Antimicrobial susceptibility testing (AST) is among the oldest and most common tasks performed by the Laboratory of Clinical Microbiology. Results of AST are essential for selection of definitive antimicrobial chemotherapeutic therapy of bacterial infections diagnosed in individual patients, including pediatric patients. Cumulative AST data are also important for compilation of surveillance reports on antimicrobial resistance epidemiology at different levels (hospital, regional, national and supranational), which serve as guidance for selection of the best empiric antimicrobial regimens in different epidemiological settings and as tools for monitoring the evolution of antimicrobial resistance trends on a broader scale. Finally, monitoring of AST patterns of clinical isolates can be useful for the early detection of outbreaks caused by antibiotic-resistant pathogens of clinical and epidemiological relevance.

The clinical value of AST has been clearly established and is further increased in settings with a high prevalence of multidrug-resistant (MDR) pathogens for which treatment options are more limited. However, the current AST practices have limitations, whereas recent technological developments in diagnostic microbiology opened new perspectives in this field. The scope of this article is to review the current AST practices and to describe and discuss the forthcoming developments in this area.

CURRENT AST PRACTICES AND THEIR LIMITATIONS

AST is currently based on testing the ability of antimicrobial agents to inhibit bacterial growth in vitro under standardized experimental conditions. The antimicrobial activity is measured either as a diameter (in mm) of the zone of bacterial growth inhibition around an antibiotic-containing disk or as a minimum inhibitory concentration (MIC, mg/L) of the antibiotic versus the tested pathogen. MIC can be determined by different methods, such as broth or agar dilution (in which bacterial growth is assessed in media containing serial dilutions of each antibiotic) and gradient diffusion (in which a zone of bacterial growth inhibition is formed around one of the extremities of a rectangular strip containing a gradient of the tested antibiotic, and the edge of the inhibition zone intersects the strip at the MIC value).

Several instruments (eg, Vitek-2 (bioMérieux, Marcy-L’Etoile, France), Phoenix (Becton-Dickinson, Franklin Lakes, NJ), Microscan (Beckman Coulter, Brea, CA)) are also available for high-throughput AST in laboratories with large workloads. These systems are based on automation of the broth dilution method for MIC testing, and in some cases they extrapolate the MIC values from growth curves recorded at different antimicrobial concentrations using dedicated algorithms. Whichever the method, the information is then translated into a susceptibility category (susceptible, resistant, intermediate or susceptible-dose dependent) referred to clinical use, based on the comparison of AST results with reference values of MIC or inhibition zone diameters, named clinical breakpoints. Clinical breakpoints are set and periodically revised by specific committees or other institutions. Definition and revision of clinical breakpoints is based on information about antibiotic dosages, pharmacokinetic and pharmacodynamic data, patterns of antimicrobial susceptibility of the bacterial species, resistance mechanisms and results of clinical studies. In Europe, the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org) has

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been officially recognized by European Centre for Disease Prevention and Control and European Medicines Agency and has become the most popular breakpoint system, whereas in North America and other non-European countries, the Clinical and Laboratory Standards Institute standard (http://www.clsi.org) remains the most popular breakpoint system.

Conventional AST based on phenotypic testing has become firmly established in the diagnostic routine, and results are the gold standard for assessment of appropriate antimicrobial chemotherapy in clinical studies. However, this approach has some limitations, which should be acknowledged by clinical users.

From the clinical standpoint, the main limitation is the relatively long time-to-response (TTR), which normally takes 18–24 hours starting from isolated bacterial colonies (which means 248–72 hours from the collection of clinical specimens). This long time frame mandates the commencement of antimicrobial chemotherapy on an empirical basis and allows the shift to definitive therapy usually no sooner than 3–4 days. In the past, attempts have been made to address this limitation by reducing the incubation times before recording results, and/or performing AST directly from positive blood culture vials or from some clinical specimens (eg, bronchoalveolar lavage).2,3 Despite some promising results in terms of TTR reduction and impact on clinical outcomes, the lack of standard procedures and the concerns with reduced accuracy have greatly limited the acceptance of similar approaches in clinical microbiology laboratories.

