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Current state of the art in rapid diagnostics for antimicrobial resistance

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Abstract

Antimicrobial resistance (AMR) is a fundamental global concern analogous to climate change threatening both public health and global development progress. Infections caused by antimicrobial-resistant pathogens pose serious threats to healthcare and human capital. If the increasing rate of AMR is left uncontrolled, it is estimated that it will lead to 10 million deaths annually by 2050. This global epidemic of AMR necessitates radical interdisciplinary solutions to better detect antimicrobial susceptibility and manage infections. Rapid diagnostics that can identify antimicrobial-resistant pathogens to assist clinicians and health workers in initiating appropriate treatment are critical for antimicrobial stewardship. In this review, we summarize different technologies applied for the development of rapid diagnostics for AMR and antimicrobial susceptibility testing (AST). We briefly describe the single-cell technologies that were developed to hasten the AST of infectious pathogens. Then, the different types of genotypic and phenotypic techniques and the commercially available rapid diagnostics for AMR are discussed in detail. We conclude by addressing the potential of current rapid diagnostic systems being developed as point-of-care (POC) diagnostic tools and the challenges to adapt them at the POC level. Overall, this review provides an insight into the current status of rapid and POC diagnostic systems for AMR.

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Conflicts of interest

There are no conflicts of interest to declare.

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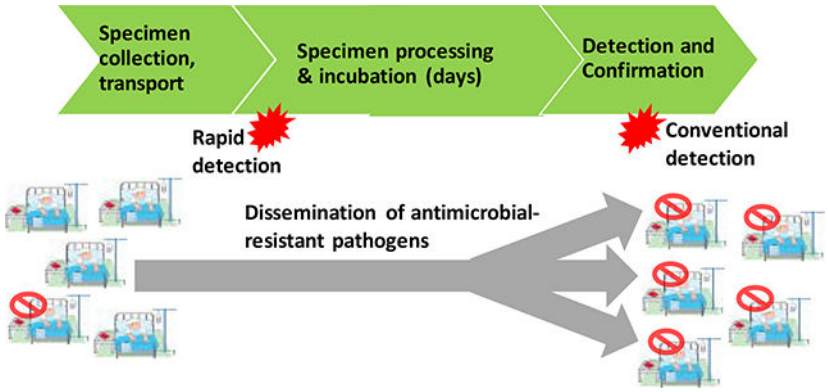

This review provides an insight into current rapid diagnostics for antimicrobial resistance, their technical aspects and benefits/limitations on their application.

1. Introduction

Since the discovery of penicillin, multiple antimicrobials have been developed for various pathogens, and these have been part of some of the major clinical and public health success stories of the last century.[1, 2] However, after the introduction new antimicrobial, resistant pathogens often emerge within a few years.[3, 4] Antimicrobial resistance (AMR) can be defined as a characteristic of any infectious pathogen that shows resistance to one or more antimicrobials, which were successfully used earlier to treat the infection. Causal and contributing factors for the emergence of AMR in pathogens towards one or more antimicrobials include extensive agricultural use, limited availability of new antimicrobials, and inappropriate prescription of antibiotics for humans, livestock, and poultry.[4–8] The spread of antimicrobial-resistant pathogens is facilitated by both environmental and healthcare factors.[9–11] The rapid dissemination of antimicrobial-resistant pathogens has adverse economic and health effects in both developed and developing nations.[12–14]

Recent estimates predict that if the current trend of increase in AMR is not controlled efficiently by 2050, it will cause 10 million deaths annually and there will be a reduction of 2–3.5% in the world's gross domestic product (GDP).[15] The United States National Strategy for Combating Antibiotic Resistance outlines several goals for addressing the issue of antimicrobial resistance, all of which essentially come down to a dual strategy of (1) slowing the emergence of antimicrobial resistance through **smarter use**; and (2) accelerating the development of **new antimicrobials**. Critical to the first of these strategies is the development of rapid "point-of-care" (POC) diagnostics that can detect antimicrobial-resistant pathogens, ensuring that the patient receives efficacious treatment, so that such targeted treatment results in a significant reduction in unnecessary use of broad-spectrum antimicrobials. POC testing is medical testing performed outside of a laboratory setting to provide accurate diagnostic results in real-time, typically within minutes at the site where the patient care is provided.[16] POC testing expedites the triage assignment at the earliest, thereby speedily initiating efficient treatment for patients.[17]

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The need for such diagnostics is further emphasized by the relatively slow development of new antimicrobials and the fact that the rate at which new antimicrobials are developed and made available to clinicians has not met the demand to efficiently combat AMR.[18–21] If the current situation prevails without any undertaking of preventive measures against the emergence and spread of AMR, it could lead to a post-antibiotic era.[21–23] In clinical laboratories, diagnosis of antimicrobial-resistant pathogens, and their antibiogram using antimicrobial susceptibility testing (AST) methods, play a key role in initiating appropriate treatment/infection control measures.[24, 25] AST is very essential to determine the right antimicrobial along with its optimal dosing regimen to be prescribed for infected patients. Due to the increasing rate of infections with antimicrobial-resistant pathogens, it becomes a predominant step in clinical laboratories to detect the antimicrobial-resistant pathogens. This detection of AMR is critical to control the dissemination of antimicrobial-resistant pathogens in the hospital and community. Since the inception of AMR, several techniques have been reported to identify antimicrobial-resistant pathogens and perform AST to determine their resistance profile.[26, 27] Improving those AMR detections and AST to be utilized at the POC will be valuable for tackling the AMR crisis. Since antimicrobial-resistant pathogens are known for the high mortality rate of infected patients, POC detection of AMR is crucial to control their hasty dissemination.[28, 29]

Even though a wide range of new and advanced technologies have been developed for their potential utility in controlling AMR, there still remains a gap in detection techniques for one that possesses all the characteristics of a robust detection system.[30, 31] Therefore, a comprehensive study of the challenges involved in the development and implementation of AMR diagnostics will be informative for enhancing the efforts to control AMR. In this review, we briefly describe the history of AMR diagnostics and their current status in clinical settings. Then, we describe about the rapid AST techniques comprising the automated and single-cell-based AST systems and their technical aspects along with their advantages and disadvantages. Next, we emphasize the diverse genotypic (PCR-based and isothermal amplification) and phenotypic technologies applicable for the rapid detection of AMR and their utility as POC diagnostics. Furthermore, the application of microfluidics for rapid AMR detection is explained elaborately. Finally, we discuss the recently introduced and commercially available rapid diagnostics and the essential requirements to improve and implement them at POC.

2. History and development of AMR diagnostics

Along with the discovery of antimicrobials and emergence of their respective resistant pathogens, diagnostics for AMR also evolved in the 20th century. Initially, the diagnostics began with the development of phenotypic AST methods which is used to determine the minimal inhibitory concentration (MIC), i.e., the lowest concentration of antimicrobial at which no visible growth of the organisms can be observed.[32] The very first AST method was the ditch plate technique introduced by Sir Alexander Fleming in 1924.[33, 34] In the ditch plate technique, a ditch is created in a solidified agar medium and filled with the antimicrobial mixed agar medium. After solidification, the ditch plate is inoculated with the bacterial culture and incubated to check the zone of inhibition for the determination of their antimicrobial susceptibility.[35] The ditch plate technique requires good handling expertise as

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it requires much handling of liquids and procedures to avoid contamination. Another AST method, the broth dilution method involves the preparation of antimicrobials at different concentrations by serial dilution and then, inoculation of them with liquid culture at 0.5 McFarland Standard. Following incubation, the turbidity of the broth is used to determine the MIC.[36] In the agar dilution AST method, different concentrations of serially-diluted antimicrobial were mixed with agar medium and solidified in petri dishes. Then, the bacterial culture at 0.5 McFarland Standard is spread over the antimicrobial-containing agar plates and incubated. The MIC of the antimicrobial is found from the plate that showed no growth of bacterial colonies.[37] Although the broth dilution and agar dilution methods are very laborious for testing several bacterial cultures for different antimicrobials simultaneously, they are still used with a broad range of modifications.

