial stewardship intervention on coagulase-negative Staphylococcus blood cultures in conjunction with rapid diagnostic testing. J Clin Microbiol 2014;52:2849-54.
31. Clerc O, Prod'hom G, Vogne C, et al. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. Clin Infect Dis 2013;56:1101-7.
32. Christner M, Rohde H, Wolters M, et al. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ionization time of flight mass spectrometry fingerprinting. J Clin Microbiol 2010;48:1584-91.
33. Munson EL, Diekema DJ, Beekmann SE, et al. Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. J Clin Microbiol 2003;41:495-7.
34. Vlek AL, Bonten MJ, Boel CH. Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One 2012;7:e32589.
35. Machen A, Drake T, Wang YF. Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. PLoS One 2014;9:e87870.
36. Verroken A, Defourny L, le Polain de WO, et al. Clinical Impact of MALDI-TOF MS Identification and rapid susceptibility testing on adequate antimicrobial treatment in sepsis with positive blood cultures. PLoS One 2016;11:e0156299.
37. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med 2013;137:1247-54.
38. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J Infect 2014;69:216-25.
39. Burrer A, Findeisen P, Jager E, et al. Rapid detection of cefotaxime-resistant Escherichia coli by LC-MS. Int J Med Microbiol 2015;305:860-4.
40. Lasserre C, De Saint ML, Cuzon G, et al. Efficient detection of carbapenemase activity in enterobacteriaceae by matrix-assisted laser desorption ionization-time of flight mass spectrometry in less than 30 minutes. J Clin Microbiol 2015;53:2163-71.
41. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 2011;49:3321-4.
42. Vella A, De CE, Vaccaro L, et al. Rapid antifungal susceptibility testing by matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis. J Clin Microbiol 2013;51:2964-9.
43. Lange C, Schubert S, Jung J, et al. Quantitative matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid resistance detection. J Clin Microbiol 2014;52:4155-62.
44. Josten M, Reif M, Szekat C, et al. Analysis of the MALDI-TOF mass spectrum of Staphylococcus aureus identifies mutations which allow differentiation of the main clonal lineages. J Clin Microbiol 2013;51:1809-17.
45. Lasch P, Fleige C, Stammler M, et al. Insufficient discriminatory power of MALDI-TOF mass spectrometry for typing of Enterococcus faecium and Staphylococcus aureus isolates. J Microbiol Methods 2014;100:58-69.
46. Christner M, Trusch M, Rohde H, et al. Rapid MALDI-TOF mass spectrometry strain typing during a large outbreak of Shiga-Toxigenic Escherichia coli. PLoS One 2014 8;9:e101924.

47. Wolters M, Rohde H, Maier T, et al. MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant Staphylococcus aureus lineages. Int J Med Microbiol 2011;301:64-8.
48. Sauget M, Mee-Marquet N, Bertrand X, et al. Matrix-assisted laser desorption ionization-time of flight Mass spectrometry can detect Staphylococcus aureus clonal complex 398. J Microbiol Methods 2016;127:20-3.
49. Gonzales R, Malone DC, Maselli JH, et al. Excessive antibiotic use for acute respiratory infections in the United States. Clin Infect Dis 2001;33:757-62.
50. Bouza E, Torres MV, Radice C, et al. Direct E-test (AB Biodisk) of respiratory samples improves antimicrobial use in ventilator-associated pneumonia. Clin Infect Dis 2007;44:382-7.
51. Barenfanger J, Drake C, Leon N, et al. Clinical and financial benefits of rapid detection of respiratory viruses: an outcomes study. J Clin Microbiol 2000;38:2824-8.
52. Zumla A, Al-Tawfiq JA, Enne VI, et al. Rapid point of care diagnostic tests for viral and bacterial respiratory tract infections—needs, advances, and future prospects. Lancet Infect Dis 2014;14:1123-35.
53. Oosterheert JJ, van Loon AM, Schuurman R, et al. Impact of rapid detection of viral and atypical bacterial pathogens by real-time polymerase chain reaction for patients with lower respiratory tract infection. Clin Infect Dis 2005;41:1438-44.
54. Timbrook T, Maxam M, Bosso J. Antibiotic discontinuation rates associated with positive respiratory viral panel and low procalcitonin results in proven or suspected respiratory infections. Infect Dis Ther 2015;4:297-306.
55. Yee C, Suarthana E, Dendukuri N, et al. Evaluating the impact of the multiplex respiratory virus panel polymerase chain reaction test on the clinical management of suspected respiratory viral infections in adult patients in a hospital setting. Am J Infect Control 2016;16:10.