Another limitation of conventional AST is represented by the poor accuracy of some testing systems with some combinations of antibiotics and pathogens (http://www.eucast.org, Breakpoint v_5.0 and http://www.clsi.org, document M100-025), which may require the use of multiple testing systems and/or the need for confirmatory testing. This issue also affects automated systems and may be particularly problematic when dealing with MDR pathogens (see for instance Arena et al4 and Lat et al4).

A further limitation of conventional AST is represented by the possibility of testing individual drugs but not of antimicrobial combinations. Although the latter is possible by different methods, these are not completely standardized, and clinical predictability has not yet been convincingly demonstrated for any of these methods.

THE FUTURE OF AST

The major efforts at improving the current AST practices are aimed at reducing the TTR and assessing the clinical predictability of results of in vitro testing of antimicrobial combinations.

Concerning TTR reduction, a number of studies and guidelines support the notion that an early and appropriate antimicrobial chemotherapy improves clinical outcomes and may reduce the risk for selection of antibiotic-resistant pathogens, including in pediatric patients.4 Indeed, a significant improvement of clinical outcomes, including in-hospital mortality, length of Intensive Care Unit stay and of mechanical ventilation, number of laboratory tests and antimicrobial consumption, has been demonstrated by studies that have evaluated the clinical impact of a faster AST.5,6

In this perspective, 2 major strategies for TTR reduction in AST have been pursued: (1) development of rapid phenotypic methods for AST, and (2) rapid detection of resistance determinants by molecular methods (molecular AST). Examples of systems for rapid phenotypic AST and for molecular AST are reported in Table, Supplemental Digital Content 1, http://links.lww.com/INF/C201.

Rapid Phenotypic AST

Methods for rapid phenotypic AST are based on the evaluation of bacterial growth curves after exposure to antimicrobial agents, over a significantly shorter period in comparison with conventional AST. MIC values and susceptibility categories are deduced from the growth curves via dedicated algorithms.

The available systems provide results in 3–5 hours. In one such system [ALFRED60 (Alfiox, Polverara, Italy)], the evaluation of growth curves is carried out by continuous recording of the turbidity (based on a light scattering technology) of cultures growing in specially formulated liquid media inoculated with either isolated bacterial colonies or with enrichment media that have been directly seeded with positive blood cultures or other clinical specimens. In another system [ACCELERATE ID/AST (Accelerate Diagnostics, Tucson, AZ)], the evaluation of growth curves is carried out at a single-colony level, by time-lapse automated microscopy, after immobilization of the bacterial cells derived from the clinical specimen into a gel matrix and exposure to different antimicrobial agents. The system is fully automated, and bases its results on separated and immobilized bacterial cells, it also works with polymicrobial specimens.

Both systems have been tested with several pathogens from positive blood cultures and other clinical specimens, and the former system has also been evaluated with pediatric patients (Table, Supplemental Digital Content 1, http://links.lww.com/INF/C201). The latter system has also been tested with MDR strains and was shown to be able to identify the presence of resistance mechanisms (eg, carbapenemase production in Klebsiella pneumoniae and methicillin-resistance in Staphylococcus aureus) on a phenotypic basis (http://acceleratediagnostics.com/our-science/publications/).

Molecular AST

Molecular AST is based on the detection of resistance determinants in bacterial isolates or directly in clinical specimens by molecular methods, whereby the presence of a resistance determinant is considered a proxy for the corresponding resistance phenotype, and the information provide a basis for clinical decisions.7

Molecular detection of resistance determinants is usually performed by polymerase chain reaction on DNA extracted from bacterial colonies or directly from clinical specimens, possibly followed by analysis of amplification products via mini- or micro-arrays, DNA sequence analysis or electro-spray mass spectrometry.

Molecular AST is usually combined with molecular identification of the infecting pathogen, providing information on both aspects, with a TTR that can vary approximately from 1 to 6 hours, depending on the system. A number of such systems are currently available based on different technologies and addressing different combinations of pathogens and resistance determinants from different clinical specimens. Examples of systems for molecular AST validated for in vitro diagnostic use are reported in Table, Supplemental Digital Content 1, http://links.lww.com/INF/C201.