The Oxford cup assay also known as the penicillin cylinder method, is another AST method inwhich the bacterial culture is spread over the agar medium and small cylinders are placed over it. Different concentrations of the antimicrobial are poured into the respective cylinders and incubated. The antimicrobials diffuse through the agar and depending on the inhibition zones formed, the susceptibility of test pathogen is decided.[34, 38] Though it is simple to perform the Oxford cup assay, it requires extra care to avoid contamination due to the usage of cylinders over the agar medium. The disk diffusion method uses a filter paper disk containing the antimicrobial at a known concentration. The bacterial inoculum is spread over the agar medium and the antimicrobial disk is placed over it followed by incubation. Then, the antimicrobial in the disk diffuses through the agar medium and the diameter of the zone of inhibition is measured to determine the susceptibility (sensitive, intermediate, resistant).[39] Disk diffusion is extensively used in clinical laboratories due to its ease of use. The gradient diffusion method applies a thin strip carrying a gradient of antimicrobial concentration from one end to the other. When this strip is placed over the agar plate containing the inoculum and incubated, an elliptical zone of inhibition is formed with the smaller part of the ellipse showing the MIC. Compared to the broth dilution and agar dilution techniques, the gradient diffusion is very simple and less laborious to perform and interpret the results.[40] Thus, from the ditch plate technique, the AST methods gradually evolved/modified from the ditch plate to the current automated systems (Fig. 1). In 1968, the National Committee for Clinical Laboratory Standards (NCCLS) now known as the Clinical and Laboratory Standards Institute (CLSI) was formed and a consensus standard, guidelines and best practices for AST in clinical laboratories were established.[41] In 1997, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was formed by the national agencies in Europe which provides a defined set of AST breakpoint values and guidelines.[42]

With the advent of the polymerase chain reaction (PCR) method, genotypic or molecular methods became indispensable for epidemiological surveillance and outbreak analysis of AMR.[43, 44] Continuing with the application of PCR, different types of nucleic acid amplification tests (NAATs) such as isothermal amplification were employed for the diagnosis of AMR towards one or multiple antimicrobials (described in detail in sections 4.1 and 5.1).[45–48] Furthermore, whole genome sequencing (WGS) aids in the detailed understanding of resistance to all known antimicrobials, new resistance mechanisms, transmission modes, and molecular epidemiology of antimicrobial-resistant pathogens.[49, 50] WGS is also used to predict the AST of pathogens towards different antimicrobials using

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bioinformatic tools; however, the accuracy when compared with the conventional phenotypic AST approach, needs to be improved.[51, 52] Though more rapid than the conventional method, the cost and expertise required for WGS are the biggest limiting factors for its implementation in clinical laboratories. In addition to these genotypic tests, phenotypic tests like enzymatic activity-based assays (e.g. Carba NP test, modified carbapenem inactivation method (mCIM), EDTA-mCIM (eCIM)), and bacterial growth-based assays (e.g. adenylate kinase (AK) bioluminescence assay) and immunoassays (e.g. lateral flow immunochromatography and latex agglutination), also have aided in the detection of resistance of pathogens towards specific antimicrobials in the past two decades (described in detail in sections 4.2 and 5.2).[53–58] Thus, a range of phenotypic and genotypic techniques were developed and applied for controlling the AMR. Figure 1 shows the different diagnostic platforms along with the timelines when they were developed or modified for AMR.

3. Rapid phenotypic AST systems

AST is performed in a clinical laboratory to determine the minimal inhibitory concentration value of different antimicrobials toward infectious agents. AST plays a key role in providing appropriate antimicrobial dosage regimens for the respective patients.[59, 60] Conventional manual AST methods are highly time-consuming, and labor-intensive, and there are possibilities for human errors during antimicrobial solution preparation as in the case of macrodilution method.[61] To reduce the time for AST result, a few of the already existing technologies were being exploited for the rapid performance of AST. For instance, apart from the utility of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology for species identification, it is also used to determine the antimicrobial susceptibility of pathogens. In the MALDI Biotyper-Selective Testing of Antibiotic Resistance-Beta-Lactamase (MBT-STAR-BL) Assay, the resistance profile of the bacteria that exhibit resistance specifically to β-lactam antibiotics with its β-lactamase activity is determined. Here, the bacterial culture is incubated with a β-lactam antibiotic and the cell-free supernatant is spotted on the MALDI plate. Then, the spectral mass shifts of the β-lactam antibiotic are analyzed automatically in the MBT-STAR-BL software module to calculate the amount of hydrolyzed β-lactam and the resistance profile is displayed for easier evaluation in a turnaround time (TAT) of 30–180 min.[62] Furthermore, the MBT-STAR-BL assay has been reported to be applicable for both pure cultured isolates and positive blood cultures.[63] MALDI Biotyper-Antibiotic Susceptibility Test Rapid Assay (MBT-ASTRA) is another application of MALDI-TOF MS to determine the susceptibility of isolated pathogens and those in positive blood cultures based on their relative growth in the presence and absence of the test antimicrobial.[62, 64, 65] After incubation with the antimicrobial, the bacterial cells are lysed and the lysate is spotted on the MALDI plate. The resulting spectra are analyzed with the web-based prototype, MBT-ASTRA where the intensity of the peaks with respect to the bacterial proteins are used to determine the susceptibility of the bacteria. The TAT to determine the resistance profile of bacteria towards any antimicrobial using the MBT-ASTRA is ~ 2 h; however, a major limitation is that it requires the culture medium be devoid of the respective amino acid that is used as a heavy marker in the analysis.[66] The identification of the pathogen and its susceptibility towards

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antimicrobials on the same day with the MALDI-based AST systems would be much more beneficial for clinical laboratories if the accuracy could be improved to a 100% match with conventional methods. For the simultaneous examination of AST for several bacterial isolates for different antimicrobials with reduced labor and minimal errors, the automated AST systems were developed and implemented at clinical laboratories (described in detail in section 3.1). Likewise, single-cell-based microfluidic AST systems with significantly less TAT than the currently available techniques were being continuously developed and reported (described in detail in section 3.2).

3.1. Automated AST systems

To reduce the labor and time required for the examination of AST of pathogens for multiple antimicrobials simultaneously, automated AST systems have been employed extensively.[61, 67] The Food and Drug Administration (FDA)-approved automated AST systems - MicroScan WalkAway (Siemens Healthcare Diagnostics, USA), BD Phoenix Automated Microbiology System (BD Diagnostics, USA), Vitek 2 System (bioMérieux, France) and Sensititre ARIS 2X (Trek Diagnostic Systems, USA) possessing different antimicrobial panels for Gram-positive and Gram-negative pathogens play a crucial role in clinical laboratories.[61] The Microscan WalkAway is an incubator-coupled reader system that can continuously monitor the result through a photometer or fluorometer. It can examine 40–96 microdilution trays, containing known concentrations of antimicrobials manually inoculated with the bacterial culture. The MIC results can be obtained in as little as 7–18 hours depending on the Gram-negative or Gram-positive pathogens.[61] The BD Phoenix Automated Microbiology System can analyze 99 test panels containing 84 wells of antimicrobial dilutions in which the growth is monitored by both the turbidometer and calorimeter. Furthermore, the BD Phoenix Automated Microbiology System can provide the MIC for a range of pathogens viz. Gram-negative, Gram-positive, *Streptococcus pneumoniae*, β-hemolytic, and viridans in 6–16 h.[61] The Vitek 2 System is an improved automated platform of miniaturized reagent cards with antimicrobials and test media in 64 wells. It can perform 30–240 tests simultaneously in 4–10 h for Gram-negative, Gram-positive, and *S. pneumoniae*.[61] The Sensititre ARIS 2X is a fluorescence-based automated system that requires 18–24 h of incubation to measure the growth for MIC determination. However, compared to the previously mentioned systems, the Sensititre ARIS 2X has an autoinoculator that further reduces human error. The Sensititre ARIS 2X can be utilized for Gram-negative, Gram-positive, *S. pneumoniae*, *Haemophilus* species, and non-fermentative Gram-negative bacilli.[61] In various reports, these automated AST systems were also shown to be applied directly for positive blood cultures.[68–71]

The Accelerate PhenoTest™ BC kit coupled with the Accelerate Pheno™ system (Accelerate Diagnostics, Inc., USA) is an FDA-approved automated system that can perform both pathogen identification and its AST directly from positive blood cultures. This system can automatically clean the sample and immobilize the organism for identification and AST. As this system can perform sample preparation, and provides the AST result in ~7 h, it is a valuable automated system for positive blood cultures.[72, 73] LifeScale (Affinity Biosensors, USA) which is yet to be commercialized employs micro-electromechanical systems (MEMS) technology for the detection of growth as an interpretation for MIC. It applies

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resonant frequency measurement of microbial biomass and the number of cells in a specific volume inside a microfluidic channel for the automated AST of Gram-negative rods directly from urine and positive blood cultures in 3–3.5 h.[59, 74] Since the LifeScale can perform AST directly on blood cultures and urine in a very short time, its development into a commercial product for a broad range of pathogens would be valuable. BacterioScan™216R (BacterioScan Inc., USA), an automated platform that is in the pipeline for FDA approval utilizes laser scattering technology along with statistical real-time analysis for AST of pathogens. It is based on the quantitative estimation of bacterial concentration by measuring the light scattered by the bacteria. The continuous measurement over time coupled with proprietary calculation algorithms offers an accurate estimation of even lower microbial concentrations. As the BacterioScan™216R system provides AST results within 6 h, it would be more attractive if it could be customized for direct clinical specimens.[75, 76] Thus, the automated systems greatly reduce the amount of labor needed to perform AST within a short period compared with conventional methods. Much effort has been undertaken on different fronts to develop a device that could perform AST in a much lower TAT along with a reduced cost than the current AST systems.