56. Gilbert D, Gelfer G, Wang L, et al. The potential of molecular diagnostics and serum procalcitonin levels to change the antibiotic management of community-acquired pneumonia. Diagn Microbiol Infect Dis 2016:10.
57. Branche AR, Walsh EE, Vargas R, et al. Serum procalcitonin measurement and viral testing to guide antibiotic use for respiratory infections in hospitalized adults: a randomized controlled trial. J Infect Dis 2015;212:1692-700.
58. Kothari A, Morgan M, Haake DA. Emerging technologies for rapid identification of bloodstream pathogens. Clin Infect Dis 2014;59:272-8.
59. Opota O, Jaton K, Greub G. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect 2015;21:323-31.
60. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36:296-327.
61. Fenollar F, Raoult D. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. Int J Antimicrob Agents 2007;30:S7-15.
62. Skvarc M, Stubljar D, Rogina P, et al. Non-culture-based methods to diagnose bloodstream infection: does it work? Eur J Microbiol Immunol 2013;3:97-104.
63. Tröger B, Hartel C, Buer J, et al. Clinical relevance of pathogens detected by multiplex PCR in blood of very-low-birth weight infants with suspected sepsis - multicentre study of the German Neonatal Network. PLoS One 2016;11:e0159821.
64. Livermore DM, Wain J. Revolutionising bacteriology to improve treatment outcomes and antibiotic stewardship. Infect Chemother 2013;45:1-10.
65. Opota O, Croxatto A, Prod'hom G, et al. Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 2015;21:313-22.
66. Holtzman C, Whitney D, Barlam T, et al. Assessment of impact of peptide nucleic acid fluorescence in situ hybridization for rapid identification of coagulase-negative staphylococci in the absence of antimicrobial stewardship intervention. J Clin Microbiol 2011;49:1581-2.
67. Wong JR, Bauer KA, Mangino JE, et al. Antimicrobial stewardship pharmacist interventions for coagulase-negative staphylococci positive blood cultures using rapid polymerase chain reaction. Ann Pharmacother 2012;46:1484-90.
68. Pulido MR, Garcia-Quintanilla M, Martin-Pena R, et al. Progress on the development of rapid methods for antimicrobial susceptibility testing. J Antimicrob Chemother 2013;68:2710-7.
69. Goff DA, Jankowski C, Tenover FC. Using rapid diagnostic tests to optimize antimicrobial selection in antimicrobial stewardship programs. Pharmacotherapy 2012;32:677-87.
70. Buchan BW, Allen S, Burnham CA, et al. Comparison of the next-generation Xpert MRSA/SA BC assay and the GeneOhm StaphSR assay to routine culture for identification of Staphylococcus aureus and methicillin-resistant S. aureus in positive-blood-culture broths. J Clin Microbiol 2015;53:804-9.
71. Gröbner S, Dion M, Plante M, et al. Evaluation of the BD GeneOhm StaphSR assay for detection of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates from spiked positive blood culture bottles. J Clin Microbiol 2009;47:1689-94.
72. Parta M, Goebel M, Thomas J, et al. Impact of an assay that enables rapid determination of Staphylococcus species and their drug susceptibility on the treatment of patients with positive blood culture results. Infect Control Hosp Epidemiol 2010;31:1043-8.
73. Carver PL, Lin SW, DePestel DD, et al. Impact of mecA gene testing and intervention by infectious disease clinical pharmacists on time to optimal antimicrobial therapy for Staphylococcus aureus bacteremia at a University Hospital. J Clin Microbiol 2008;46:2381-3.
74. Brown J, Paladino JA. Impact of rapid methicillin-resistant Staphylococcus aureus polymerase chain reaction testing on mortality and cost effectiveness in hospitalized patients with bacteraemia: a decision model. Pharmacoeconomics 2010;28:567-75.
75. Bauer KA, West JE, Balada-Llasat JM, et al. An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant Staphylococcus aureus/S. aureus blood culture test in patients with S. aureus bacteremia. Clin Infect Dis 2010;51:1074-80.
76. Davies J, Gordon CL, Tong SY, et al. Impact of results of a rapid Staphylococcus aureus diagnostic test on prescribing of antibiotics for patients with clustered gram-positive cocci in blood cultures. J Clin Microbiol 2012;50:2056-8.
77. Emonet S, Charles PG, Harbarth S, et al. Rapid molecular determination of methicillin resistance in staphylococcal bacteraemia improves early targeted antibiotic prescribing: a randomized clinical trial. Clin Microbiol Infect 2016:10.
