# New Technologies for the Rapid Identification of Drug-Resistant Bacteria

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## ABSTRACT

The extensive use of antibiotics to treat infections, without first specifically identifying the underlying cause, has resulted in a strong natural selection for antibiotic resistant organisms. Conservative estimates from the CDC indicate that more than two million people are sickened by antibiotic-resistant infections in the US each year, leading to more than 23,000 deaths. Because it takes days by traditional methods to identify the etiological organism and its drug-susceptibility profile, up to 50% of all antibiotics prescribed are not needed or are not optimally effective. This significant threat to human health is starting to be addressed technologically through the development and commercialization of novel rapid diagnostics. The approaches include phenotypic and genotypic methodologies that target a variety of direct and indirect biomarkers, including protein, lipid, and cell-based phenotypic assays as well as molecular diagnostic approaches. One of the key area of innovation is the development and integration of appropriate and easy-to-use sample preparation that is tailored to the target analyte, the specimen, and the diagnostic modality. A survey of key innovations in the field will be presented.

***Keywords***: microfluidics, antibiotic susceptibility, phenotypic testing, diagnostics

## 1 INTRODUCTION

Antimicrobial resistance (AMR) is a serious and growing problem that impacts the world's healthcare, veterinary and agricultural industries. In the United States alone, antibiotic-resistant bacteria infect more than 2 million people and cause over 23,000 deaths.[1] The continued spread of AMR is a multi-factorial problem, including inappropriate prescription practices, lack of antimicrobial stewardship programs, insufficient patient education, non-human use of antimicrobials and limitations of existing diagnostics.[2] The criticality of administering appropriate antibiotics has been studied in the case of septic shock, where every hour of delay is associated with an average decrease in survival of 7.6%.[3] However, the standard methods for antibiotic susceptibility determination require multiple days and therefore physicians are left with no choice but to prescribe antibiotics in the absence of this information, which further contributes to proliferation of AMR.

To address this threat, the U.S. government developed a five-year National Action Plan in 2015 for Combating Antibiotic Resistant Bacteria, which specifically included a goal to "Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria".[4] Several other organizations have also recognized the need for improved antibiotic susceptibility testing (AST), and introduced prize-based incentive programs including the Longitude Prize (UK) [5] and the Point-of-Care Diagnostic Challenge Prize (USA) [6]. These incentives intended to hasten the development of novel rapid diagnostics, although significant challenges have yet to be overcome to meet the clinical need.

## 2 STATE-OF-THE-ART DIAGNOSTICS

Traditionally, diagnosis and determination of antibiotic susceptibility has been based on culture methods. Typically, a specimen is taken from the patient and cultured to grow the pathogens to a detectable level and to isolate pure colonies. Once isolated, a second culture step (e.g. disk diffusion, broth dilution or E-test)[7], develops an overall profile of pathogen antibiotic susceptibility, known as an antibiogram. Generation of the antibiogram typically takes days, and, while the methods are accurate and inexpensive, they require strict adherence to standardized protocols.[8] Commercial automated systems are widely used in larger clinical labs (e.g. BD Phoenix, Beckman Coulter MicroScan), which decrease the operator time and overall turn-around-time, but also increase the cost of the analysis.

Recently, several new rapid diagnostics have entered the marketplace and are gaining traction in clinical microbiology laboratories. In 2007, the Cepheid Xpert MRSA test became one of the first rapid fully automated diagnostics for detection of antibiotic resistant organisms cleared by the FDA.[9] The test is PCR-based and detects both bacteria-specific and methicillin-resistance genetic markers. While useful for screening for outbreaks, the utility of this test is quite limited as it only detects a single bacterial species and resistance marker. More recent entries to the market, such as the Biomerieux BioFire® FilmArray® and Accelerate Pheno, have broader panels. The BioFire® FilmArray®, based on a multiplexed PCR technology, has FDA cleared panels for respiratory (RP2, 2017), blood culture ID (2016), gastrointestinal (2016), meningitis/encephalitis (2016), and pneumonia (2018) pathogens and resistance markers.[10, 11] While these molecular diagnostic panels are useful, they remain more limited than phenotypic tests because genetic

markers have not been identified for all bacterial strains, susceptibility is not always correlated with genetic markers, the rapid evolution of bacteria can lead to false negatives when mutations arise in the target region, and they cannot provide minimum inhibitory concentration information.[8] In contrast, phenotypic tests tend to provide results that are more robust in that the response to the antibiotics is directly observed. In 2017, the FDA granted the de novo request (DEN160032) of Accelerate Diagnostics to market their pathogen identification and phenotypic AST directly from positive blood culture tests.[12] This test has combined qualitative nucleic acid fluorescence in situ hybridization (FISH) identification of 29 species and quantitative AST that yields MICs. After the initial blood culture (8-24 hrs.), the PhenoTest™ provides average identification times in less than 90 minutes and AST results in less than 7 hours.[13] While this is a significant improvement over the traditional methods, other technologies in development hold the promise of even shorter turn-around-times.

# 3 DEVELOPMENT OF NEW RAPID DIAGNOSTICS AND THE IMPACT OF MICROFLUIDCS

A number of key challenges in reducing the time to results exist, including fast, integrated, and customized sample preparation, rapid bacterial identification to select the appropriate antibiotics to test, and the development of phenotypic AST that circumvents the need for long culture times. Several groups are leveraging the advantages of microfluidics and miniaturized volumes to address these technological hurdles.