3.2. Singe-cell-based microfluidic AST systems

The application of single-cell technologies for AST took the development of rapid AST systems to the next level with a reduced TAT.[77] Various reports have noted the reliability of nanotechnology and microfabrication coupled with imaging algorithms for the development of rapid phenotypic AST methodologies using single cells.[78–81] Microfluidics technology enables the performance of AST on a single cell by confining it into a micrometer scale environment.[82–84] In the single-cell-based AST, the cell division is observed earlier than conventional methods at the single-cell level, thereby developing rapid AST systems. Different parameters such as biomass, morphological changes, viability, growth rate, metabolites, etc. of the single cell were used to determine the MIC using different technologies.[78–84] This section elaborates the different technologies applied with microfluidics for the development of rapid AST systems based on single-cell analysis. Recently, several studies have illustrated the capability of single-cell AST within a few hours through direct and indirect monitoring of cell growth.[78, 80, 82, 84, 85] Baltekin *et al.* developed a custom-designed microfluidic chip to capture bacterial cells and monitor their growth rates using microscopy.[78] They demonstrated the chip's ability to detect changes in growth rate in response to each of nine antimicrobials used to treat urinary tract infections with a TAT of approximately 30 min. The captured images were processed and analyzed using an algorithm developed in MATLAB to determine the susceptibility of the pathogen. In conjugation with a microfluidic channel, a nanotube assisted microwave electroporation (NAME) technology was deployed for the single-cell AST by Gao *et al.*[80] NAME applies the intracellular injection of a fluorescent double-stranded nucleic acid probes to target the specific DNA region in a single cell. When the fluorescent probe binds to the target region in the bacteria it emits fluorescence. The AST result of the test pathogen is determined in ~ 90 min based on the fluorescence emitted. Since only a few specific species were examined, a study on a wide range of pathogens evaluated with NAME technology is warranted to implement its use in clinical laboratories.

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A miniaturized microfluidic device was developed to generate a serial dilution of antimicrobials similar to the conventional AST.[81] By combining the concentration gradient generator with the cell culture chambers, this miniaturized device allows for the continuous monitoring of the response of *Escherichia coli* cells to the antimicrobial administered. Images of the bacteria responding to the antimicrobial were captured with an electron-multiplying charge-coupled device and processed using the *ImageJ* software. The major advantages of this miniature device include the fact that it is inexpensive, easy to fabricate with polydimethylsiloxane and a TAT of ~3 h. However, no data were available to substantiate its applicability for clinical isolates. Single Cell Morphological Analysis (SCMA) is a microfluidic chip-based method in which the bacteria are mixed with agarose and immobilized inside the microfluidic chip.[82] Then, the bacterial cells are supplied with the liquid medium containing antimicrobial through the microfluidic channel. Unlike other single-cell AST techniques, SCMA determines the morphological changes in the bacterial cell by capturing the images with an optical microscope. The images are processed using MATLAB R2013a and AST results are provided in ~ 4h. Although the results of SCMA are shown to be in 91% agreement with broth microdilution, it requires a huge expertise for image analysis and data interpretation. Kaushik *et al.* developed a droplet-based Fluorescent Antimicrobial Susceptibility Test (dropFAST) in which a single bacterial cell is encapsulated in a picoliter-sized droplet and its growth is measured by a fluorescence-based assay.[83] The bacterial cell is grown in the presence of a culture medium mixed with an antimicrobial of known concentration and resazurin dye. The droplet is continuously excited with a laser source and the emission is detected using a silicon avalanche photodiode detector followed by data analysis using a customized LabVIEW program. The dropFAST has been shown to perform the AST for *E. coli* in ~1 h; however, several modifications including the ability to test multiple bacteria/antimicrobials in a single reaction and automated sample-to-answer workflow is warranted.

A microfluidic-based technique monitoring the cell division and viability of a single bacterial cell for AST was evaluated by Peitz *et al.*[84] Here the bacterial suspension along with the antimicrobial is injected into the microfluidic channel mounted on the microscopic stage and incubated. The signal from a function generator connected with the electrode arrays in the microfluidic chip is analyzed using the LabVIEW software. Even though this approach can provide the AST in ~ 5 h, an automated system for data analysis and result interpretation would make this approach much simpler. A single-cell AST method was examined with *E. coli* using confined microchannels coupled with electrokinetic loading.[85] Here the *E. coli* cells mixed with culture medium containing the antimicrobial are confined into a gas-permeable microchannel in which the microelectrodes position the bacteria with dielectrophoresis for monitoring the growth of a single cell. The microfluidic device is coupled with an inverted fluorescent microscope to determine the AST results within 1 h. In another method, a drug susceptibility testing microfluidic (DSTM) device, consisting of an array of microfluidic channels fabricated by soft lithography, can run AST for *Pseudomonas aeruginosa* within 3 h.[86] The DSTM approach microscopically evaluates drug susceptibility based on differences in cell numbers and shapes between antimicrobial-treated and control cells, using dedicated software. Despite the 3 h TAT, this DSTM method requires expertise for post-experimental analysis with the images obtained. Furthermore, the utility of the

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DSTM approach for other pathogenic species needs to be clarified. Microfluidic, plug-based stochastic confinement of single cells into a nanoliter-sized antimicrobial plugs has been applied to determine the AST of methicillin-resistant *Staphylococcus aureus* (MRSA) to several antimicrobials by measuring their viability with a fluorescence indicator without requiring a preincubation step.[79] This stochastic confinement reduced the AST time significantly and aids in distinguishing the sensitive and resistant cells. The fluorescence of the plugs was measured with an epi-fluorescent microscope and images were analyzed with the Metamorph Imaging Software. This plug-based technique has a TAT of 8 h but requires further testing to implement it in clinical laboratories.

Similarly, various reports state the utility of single-cell-based AST systems applying different technologies (Table 1). Regardless of the applicability of emerging technologies such as microfluidics and single-cell analysis, their equipment and/or expertise requirements preclude their utility as POC AST systems. Thus, research into the development of a rapid AST system, which could be used at POC in clinical settings is still ongoing.

4. Rapid diagnostics for AMR

Although phenotypic AST techniques are indispensable for the treatment of infections, they do not provide any information on molecular epidemiology such as the presence of drug resistance genes. Furthermore, early detection of AMR in the pathogens would be beneficial to implement infection control measures to abrogate their spreading within healthcare settings.[87, 88] The conventional method for AMR diagnosis involves a series of stepwise procedures in clinical laboratories: isolation of the pathogen from clinical specimen, identification, and AST.[89] Due to the emergence of advanced technologies, different types of genotypic and phenotypic systems for the rapid diagnosis of AMR are being reported for a wide range of target pathogens. Genotypic techniques for AMR diagnosis detect the presence of resistance genes that are responsible for the expression of resistance characteristics towards the respective antimicrobial. Several genotypic NAATs evolved and are frequently cited for their usefulness in molecular diagnosis.[90–93] Also, various reports state the utility of NAATs for the rapid detection of antimicrobial-resistant pathogens.[94, 95] On the other hand, phenotypic tests detect AMR by phenotypic characteristics of the organisms such as their growth, expression of specific proteins and their enzymatic activities. [96–98] Table 2 describes the various genotypic and phenotypic techniques that are designed or customized for rapid diagnosis of AMR.