78. Christner M, Rohde H, Wolters M, et al. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ioniza-

tion time of flight mass spectrometry fingerprinting. J Clin Microbiol 2010;48:1584-91.
79. Romero-Gomez MP, Munoz-Velez M, Gomez-Gil R, et al. Evaluation of combined use of MALDI-TOF and Xpert MRSA/SA BC assay for the direct detection of methicillin resistance in Staphylococcus aureus from positive blood culture bottles. J Infect 2013:10.
80. Clerc O, Prod'hom G, Senn L, et al. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry and PCR-based rapid diagnosis of Staphylococcus aureus bacteraemia. Clin Microbiol Infect 2014;20:355-60.
81. Blanc DS, Basset P, Nahimana-Tessemo I, et al. High proportion of wrongly identified methicillin-resistant Staphylococcus aureus carriers by use of a rapid commercial PCR assay due to presence of staphylococcal cassette chromosome element lacking the mecA gene. J Clin Microbiol 2011;49:722-4.
82. Gastmeier P, Schroder C, Behnke M, et al. Dramatic increase in vancomycin-resistant enterococci in Germany. J Antimicrob Chemother 2014;69:1660-4.
83. Tacconelli E, Cataldo MA. Vancomycin-resistant enterococci (VRE): transmission and control. Int J Antimicrob Agents 2008;31:99-106.
84. Sango A, McCarter YS, Johnson D, et al. Stewardship approach for optimizing antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for Enterococcus species. J Clin Microbiol 2013;51:4008-11.
85. MacVane SH, Hurst JM, Boger MS, et al. Impact of a rapid multiplex polymerase chain reaction blood culture identification technology on outcomes in patients with vancomycin-resistant Enterococcal bacteremia. Infect Dis (Lond) 2016;48:732-7.
86. Tuite N, Reddington K, Barry T, et al. Rapid nucleic acid diagnostics for the detection of antimicrobial resistance in Gram-negative bacteria: is it time for a paradigm shift? J Antimicrob Chemother 2014;69:1729-33.
87. Dodemont M, De MR, Nonhoff C, et al. Performance of the verigene Gram-negative blood culture assay for rapid detection of bacteria and resistance determinants. J Clin Microbiol 2014;52:3085-7.
88. Han E, Park DJ, Kim Y, et al. Rapid detection of Gram-negative bacteria and their drug resistance genes from positive blood cultures using an automated microarray assay. Diagn Microbiol Infect Dis 2015;81:153-7.
89. Ward C, Stocker K, Begum J, et al. Performance evaluation of the Verigene(R) (Nanosphere) and FilmArray(R) (BioFire(R)) molecular assays for identification of causative organisms in bacterial bloodstream infections. Eur J Clin Microbiol Infect Dis 2015;34:487-96.
90. McCoy MH, Relich RF, Davis TE, et al. Performance of the FilmArray(R) blood culture identification panel utilized by non-expert staff compared with conventional microbial identification and antimicrobial resistance gene detection from positive blood cultures. J Med Microbiol 2016;65:619-25.
91. Ledeboer NA, Lopansri BK, Dhiman N, et al. Identification of Gram-Negative bacteria and genetic resistance determinants from positive blood culture broths by use of the Verigene Gram-negative blood culture multiplex microarray-based molecular assay. J Clin Microbiol 2015;53:2460-72.
92. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J Infect 2014;69:216-25.
93. Altun O, Almuhayawi M, Ullberg M, et al. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol 2013;51:4130-6.
94. Beal SG, Ciurca J, Smith G, et al. Evaluation of the nanosphere verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. J Clin Microbiol 2013;51:3988-92.
95. Southern TR, VanSchooneveld TC, Bannister DL, et al. Implementation and performance of the BioFire FilmArray(R) Blood Culture Identification panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. Diagn Microbiol Infect Dis 2015;81:96-101.
96. Walker T, Dumadag S, Lee CJ, et al. Clinical impact of laboratory implementation of Verigene BC-GN Microarray-based assay for detection of gram-negative bacteria in positive blood cultures. J Clin Microbiol 2016;54:1789-96.
97. Banerjee R, Teng CB, Cunningham SA, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. Clin Infect Dis 2015;61:1071-80.
98. Nguyen DT, Yeh E, Perry S, et al. Real-time PCR testing for mecA reduces vancomycin usage and length of hospitalization for patients infected with methicillin-sensitive staphylococci. J Clin Microbiol 2010;48:785-90.