## 3.1 Sample preparation

The sample preparation method must be developed in light of the concentration of the target analyte in the specimen, the lower limit of detection of the sensing technology, and the sample matrix. Two common applications rapid diagnostics developers are targeting are urinary tract infections (UTI) and blood-stream infections (BSI). In UTIs, the threshold for diagnosing a clinically significant concentration of bacteria in urine is typically set at $> 10^5$ colony forming units per milliliter (CFU/mL), but some can be as low as $10^3$ CFU/mL.[14] In BSIs, the bacterial concentration is extremely low, in the range of 1-100 CFU/mL.[15] Traditional culture based diagnostics have long turnaround times because the bacterial concentration in the specimen is much lower than the limit of detection (visual inspection or optical density measurements), and therefore the methods must incorporate sufficient time for the bacteria to multiply in culture to a high enough concentration to detect. Thus, detection methods that have lower limits of detection can reduce or eliminate the requirement for culture. Methodologies must also be able to address any matrix effects that can introduce bias to the assay by either impeding detection or causing false positives. For example, PCR is known to be highly sensitive to inhibition by blood.[16] Furthermore, other pre-analytical factors, including leachates from containers can also interfere with downstream assays.[17] Thus, integrated and optimized sample preparation is an important determinant in reducing the total assay time, while maintaining high diagnostic accuracy.

## 3.2 Bacterial identification

An often overlooked factor to consider in the development of rapid AST technologies is the decision process of which antibiotics to test against the isolated pathogen. In the standard clinical workflow, bacteria are isolated from the matrix, identified, and then specific choices are made as to which antibiotics should be tested. Several factors go into this decision making process, including the mechanism of action and effectiveness of a given antibiotic, the availability of certain antibiotics at the hospital, known resistance patterns, and guidelines from the institution's antibiotic stewardship program. These traditional methodologies allow for nearly complete freedom in this antibiotic selection process, however, automating and integrating sample-to-answer technologies may necessitate a narrowing of the choices to a specific set. For example, in Accelerate Diagnostic's PhenoTest™ BC Kit, only certain antibiotics are tested based on the bacterial species identified [18] (Table 1).

## 3.3 Microfluidic Phenotypic AST

Several innovative approaches have been published describing methods to reduce or eliminate the need for culturing bacteria in the presence of antibiotics while still providing a phenotypic assessment of the antibiotic susceptibility. One approach is to minimize the culture interrogation area such that a susceptibility can be detected on the single cell divisions and/or morphological changes. These approaches include microfluidic devices that trap cells into compartments [19], isolate bacteria into droplets [20], or immobilize them in microwells [21] and morphological changes are assessed by microscopy. Other microfluidic techniques measure biochemical response to the antibiotics, which allows a phenotypic readout without requiring cell division.[22-25] Changes in the nanomechanical movements of bacteria have also been explored as a signature for antibiotic susceptibility.[26, 27] These technologies and others like them have the potential to eliminate the need for culture-based AST if they can properly be integrated with optimized sample preparation and bacterial identification technologies.

Table 1: Antibiotic – bacteria pairings in Accelerate Diagnostic's PhenoTest™ BC Kit [18]. Gray columns indicate Gram positive bacteria and white columns indicate Gram negative bacteria.

| | *S. aureus* | *S. lugdunensis* | CNS spp. | *E. faecalis* | *E. faecium* | *E. coli.* | *Klebsiella* spp. | *Enterobacter* spp. | *Proteus* spp. | *Citrobacter* spp. | *S. marcescens* | *P. aeruginosa* | *A. baumannii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ampicillin | | | | * | * | | | | | | | | |
| Ceftaroline | * | | | | | | | | | | | | |
| Erythromycin | * | | | | | | | | | | | | |
| Daptomycin | * | | * | * | * | | | | | | | | |
| Linezolid | * | | | * | * | | | | | | | | |
| Vancomycin | * | * | * | * | * | | | | | | | | |
| Ampicillin-Sulbactam | | | | | | * | * | | * | | | | |
| Piperacillin-Tazobactam | | | | | | * | * | * | * | * | * | * | * |
| Cefepime | | | | | | * | * | * | * | * | * | * | |
| Ceftazidime | | | | | | * | * | * | * | * | * | * | |
| Ceftriaxone | | | | | | * | * | * | * | * | * | | |
| Ertapenem | | | | | | * | * | * | * | * | * | | |
| Meropenem | | | | | | * | * | * | * | * | * | * | |
| Amikacin | | | | | | * | * | * | * | * | * | * | * |
| Gentamicin | | | | | | * | * | * | * | * | * | * | |
| Tobramycin | | | | | | * | * | * | * | * | * | * | |
| Ciprofloxacin | | | | | | * | * | * | * | * | * | * | |
| Aztreonam | | | | | | * | * | * | * | * | * | | |

## 4 CONCLUSIONS

The ideal AST would be a sample-to-answer assay that provided actionable results to the physicians and enable optimized antibiotic therapies within an hour. While such a technology is not yet commercially available, advances in sample preparation, rapid bacterial identification and phenotypic testing that do not require culture are bringing this goal within reach. Several systems appear to be poised to meet the clinical needs, including the finalists in the AMR challenge (Affinity Biosensors, Klaris Diagnostics, GeneFluidics, Click Diagnostics, and Predigen, Inc.)[6]. It seems only a matter of time before these truly rapid AST technologies are available to make a major impact on clinical practice.

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