4.1. Genotypic techniques for the rapid diagnosis of AMR

4.1.1. PCR-based NAATs—The conventional PCR technique relies on agarose gel electrophoresis for result interpretation.[99, 100] With the development of rapid result interpretation strategies for PCR-based assays, the time to result of these genotypic techniques have been reduced considerably.[101–104] Such rapid PCR-based assays have been applied for the rapid detection of antimicrobial-resistant pathogens by targeting their respective AMR genes (Table 2). Most importantly, in the real-time PCR systems the PCR products can be detected during the reaction itself.[102] These systems offer a significant advantage of result interpretation, without needing to do any additional procedures on the

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workbench. Moreover, multiplex detection of several target genes in a single reaction can be done easily. Real-time PCR is applicable for the detection of a wide range of AMR genes in different pathogen types including carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *Enterococci* (VRE), MRSA, multidrug-resistant tuberculosis, etc.[105–108] The PCR-dipstick chromatography technique involves visualization of the PCR products on a nucleic acid-based dipstick for differential detection of carbapenemase/*mcr-1* genes that confer resistance to carbapenems/colistin directly from stool specimens.[109, 110] Here, the PCR is performed with the biotin- and single-strand tag-linker-labeled primers. The single-strand tag-linker allows for the hybridization to its complementary probe in the dipstick without denaturation which is a necessary step for other hybridization techniques. The dipstick assay can detect the PCR amplicons in less than 15 min with the naked eye and without any equipment, whereas the agarose gel electrophoresis requires ~ 1–1.5 h for result interpretation using the electrophoresis and gel documentation system. Furthermore, the PCR-dipstick offers the multiplex detection of up to 8 targets in a single reaction thereby reducing the cost per test with a TAT of ~2 h from sample preparation to result interpretation. Thus, it can be easily implemented in the clinical laboratories for the rapid detection of AMR. The main limitation of the PCR-dipstick is that it can detect only the specific target genes, and so there is a chance that the genes involved in other resistance mechanisms could be missed.

The line probe assay which is based on DNA strip technology and evaluated by Meaza *et al.* has been shown to be effective for the rapid detection of *Mycobacterium tuberculosis* (Mtb) and its resistance to rifampin (RIF) and/or isoniazid (INH).[111] The line probe assay includes DNA extraction from decontaminated samples and amplification by PCR, followed by reverse hybridization to single-stranded, membrane-bound probes on the strip. Unlike the PCR-dipstick, the line probe assay requires the denaturation of the PCR product, an additional step and with a long TAT of 5 h. The microarray technology offers an advantage of the simultaneous detection of several target AMR genes in many samples along with the identification of pathogens within a short period.[112–114] For the microarray, DNA is extracted from the specimens followed by PCR of the target genes. The PCR amplicons are labeled and added to the microarray for hybridization. Based on the signal intensities, the presence or absence of the AMR genes is determined.[114] Various reports stated the application of microarray technique for antimicrobial-resistant pathogens including extended-spectrum β-lactamase producers, carbapenemase producers, VRE and MRSA.[112–114] However, the TAT varies across each methodology that applies the microarray technique. Although the microarray has the advantage of performing the assay simultaneously for many specimens to detect multiple targets, the expensive price and expertise requirement limits its application at the POC.

4.1.2. Isothermal amplification-based NAATs—PCR-based methods are further simplified by the introduction of isothermal amplification methods. PCR-based methods require a thermal cycler or real-time PCR equipment for thermal cycling between temperatures whereas the isothermal amplification can be performed just with a heating block which is much easier to operate and requires less energy. Additionally, isothermal amplification can be performed in a considerably shorter duration than PCR-based methods,

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enhancing its implementation at POC. A loop-mediated isothermal amplification (LAMP) method for detecting *bla*$_{OXA-23}$-positive carbapenem-resistant *Acinetobacter baumannii* (CRAb) has been reported by Yamamoto *et al.*[115] LAMP uses six primers for the amplification of a single target and forms multiple amplicons of different sizes, unlike PCR which forms a single amplicon. LAMP can be performed in a heating block with a TAT of 40 min and the results can be interpreted with the naked eye by visualizing the turbidity. It is highly reliable for poorly resourced clinical settings that lack minimal expertise to work with a thermal cycler and electrophoresis for result interpretation. However, due to the use of six primers in LAMP, it is difficult to implement multiplexity and there is a greater chance of obtaining false positives with direct clinical specimens. Helicase-dependent amplification (HDA) is a unique isothermal amplification technique, which has been developed by employing the DNA helicase enzyme to unwind double-stranded DNA without the need for thermocycling.[116, 117] HDA employs two primers similar to PCR but uses two enzymes - helicase and DNA polymerase together for unwinding the double-stranded DNA and amplification, respectively. Like PCR, HDA also forms a single amplicon which can be determined using fluorescent probes when performed in a real-time PCR machine aiding the utmost utility of HDA. Pasko *et al.* described the Staph ID/R, a rapid test which couples HDA with a chip-based array to detect the presence of *mecA* gene that confers resistance to β-lactams (except ceftaroline and ceftobiprole) in *S. aureus* within 75 min.[118–120] HDA also possesses the advantage of detecting multiple targets in a single reaction under an isothermal condition.

Rolling circle amplification (RCA) is another isothermal amplification technique which involves amplification of a short DNA/RNA primer to form a long single-stranded DNA/RNA using a circular DNA template and DNA/RNA polymerase (Phi29, Bst or Vent *exo*-DNA polymerase for DNA and T7 RNA polymerase for RNA).[121] The RCA method has been reported by Chen *et al.* to accurately detect RIF-resistant Mtb directly from clinical specimens by detecting the RIF resistance determining region of the *rpoB* gene.[122] The RCA results can be interpreted with agarose gel electrophoresis, however fluorescence-based detection of target amplicons is also feasible to reduce the TAT. Recombinase polymerase amplification (RPA) is an isothermal amplification technique, in which three different enzymes - recombinase, single-stranded binding protein, and DNA polymerase, altogether in a single tube are used for the detection of the target gene. RPA offers the feasibility of multiplex detection of more than one target in a single reaction. RPA has been employed for the detection of macrolide resistance by targeting the *mef(A)*, macrolide efflux gene.[123] RPA coupled with a probe incorporating fluorophore and quencher offers real-time detection in a TAT of ~ 1 h. The main disadvantage of RPA is that it cannot be applied to direct clinical specimens. Multiple Cross Displacement Amplification (MCDA) is a unique isothermal amplification in which ten primers are used to amplify the target sequence with a polymerase having strand displacement activity. Though it is very difficult to apply multiplexity with ten primers, Wang et al. developed a multiplex-MCDA for the detection of MRSA by targeting the *nuc* (*S. aureus*-specific gene) and *mecA* gene together in a single reaction. A lateral flow biosensor was used to interpret the results visually with the naked eye providing a shorter TAT of ~ 1 h. However, the MCDA has not shown to be applicable to direct clinical specimens.[124] Different types of NAATs both PCR- and isothermal

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amplification-based techniques which play a major role in the development of rapid diagnostics for AMR are shown in Table 2.

4.2. Phenotypic techniques for the rapid diagnosis of AMR

The determination of the presence or enzymatic activity of the proteins responsible for AMR characteristics in the pathogens can serve as a rapid phenotypic detection of AMR. The different types of rapid phenotypic detection techniques are listed in Table 2. Lateral flow or immunochromatographic assay is a paper-based test for the detection of target proteins using the antigen-antibody interaction without the need for any specialized equipment and with a TAT of less than 30 minutes.[125] Multiplex immunochromatographic assays have been reported for carbapenem-resistant organisms by detecting the presence of carbapenemase enzymes in ~15–45 min. from overnight grown bacterial cultures and positive blood cultures.[55, 126] Similarly, the immunochromatographic assay is applied for the detection of MRSA from bacterial cultures by targeting the PBP2a proteins.[127] Since it can be applied for direct clinical specimens and the results can be interpreted easily with the naked eye, it is easy to implement at POC, even in poorly resourced settings.

Carba NP test, a novel carbapenemase activity detection test based on the principle of acidimetry has been recommended by CLSI as a confirmatory test for carbapenemase-producing organisms. The first report on the introduction of Carba NP test showed both 100% sensitivity and specificity.[56] Even though the latter studies showed a varied sensitivity/specificity depending on the type of carbapenemase enzyme, it is very helpful in clinical laboratories for the detection of carbapenem-resistant organisms.[128–130] Although it is very rapid in determining the carbapenemase activity to detect carbapenem-resistant organisms in less than 2 h, it can’t be applied to determine resistance towards other antimicrobials. The AK bioluminescence assay employs the AK enzyme activity, which is related to the growth of bacterial cells to determine their resistance towards the antimicrobial with which they are treated. Initially, the bacterial cells from the clinical specimen are selectively enriched in a medium with the antimicrobial and the bacterial cells are lysed to release the AK. When adenosine triphosphate is added, the AK catalyzes the production of adenosine triphosphate from adenosine diphosphate which is then quantified using luciferase. Based on the bioluminescence intensity, the pathogen is determined to be resistant or sensitive to the tested antimicrobial within 5 h.[131] The Slide latex agglutination test is another phenotypic AMR detection test for MRSA in which the PBP2a proteins from MRSA is detected by the visible agglutination of latex particles conjugated with the monoclonal antibodies for PBP2a in ~20 min.[132] Both the adenylate kinase bioluminescence and latex agglutination assays have been reported for the detection of MRSA; however, it would be more reliable if the assays could be applicable for other antimicrobial-resistant pathogens. Although the phenotypic techniques for AMR detection are very simple and rapid, their utility as POC diagnostics is limited by their applicability only to cultured bacterial cells.