The major advantages offered by molecular AST are represented by: (1) the much shorter TTR in comparison with conventional AST; (2) a higher sensitivity, especially with patients who have already received antimicrobial agents before microbiological testing, a situation particularly common among pediatric patients; (3) the possibility to perform testing directly from clinical specimens; (4) the possibility to perform testing also with polymicrobial samples; (5) the possibility of process automation and (6) a higher reliability in the detection of some resistance phenotypes (eg, detection of mec gene in methicillin resistance in staphylococci based on the detection of the mec gene, which is now considered the gold standard for detection of this resistance phenotype). Major drawbacks of molecular AST are represented by the high costs and by the risk of underestimating resistance. In fact, these systems are only able to detect the resistance genes targeted by the probes and will not detect other resistance determinants. Moreover, using these systems, there is also the possibility of overestimating resistance because the presence of a resistance gene is not necessarily associated with the expression of a resistance phenotype (the gene could be inactivated or not expressed).

Because of these limitations, molecular identification and AST systems are usually regarded as an add-on to the conventional identification and AST procedures, and because of their cost, implementation in the diagnostic

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microbiology workflow requires a correct positioning. In principle, positioning of such systems must consider (1) selection of target patient categories for whom the system is used (the added costs and labor do not allow to use these systems with any type of clinical specimen); (2) the local epidemiology of resistance determinants (the selected system must be able to cover the most prevalent and relevant resistance determinants circulating in the local epidemiological setting); (3) the need to develop antimicrobial stewardship algorithms that account for the results of molecular AST to rapidly revise therapeutic regimens. Concerning patient categories, molecular AST could primarily be recommended for patients in the Intensive Care Unit/Neonatal Intensive Care Unit settings, for patients in onco-hematology wards and for high-risk patients known to be colonized by an MDR pathogen. Concerning antimicrobial stewardship algorithms, the information acquired by molecular AST should prompt for rapid revisions of empiric antimicrobial regimens to improve appropriateness. As an example, we describe one of the algorithms implemented in our antimicrobial stewardship program based on the results of molecular AST of positive blood cultures. All blood cultures positive for Gram-negative bacilli from Intensive Care Unit patients are tested by molecular methods for bacterial identification and for the presence of resistance genes encoding the CTX-M-type extended spectrum β-lactamases and the carbapenemases of the KPC, OXA-48, VIM and NDM types. If molecular testing is positive for *Escherichia coli* (which is the most common cause of bacteremia among Gram-negative bacilli) but negative for the searched resistance genes, the physician is advised that treatment with piperacillin–tazobactam could be appropriate and an empiric treatment commenced with a carbapenem (which is a likely choice in a setting of high extended spectrum β-lactamases endemicity) could be rapidly downgraded. This strategy is relevant for carbapenem-sparing policies, also considering the recent clearance of meropenem for treatment of complicated intra-abdominal infections in children younger than 3 months (http://www.fdanews.com/articles/171393-fda-gives-meropenem-a-new-pediatric-indication?view=preview), which is expected to increase the use of carbapenems in this pediatric population.

**CONCLUDING REMARKS AND PRACTICAL RECOMMENDATIONS**

AST data remain an essential support to the selection of antimicrobial chemotheraphy of bacterial infections. Although assessment of clinical predictability of testing antimicrobial combinations awaits the results of ad hoc clinical studies, recent technological advances may allow a significant reduction of the TTR of AST, and there is an increasing demand for the implementation of these technologies also in the pediatric setting. In fact, because of the relative immaturity of the immune system and the possibility of rapid progression of bacterial infections, the use of rapid AST systems is expected to be of particular interest in this setting.

To maximize efficiency, positioning of rapid AST technologies in the diagnostic workflow must be discussed by clinical microbiologists together with clinical users and the Antimicrobial-Stewardship-Team. Studies designed to quantify the impact of these technologies in clinical and economic outcomes, and to assess their best positioning in the diagnostic workflow (diagnostic stewardship), are still very limited and will be needed. With pediatric patients, in particular, published studies were mostly designed to assess the reliability of rapid AST methods with positive blood cultures, and prospective studies focused on the clinical usefulness of these methods in the pediatric setting are needed.

**REFERENCES**


