The Changing Role of the Clinical Microbiology Laboratory in Defining Resistance in Gram-negatives

Andrea Endimiani, MD, PhD[a,*], Michael R. Jacobs, MD, PhD[b]

KEYWORDS

• AST • PCR • Real time • LAMP • Microarray • MALDI-TOF • Sequencing • Rapid

KEY POINTS

- Antimicrobial resistance in Gram-negatives is a major challenge.
- Multidrug-resistant strains, including carbapenem-resistant strains, are increasing.
- More rapid methods are needed.
- Considerable advances have been made with rapid genotypic methods.
- Advances have also been made with rapid phenotypic methods.

INTRODUCTION

The role of the clinical microbiology laboratory in defining resistance in Gram-negatives has been challenged by the evolution of resistance to antimicrobial agents because we are no longer able to rely on the efficacy of the empiric use of "broad-spectrum" agents. In particular, development and spread of extended-spectrum β-lactamases (ESBLs; eg, CTX-Ms) and carbapenemases have presented major challenges. The mutation of narrow-spectrum β-lactamases (which degrade penicillins) into ESBLs (which add cephalosporins and monobactams to their spectrum) has limited the activity of advanced generation cephalosporins. Acquisition of carbapenemases such as KPC, NDM, IMP, and VIM results in resistance to virtually all available β-lactams in common use. Moreover, these strains are frequently resistant to many other drug classes, rendering them resistant to typical empiric therapy combinations.[1–3]

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[a] Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Friedbühlstrasse 51, Bern CH-3010, Switzerland; [b] Department of Pathology, University Hospitals Case Medical Center, Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106, USA
* Corresponding author.
E-mail addresses: andrea.endimiani@ifik.unibe.ch; aendimiani@gmail.com

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These challenges have led to the realization of the need for more rapid diagnosis, particularly of bloodstream infections, and for more rapid antimicrobial susceptibility testing (AST). A mean decrease in survival of 7.6% for each hour after onset of infection until effective antibiotics are administered has been reported, as well as a 5-fold increase in mortality when inappropriate antimicrobials were administered within 6 hours after recognition of septic shock.[4] Recent studies have also documented the value of more rapid diagnosis, which allows earlier appropriate, targeted antimicrobial use.[5] This has been shown to improve patient outcomes, lower mortality, decrease hospital length of stay, lower superinfection and adverse drug reaction rates, and decrease costs.

Although the rapid detection of bacteria and their resistance mechanisms directly from blood specimens is still an elusive target, this has been achieved on growing blood cultures, which typically become positive after 18 to 24 hours of incubation. Many systems for rapid bacterial identification from growing blood cultures have been developed, such as fluorescence in situ hybridization (FISH) tests, mass spectroscopy (MS), and automated polymerase chain reaction (PCR) systems.[6] Many of these systems can also detect antimicrobial resistance genes. For instance, a recent study of an automated molecular system documented the value of one such system, the Verigene Gram-negative blood culture nucleic acid test (BC-GN; Nanosphere), a multiplex, automated test for the identification of 8 Gram-negative organisms and 6 resistance markers from blood cultures with a turnaround time (TAT) of approximately 2 hours. The test correctly identified 95.6% of isolates and detected CTX-M and OXA resistance determinants, with an intervention group having a significantly shorter duration to both effective (3.3 vs 7.0 h; $P<.01$) and optimal (23.5 vs 41.8 h; $P<.01$) antibiotic therapy.[7]

AVAILABLE METHODS

Standard Antimicrobial Susceptibility Test Methods

Conventional AST procedures have been in use for many decades and follow methods and interpretations of various organizations such as European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute,[8,9] as well as regulatory agencies such as US Food and Drug Administration and European Agency for the Evaluation of Medicinal Products. These organizations and agencies have established "reference" AST methods based on minimum inhibitory concentration (MIC) determination by microdilution and agar dilution, with incubation times ranging from 18 to 48 hours. Disk diffusion methods have also been standardized by these groups.

Many commercial methods for AST are available and are based on using these reference methods directly, or by methods correlated to reference methods and providing comparable results. Commercial methods using reference microdilution methods include MicroScan WalkAway (Siemens Healthcare Diagnostics, Erlangen, Germany) and Sensititre (Trek Diagnostic Systems, Independence, OH). Methods providing results comparable with reference testing include gradient diffusion MIC determination (Etest), Vitek (bioMérieux, Marcy l'Etoile, France), Phoenix (BD Diagnostic Systems, Franklin Lake, NJ), as well as rapid versions of MicroScan and Sensititre. Several of the methods have faster TAT than reference methods, and many are automated with machine-generated results. Instruments that record and interpret disk diffusion zone are also available (eg, BIOMIC V3, Giles Scientific, Santa Barbara, CA; ADAGIO, Bio-Rad, Hercules, CA; Scan 1200, Interscience, Boston MA; SirSCAN, i2a Diagnostics, Montpellier Cedex 2, France).

These reference AST methods also include methods for determination of resistance mechanisms, such as the presence of ESBLs in some *Enterobacteriaceae* using