4.3. Microfluidics for the rapid diagnosis of AMR

Microfluidics technology deals with small volume of fluids using channels with dimensions in micrometers. With the advantage of small sample volume requirement and shorter processing time, microfluidics is being applied in diverse fields.[133, 134] In recent years,

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several studies have reported that microfluidics can be applied to the molecular diagnosis of AMR.[135–137] Microfluidic devices can also be employed for the simultaneous analysis of multiple target genes in different pathogens. Sandberg *et al.* developed a microfluidic quantitative PCR (MF-qPCR) method for the detection of different types of AMR genes directly from environmental samples.[138] MF-qPCR is performed in the BioMark real-time PCR system in which different arrays are used to perform thousands of singlet qPCR each in a 10 nL reaction volume with a TAT of 4 h. For the specific target amplification to improve the sensitivity of qPCR, MF-qPCR requires a preamplification step. Although this technique can perform qPCR for 39 genes simultaneously, the reaction conditions for amplification of all the individual genes need to be standardized at the same thermal cycling program. A multiplex PCR-based microfluidics platform has been described for the detection of resistance genes in vancomycin- and β-lactam-resistant pathogens from perianal swab specimens with excellent sensitivity and specificity.[139] This microfluidic technique also requires two PCR amplifications instead of a single reaction. In the first step, a nested PCR is performed in three multiplex reactions of which amplicons will serve as targets for the second PCR. The second PCR detection is performed in a BioMark HD system and 192.2.4 Dynamic Array Integrated Fluidic Circuit array that can analyze 192 samples with 24 separate PCR arrays. Though it showed 100% gene specificity, much care is needed to avoid cross-contamination as it requires double PCR amplifications. Therefore, a single reaction providing an optimal output would be very promising.

In addition to PCR-based techniques, isothermal amplification is also integrated with microfluidics for the rapid detection of AMR. A programmable digital platform coupled with droplet microfluidics that utilizes the isothermal RPA assay has been shown to be promising for the detection of $bla_{\text{CTX-M-15}}$, a β-lactam resistance gene.[140] This device constitutes an impedance sensor for real-time detection of droplet position and its size, an automatic droplets dispenser, an on-chip thermistor, and an integrated heater for regulating the droplet temperature. Real-time monitoring of the RPA reaction is done using exonuclease fluorescent probes in a TAT of 60 min. It was shown to be applicable for purely isolated DNA from the bacterial culture, however, it will be more beneficial as a POC device if this technology can be upgraded for direct specimens. Likewise, LAMP is employed in a microfluidic system for the diagnosis of VRE by detecting the presence of the *vanA* gene.[141] In this integrated microfluidic system, the joint fluid specimen is administered into the reaction chamber in which ethidium monoazide is used to distinguish the live and dead bacteria. Then, low-temperature chemical lysis reagents and 16s rRNA probe-coated magnetic beads are added. After the chemical lysis of bacterial cells, the 16s rRNA probe binds only to the dsDNA of the live bacteria and is separated using a magnet underneath the microfluidic chip and washed. Following DNA isolation from live cells, LAMP reagents along with the fluorescent dye, are pumped into the reaction chamber. The fluorescence signal of the LAMP reaction is captured with the photomultiplier tube sensor and the results can be visualized in the monitor. The TAT to detect the *vanA* gene directly from clinical specimens using this integrated microfluidic system is 1 h. With a detection limit of 10 colony forming units, low TAT, and small equipment size, an incorporation of multiplex detection will bring this integrated microfluidic system as an efficient POC diagnostics for

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AMR. Thus, microfluidics technology aids in the adaptation of molecular diagnostics towards the rapid detection of AMR.

5. Commercially available rapid diagnostics for AMR

A broad range of commercial diagnostic tools are available to enable healthcare practitioners to identify AMR. Despite the large number of reports on rapid diagnostics for AMR, there are several criteria, not limited to specimen-direct utility, sensitivity, specificity, ease of use, equipment and expertise requirements, to determine whether a detection system could serve as a POC diagnostic tool. Furthermore, the regulatory hurdles and return on investment issues remain limiting factors for any diagnostics to be implemented at POC.[142, 143] Therefore, a diagnostic system that meets all the requirements of POC diagnostics will be valuable for both clinicians and patients. Table 3 shows the commercially available genotypic/phenotypic rapid diagnostic kits and their utilities as POC testing systems for AMR.

5.1. Commercial genotypic diagnostics for AMR

Xpert® MTB/RIF (Cepheid, Inc., USA) is an automated real-time PCR-based assay with a detection time of 2 h, to be performed on sputum specimens for simultaneous detection of both Mtb and RIF resistance mutations. The World Health Organization (WHO) endorsed the Xpert® MTB/RIF in 2011 for use in TB endemic countries. The accuracy of Xpert® MTB/RIF in adults has been summarized by a Cochrane review.[144] Despite the limitations of Xpert® MTB/RIF including the requirement of stable and uninterrupted power supply, trained staff, regular maintenance, and annual calibration of modules, it is implemented in high TB burden countries.[145] However, the cost of Xpert® MTB/RIF precludes its expanded utility in endemic settings as a POC device.[146] GenoType MTBDR*plus* ver 2.0 (Hain Lifescience GmBH, Germany) is a PCR-based technique that applies the DNA strip technology for the detection of multidrug-resistant tuberculosis from pulmonary specimens within 5 h. GenoType MTBDR*plus* ver 2.0 can provide multiplex detection of the Mtb complex and its resistance to RIF and INH simultaneously in a single reaction. Also, the test results can be interpreted visually by comparing the specific band pattern on the DNA strip without the need for any additional equipment.[111, 147] Due to the longer TAT, it is difficult to implement the GenoType MTBDR*plus* ver 2.0 at POC.

Anyplex™ vanR Real-time Detection (Seegene, Inc., USA) is a diagnostic kit for the detection of VRE by targeting the *vanA*, *vanB* and *vanC* genes using real-time PCR technique. With the Anyplex™ vanR Real-time Detection, multiplex amplification of all three resistance genes is accomplished and upon interlocking with the Seegene Viewer, data interpretation can be performed automatically. The major limitation of the Anyplex™ vanR Real-time Detection at the POC level is that the DNA extraction must be done manually with the kit reagents and there are no published reports stating its sensitivity and specificity. BD GeneOhm™ MRSA ACP (BD Diagnostics, USA) is a real-time PCR-based genotypic technique for the detection of MRSA directly from nasal swabs. As it is directly applicable to clinical specimens, it reduces the sample processing time by the faster lysis of specimens using achromopeptidase.[148] Following lysis, the specimen is subjected to real-time PCR,

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with MRSA detected in a shorter TAT of ~3 h with high sensitivity (92%) and specificity (94%).[149] Furthermore, BD GeneOhm™ MRSA ACP is compatible with the Smartcycler instrument, so that it could be easily implemented in clinical laboratories.

The VERIGENE® BC-GP and VERIGENE® BC-GN (Luminex, USA) are FDA-approved, hybridization-based detection systems that can identify a range of Gram-positive and Gram-negative pathogens along with their AMR genes without any amplification directly from positive blood cultures, respectively. The VERIGENE® system is a highly automated sample-to-result system without the need for manual sample processing, DNA extraction, and detection. Furthermore, it requires only ~ 5 min of hands-on time and ~ 2.5 h of run time. As amplification of the target genes is not required for VERIGENE® BC-GP and VERIGENE® BC-GN, it greatly reduces the detection time of AMR in positive blood cultures compared to conventional methods, while providing excellent sensitivity and specificity.[150, 151] However, they are relatively expensive due to the requirement of VERIGENE® system. FilmArray® BCID (BioFire Diagnostics, USA) is an amplification-based diagnostic system that has obtained FDA clearance for employing them in clinical laboratories. FilmArray® BCID applies the real-time PCR technique for the multiplex detection of different pathogen types and their resistance genes, including *mecA*, *vanA*, *vanB*, and bla_{KPC} directly from positive blood cultures.[152, 153] The main advantage of FilmArray® BCID is that it automatically performs nucleic acid extraction, multiplexed nested PCR and melt curve analysis all within 1 h. The ePlex® BCID system (GenMark Diagnostics, USA) is another FDA-cleared real-time PCR-based technique that can identify the Gram-positive and Gram-negative pathogens and their resistance genes in positive blood cultures in ~1.5 h.[154] Like the FilmArray® BCID, the ePlex® BCID system works on a fully automated technology in which nucleic acid extraction, amplification, and digestion are performed with the electrowetting technology and target detection using eSensor technology. With a short TAT and multiplex detection, the ePlex® BCID system could serve as an efficient rapid diagnostic for clinical laboratories.

In addition to the PCR-based genotypic detection systems, a few isothermal NAATs-based commercial diagnostics are also available for AMR. The NUCLISENS® EASYQ® platform (bioMérieux, France) utilizes a nucleic acid sequence-based amplification technique coupled with sequence-specific fluorescent probes for the detection of target DNA in MRSA and bla_{KPC} carriers.[155, 156] The specimen lysates are used for isothermal amplification and the qualitative results are analyzed with fluorescence signal curves measured by the NUCLISENS® EASYQ® Analyzer with a TAT of 3 h. Since the NUCLISENS® EASYQ® uses nucleic acid sequence-based amplification, it cannot be used for the detection of multiple targets in a single reaction. Thus, each of the currently existing genotypic techniques have their own advantages and disadvantages in implementing them at the POC level. Table 3 lists the different types of genotypic commercial diagnostic products for AMR, their targets, TAT, POC applicability and other characteristics. There are several other products from different companies that use similar technologies as listed in Table 3.

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5.2. Commercial phenotypic diagnostics for AMR

The phenotypic diagnostic systems that apply either an antigen-antibody interaction or enzymatic activity assessment are very easy and rapid compared to genotypic systems. RAPIDEC® CARBA NP (bioMérieux, France) is an FDA-cleared test for the detection of carbapenemase-producing bacteria grown in culture with time- to-results ranging from 30 min to 2 h. The results of RAPIDEC® CARBA NP can be interpreted visually by the color change of the pH indicator from phenol red to yellow/orange upon hydrolysis of imipenem by carbapenemase producers.[157, 158] As the RAPIDEC® CARBA NP rapidly detects the transmissible carbapenem resistance, it is very helpful for controlling the spread of carbapenemase-producing organisms in healthcare settings. NG-Test® CARBA 5 (NG Biotech, France) is an immunochromatographic assay, which provides multiplex detection of five carbapenemase enzymes viz. KPC, OXA-48-like, NDM, VIM, and IMP.[159, 160] It does not require any special equipment and the cost per test is incredibly low compared to genotypic tests. Furthermore, its TAT of ~ 15 min, directly from both bacterial cultures and positive blood cultures, the NG-Test® CARBA 5 is easy to implement as a rapid diagnostic in clinical laboratories. NG-Test® CTX-M MULTI (NG Biotech, France) is another immunochromatographic test, which is designed to detect multiple variants of CTX-M, an extended-spectrum β-lactamase with a single antibody. Recently, Bianco *et al.* showed that NG-Test® CTX-M MULTI could detect CTX-M in blood cultures with 100% sensitivity and specificity in a TAT of ~ 15 min.[161]

The Alere™ PBP2a SA culture colony test (Abbott, USA) is a qualitative immunochromatography membrane assay for identifying MRSA, based on the detection of penicillin-binding protein 2a directly from isolated bacterial colonies that are identified as *S. aureus*.[127] The results of the PBP2a SA test can be read visually in ~ 6 min. It is very simple and easy to perform without the requirement of any expertise, in a very short time compared to conventional methods to differentiate the MRSA from methicillin-sensitive *S. aureus*. Slidex® MRSA kit (bioMérieux, France) works on latex agglutination in which the agglutination of latex beads coated with antibodies for the target protein in MRSA can be interpreted visually.[162] Here the bacterial culture is subjected to boiling and centrifugation followed by mixing the supernatant with two drops of latex particles. Although the results can be interpreted in 20 min, it requires an extraction step, unlike the PBP2a SA which can be tested directly on bacterial colonies. The 3M™ BacLite™ Rapid MRSA Test (3M, USA) is a bacterial growth-based assay which detects the presence of MRSA in a broth containing antimicrobial by assessing the activity of the AK enzyme in generating ATP as a measure of the growth of *S. aureus*.[53] It can be used for the direct detection of MRSA from nasal and groin swabs. The 3M™ BacLite™ Rapid MRSA Test is performed in the automated 3M BacLite system and the result is more sensitive than traditional optical techniques. However, it has a TAT time of ~5 h that is very long compared to other commercial phenotypic tests. Despite the simplicity and rapidity of phenotypic tests for AMR, all of them have a common limitation of their applicability only to pure bacterial cultures which predominantly preclude their usage as a POC tool for AMR.

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6. Challenges with AMR diagnostics at the POC level

Despite the advances in the field of medical diagnostics, the development and application of AST and AMR diagnostics at the POC level is still ongoing. Different types of the above-mentioned diagnostic platforms have been introduced for the rapid detection of AMR and determination of AST; however, each method faces certain limitations to reach POC utility. For instance, even though the single-cell-based AST systems can reduce labor and time to result, they require sophisticated equipment for test performance and result interpretation. Furthermore, the application of these single-cell-based AST systems in the clinical laboratory requires expertise. In addition, the single-cell-based AST techniques can examine the resistance profile of test pathogens against a limited number of antimicrobials. Given that most of the reports on single-cell AST techniques were examined with standard strains and/or previously characterized drug-resistant pathogens, there is a critical step to be achieved, pending prior to utilizing them for direct clinical specimens.

PCR-based technologies play a vital role in clinical laboratories for the identification of drug resistance genes. Although minimal expertise is required to perform PCR, the automated and real-time PCR platforms reduce the expertise requirement considerably. However, only the known targets specified for detection can be checked with the PCR-based diagnostic techniques and thus, a new variant of a resistance gene or a new resistance gene could not be detected.[99, 163] Isothermal NAATs are easier to work without any specialized equipment; however, they expertise is needed to prevent contamination, that can lead to false positive results. Regardless of the rapidity of isothermal NAATs, it is challenging to design them for detecting multiple targets in direct clinical specimens. DNA microarrays offer the advantage of examining many specimens in a single reaction time; however, they are very expensive and require specialized equipment and expertise, which taken together, limits their implementation in routine clinical examination.[164, 165] With WGS, it is feasible to both predict the resistance profile of bacteria and determine novel resistance mechanisms and genomic epidemiology of AMR, but the time to result and cost are the major disadvantages that preclude their POC utility.[166] Since WGS requires technical expertise for data analysis, an automated data analysis system is warranted.[167]

The major limitation of the immunoassays is that commercially available diagnostic kits are applicable mostly for pure cultured pathogens and not for direct clinical specimens. Although the mechanism-based tests, such as the Carba NP test and bacterial growth-based tests like AK bioluminescence assay, do not require expertise, they can be applied only to determine the resistance of pathogens towards specific antimicrobials. Overall, improvements such as specimen-direct compatibility, easier methodology, shorter TAT, multiplexity, cost-effectiveness, and visual interpretation of results, need to be incorporated into rapid detection techniques to translate them into POC platform for AMR. In addition to the various specific limitations for each diagnostic approach, overall cost and regulatory bottlenecks are common factors hindering the implementation of POC diagnostics.

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7. Conclusions

In this review, we described various rapid diagnostic techniques and commercial diagnostics available for AMR detection. Single-cell-based AST systems seem more promising in reducing the time to result, but the cost, expertise, and equipment highly precludes their application to clinical settings currently. With respect to the diagnostics for AMR, molecular technologies have taken a lead in the detection of AMR, with the invention of PCR and its allied techniques. From agarose gel electrophoresis to real-time visualization, several result-interpretation strategies, such as dipstick and line probes, have been developed for PCR-based techniques to implement them at POC level. However, the molecular techniques still need improvement of different facets to efficiently apply them for the POC diagnosis of AMR. On the other hand, the immunoassays and mechanism-based assays are not applicable for direct clinical specimens. The commercially available AMR diagnostics are efficient as rapid detection systems, but they also have different limitations when used for POC diagnostics at a patient's bedside. Therefore, a successful diagnostic system for AMR must address all the above limitations to be implemented at the POC, a much-needed development to fight the ongoing AMR crisis.

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References

1. Gould K, The Journal of antimicrobial chemotherapy, 2016, 71, 572–575. [PubMed: 26851273]
2. Zaffiri L, Gardner J and Toledo-Pereyra LH, Journal of investigative surgery : the official journal of the Academy of Surgical Research, 2012, 25, 67–77. [PubMed: 22439833]
3. Lobanovska M and Pilla G, The Yale journal of biology and medicine, 2017, 90, 135–145. [PubMed: 28356901]
4. Ventola CL, P & T : a peer-reviewed journal for formulary management, 2015, 40, 277–283. [PubMed: 25859123]
5. Aminov RI, Frontiers in microbiology, 2010, 1, 134. [PubMed: 21687759]
6. Singer AC, Shaw H, Rhodes V and Hart A, Frontiers in microbiology, 2016, 7, 1728. [PubMed: 27847505]
7. Tangcharoensathien V, Chanvatik S and Sommanustweechai A, Bull World Health Organ, 2018, 96, 141–144. [PubMed: 29403119]
8. Golding SE, Ogden J and Higgins HM, Front Vet Sci, 2019, 6, 132. [PubMed: 31106216]
9. Martinez JL and Baquero F, Upsala journal of medical sciences, 2014, 119, 68–77. [PubMed: 24678768]
10. Zhang R, Eggleston K, Rotimi V and Zeckhauser RJ, Globalization and health, 2006, 2, 6. [PubMed: 16603071]
11. Podolsky SH, Palgr Commun, 2018, 4.
12. Brogan DM and Mossialos E, Globalization and health, 2016, 12, 8. [PubMed: 27000847]
13. Founou RC, Founou LL and Essack SY, PloS one, 2017, 12, e0189621. [PubMed: 29267306]
14. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM and Robotham JV, Antimicrobial resistance and infection control, 2018, 7, 58. [PubMed: 29713465]
15. Allcock S, Young EH, Holmes M, Gurdasani D, Dougan G, Sandhu MS, Solomon L and Torok ME, Global health, epidemiology and genomics, 2017, 2, e4.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

16. Nichols JH, Clinics in laboratory medicine, 2007, 27, 893–908, viii. [PubMed: 17950904]
17. Price CP, Bmj, 2001, 322, 1285–1288. [PubMed: 11375233]
18. Kallberg C, Ardal C, Salvesen Blix H, Klein E, M. M. E, Lindbaek M, Outterson K, Rottingen JA and Laxminarayan R, PloS one, 2018, 13, e0205166. [PubMed: 30325963]
19. Marston HD, Dixon DM, Knisely JM, Palmore TN and Fauci AS, Jama, 2016, 316, 1193–1204. [PubMed: 27654605]
20. Conly J and Johnston B, The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale, 2005, 16, 159–160. [PubMed: 18159536]
21. Fair RJ and Tor Y, Perspectives in medicinal chemistry, 2014, 6, 25–64. [PubMed: 25232278]
22. Fiore DC, Fettic LP, Wright SD and Ferrara BR, The Journal of family practice, 2017, 66, 730–736. [PubMed: 29202142]
23. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT and Hossain N, Cureus, 2017, 9, e1403. [PubMed: 28852600]
24. van Belkum A, Bachmann TT, Ludke G, Lisby JG, Kahlmeter G, Mohess A, Becker K, Hays JP, Woodford N, Mitsakakis K, Moran-Gilad J, Vila J, Peter H, Rex JH, Dunne WM Jr., J. A.-R. W. G. o. A. Resistance and T. Rapid Diagnostic, Nature reviews. Microbiology, 2019, 17, 51–62. [PubMed: 30333569]
25. Woodford N and Sundsfjord A, The Journal of antimicrobial chemotherapy, 2005, 56, 259–261. [PubMed: 15967769]
26. Kelley SO, SLAS technology, 2017, 22, 113–121. [PubMed: 27879409]
27. McLain JE, Cytryn E, Durso LM and Young S, Journal of environmental quality, 2016, 45, 432–440. [PubMed: 27065389]
28. Dadgostar P, Infection and drug resistance, 2019, 12, 3903–3910. [PubMed: 31908502]
29. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF and Baloch Z, Infection and drug resistance, 2018, 11, 1645–1658. [PubMed: 30349322]
30. Burnham CD, Leeds J, Nordmann P, O'Grady J and Patel J, Nature reviews. Microbiology, 2017, 15, 697–703. [PubMed: 29021600]
31. Mitsakakis K, Kaman WE, Elshout G, Specht M and Hays JP, Future microbiology, 2018, 13, 1157–1164. [PubMed: 30113214]
32. Wiegand I, Hilpert K and Hancock RE, Nat Protoc, 2008, 3, 163–175. [PubMed: 18274517]
33. Poupard JA, Rittenhouse SF and Walsh LR, Advances in experimental medicine and biology, 1994, 349, 3–14. [PubMed: 8209807]
34. Wheat PF, J Antimicrob Chemoth, 2001, 48, 1–4.
35. Rice WG and Lonergan AM, Am J Clin Pathol, 1950, 20, 68–70, illust. [PubMed: 15408846]
36. Stalons DR and Thornsberry C, Antimicrobial agents and chemotherapy, 1975, 7, 15–21. [PubMed: 1137356]
37. European M Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical and D. Infectious, Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 2000, 6, 509–515.
38. Dai K, Wen X, Chang YF, Cao S, Zhao Q, Huang X, Wu R, Huang Y, Yan Q, Han X, Ma X and Wen Y, PeerJ, 2018, 6, e4253. [PubMed: 29340249]
39. Bauer AW, Perry DM and Kirby WM, AMA Arch Intern Med, 1959, 104, 208–216. [PubMed: 13669774]
40. Charteris WP, Kelly PM, Morelli L and Collins JK, J Food Prot, 2001, 64, 2007–2014. [PubMed: 11770631]
41. Jones RJ, Jama, 1980, 243, 1748. [PubMed: 6988614]
42. Kassim A, Omuse G, Premji Z and Revathi G, Annals of clinical microbiology and antimicrobials, 2016, 15, 21. [PubMed: 27068515]
43. Anjum MF, Zankari E and Hasman H, Microbiology spectrum, 2017, 5.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

44. Templeton NS, Diagnostic molecular pathology : the American journal of surgical pathology, part B, 1992, 1, 58–72.
45. Ao W and Jenison R, Methods in molecular biology, 2013, 1039, 89–98. [PubMed: 24026688]
46. Deiman B, Jay C, Zintilini C, Vermeer S, van Strijp D, Venema F and van de Wiel P, Journal of virological methods, 2008, 151, 283–293. [PubMed: 18514336]
47. Rivoarilala OL, Garin B, Andriamahery F and Collard JM, PloS one, 2018, 13, e0200421. [PubMed: 30021007]
48. Xu J, Wang X, Yang L, Kan B and Lu X, Journal of medical microbiology, 2018, 67, 1682–1688. [PubMed: 30355422]
49. Koser CU, Ellington MJ and Peacock SJ, Trends in genetics : TIG, 2014, 30, 401–407. [PubMed: 25096945]
50. Oniciuc EA, Likotrafiti E, Alvarez-Molina A, Prieto M, Santos JA and Alvarez-Ordonez A, Genes, 2018, 9.
51. Tamma PD, Fan Y, Bergman Y, Pertea G, Kazmi AQ, Lewis S, Carroll KC, Schatz MC, Timp W and Simner PJ, Antimicrobial agents and chemotherapy, 2019, 63.
52. Doyle RM, O'Sullivan DM, Aller SD, Bruchmann S, Clark T, Coello Pelegrin A, Cormican M, Diez Benavente E, Ellington MJ, McGrath E, Motro Y, Phuong Thuy Nguyen T, Phelan J, Shaw LP, Stabler RA, van Belkum A, van Dorp L, Woodford N, Moran-Gilad J, Huggett JF and Harris KA, Microb Genom, 2020, 6.
53. von Eiff C, Maas D, Sander G, Friedrich AW, Peters G and Becker K, The Journal of antimicrobial chemotherapy, 2008, 61, 1277–1280. [PubMed: 18359757]
54. Brown DF and Walpole E, The Journal of antimicrobial chemotherapy, 2001, 47, 187–189. [PubMed: 11157904]
55. Hamprecht A, Vehreschild JJ, Seifert H and Saleh A, PloS one, 2018, 13, e0204157. [PubMed: 30216371]
56. Nordmann P, Poirel L and Dortet L, Emerging infectious diseases, 2012, 18, 1503–1507. [PubMed: 22932472]
57. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB Jr., Jenkins SG, Limbago BM and Das S, Journal of clinical microbiology, 2017, 55, 2321–2333. [PubMed: 28381609]
58. Sfeir MM, Hayden JA, Fauntleroy KA, Mazur C, Johnson JK, Simner PJ, Das S, Satlin MJ, Jenkins SG and Westblade LF, Journal of clinical microbiology, 2019, 57.
59. Maugeri G, Lychko I, Sobral R and Roque ACA, Biotechnology journal, 2019, 14, e1700750. [PubMed: 30024110]
60. Tan TY, McNulty C, Charlett A, Nessa N, Kelly C and Beswick T, The Journal of antimicrobial chemotherapy, 2003, 51, 379–384. [PubMed: 12562706]
61. Jorgensen JH and Ferraro MJ, Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2009, 49, 1749–1755. [PubMed: 19857164]
62. Vrioni G, Tsiamis C, Oikonomidis G, Theodoridou K, Kapsimali V and Tsakris A, Annals of translational medicine, 2018, 6, 240. [PubMed: 30069442]
63. Lee AWT, Lam JKS, Lam RKW, Ng WH, Lee ENL, Lee VTY, Sze PP, Rajwani R, Fung KSC, To WK, Lee RA, Tsang DNC and Siu GKH, Frontiers in microbiology, 2018, 9, 334. [PubMed: 29527202]
64. Axelsson C, Rehnstam-Holm AS and Nilson B, Infect Dis (Lond), 2020, 52, 45–53.
65. Lange C, Schubert S, Jung J, Kostrzewa M and Sparbier K, Journal of clinical microbiology, 2014, 52, 4155–4162. [PubMed: 25232164]
66. Sparbier K, Schubert S and Kostrzewa M, Methods, 2016, 104, 48–54. [PubMed: 26804565]
67. Zhou M, Wang Y, Liu C, Kudinha T, Liu X, Luo Y, Yang Q, Sun H, Hu J and Xu YC, Infection and drug resistance, 2018, 11, 1347–1358. [PubMed: 30214255]
68. Waites KB, Brookings ES, Moser SA and Zimmer BL, Journal of clinical microbiology, 1998, 36, 2052–2056. [PubMed: 9650961]
69. Ling TK, Liu ZK and Cheng AF, Journal of clinical microbiology, 2003, 41, 4705–4707. [PubMed: 14532207]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

70. de Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ and Pascual A, Journal of clinical microbiology, 2004, 42, 3734–3738. [PubMed: 15297523]
71. Chapin KC and Musgnug MC, Journal of clinical microbiology, 2003, 41, 4751–4754. [PubMed: 14532215]
72. Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, Granato PA, Harrington AT, Hernandez DR, Humphries RM, Jindra MR, Ledeboer NA, Miller SA, Mochon AB, Morgan MA, Patel R, Schreckenberger PC, Stamper PD, Simner PJ, Tucci NE, Zimmerman C and Wolk DM, Journal of clinical microbiology, 2018, 56.
73. Humphries R and Di Martino T, The Journal of antimicrobial chemotherapy, 2019, 74, i40–i43. [PubMed: 30690541]
74. Li Y, Yang X and Zhao W, SLAS technology, 2017, 22, 585–608. [PubMed: 28850804]
75. Hayden RT, Clinton LK, Hewitt C, Koyamatsu T, Sun Y, Jamison G, Perkins R, Tang L, Pounds S and Bankowski MJ, Journal of clinical microbiology, 2016, 54, 2701–2706. [PubMed: 27558176]
76. Idelevich EA, Hoy M, Gorlich D, Knaack D, Grunastel B, Peters G, Borowski M and Becker K, Frontiers in microbiology, 2017, 8, 1064. [PubMed: 28659889]
77. Kaminski TS, Scheler O and Garstecki P, Lab on a chip, 2016, 16, 2168–2187. [PubMed: 27212581]
78. Baltekin O, Boucharin A, Tano E, Andersson DI and Elf J, Proceedings of the National Academy of Sciences of the United States of America, 2017, 114, 9170–9175. [PubMed: 28790187]
79. Boedicker JQ, Li L, Kline TR and Ismagilov RF, Lab on a chip, 2008, 8, 1265–1272. [PubMed: 18651067]
80. Gao J, Li H, Torab P, Mach KE, Craft DW, Thomas NJ, Puleo CM, Liao JC, Wang TH and Wong PK, Nanomedicine : nanotechnology, biology, and medicine, 2019, 17, 246–253.
81. Kim SC, Cestellos-Blanco S, Inoue K and Zare RN, Antibiotics, 2015, 4, 455–466. [PubMed: 27025635]
82. Choi J, Yoo J, Lee M, Kim EG, Lee JS, Lee S, Joo S, Song SH, Kim EC, Lee JC, Kim HC, Jung YG and Kwon S, Science translational medicine, 2014, 6, 267ra174.
83. Kaushik AM, Hsieh K, Chen L, Shin DJ, Liao JC and Wang TH, Biosensors & bioelectronics, 2017, 97, 260–266. [PubMed: 28609716]
84. Peitz I and van Leeuwen R, Lab on a chip, 2010, 10, 2944–2951. [PubMed: 20842296]
85. Lu Y, Gao J, Zhang DD, Gau V, Liao JC and Wong PK, Analytical chemistry, 2013, 85, 3971–3976. [PubMed: 23445209]
86. Matsumoto Y, Sakakihara S, Grushnikov A, Kikuchi K, Noji H, Yamaguchi A, Iino R, Yagi Y and Nishino K, PloS one, 2016, 11, e0148797. [PubMed: 26872134]
87. Bhattacharya S, Virulence, 2013, 4, 172–184. [PubMed: 23302786]
88. Simoes AS, Couto I, Toscano C, Goncalves E, Povoa P, Viveiros M and Lapao LV, Frontiers in microbiology, 2016, 7, 855. [PubMed: 27375577]
89. Leonard H, Colodner R, Halachmi S and Segal E, ACS sensors, 2018, 3, 2202–2217. [PubMed: 30350967]
90. Chitnis AS, Davis JL, Schecter GF, Barry PM and Flood JM, Infection control and hospital epidemiology, 2015, 36, 1215–1225. [PubMed: 26166303]
91. Gullett JC and Nolte FS, Clinical chemistry, 2015, 61, 72–78. [PubMed: 25403817]
92. Zhao Y, Chen F, Li Q, Wang L and Fan C, Chemical reviews, 2015, 115, 12491–12545. [PubMed: 26551336]
93. Kaur N and Toley BJ, Analyst, 2018, 143, 2213–2234. [PubMed: 29683153]
94. Tuite N, Reddington K, Barry T, Zumla A and Enne V, The Journal of antimicrobial chemotherapy, 2014, 69, 1729–1733. [PubMed: 24677160]
95. Williams MR, Stedtfeld RD, Waseem H, Stedtfeld T, Upham B, Khalife W, Etchebarne B, Hughes M, Tiedje JM and Hashsham SA, Analytical methods : advancing methods and applications, 2017, 9, 1229–1241. [PubMed: 29657581]
96. Boutal H, Naas T, Devilliers K, Oueslati S, Dortet L, Bernabeu S, Simon S and Volland H, Journal of clinical microbiology, 2017, 55, 2018–2029. [PubMed: 28404680]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

97. Shin KS, Song HG, Kim H, Yoon S, Hong SB, Koo SH, Kim J, Kim J and Roh KH, Diagnostic microbiology and infectious disease, 2010, 67, 301–303. [PubMed: 20542210]

98. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ and Schouls LM, PloS one, 2015, 10, e0123690. [PubMed: 25798828]

99. Garibyan L and Avashia N, The Journal of investigative dermatology, 2013, 133, 1–4.

100. Lee PY, Costumbrado J, Hsu CY and Kim YH, Journal of visualized experiments : JoVE, 2012, DOI: 10.3791/3923.

101. Curran R, Talbot DC and Towner KJ, Journal of medical microbiology, 1996, 45, 76–78. [PubMed: 8667416]

102. Deepak S, Kottapalli K, Rakwal R, Oros G, Rangappa K, Iwahashi H, Masuo Y and Agrawal G, Current genomics, 2007, 8, 234–251. [PubMed: 18645596]

103. Monden Y, Takasaki K, Futo S, Niwa K, Kawase M, Akitake H and Tahara M, Journal of biotechnology, 2014, 185, 57–62. [PubMed: 24954682]

104. Higuchi R, Fockler C, Dollinger G and Watson R, Biotechnology (N Y), 1993, 11, 1026–1030. [PubMed: 7764001]

105. He YH, Ruan GJ, Hao H, Xue F, Ma YK, Zhu SN and Zheng B, J Microbiol Immunol Infect, 2019, DOI: 10.1016/j.jmii.2019.02.002.

106. Smiljanic M, Kaase M, Ahmad-Nejad P and Ghebremedhin B, Annals of clinical microbiology and antimicrobials, 2017, 16, 48. [PubMed: 28693493]

107. Huletsky A, Giroux R, Rossbach V, Gagnon M, Vaillancourt M, Bernier M, Gagnon F, Truchon K, Bastien M, Picard FJ, van Belkum A, Ouellette M, Roy PH and Bergeron MG, Journal of clinical microbiology, 2004, 42, 1875–1884. [PubMed: 15131143]

108. Ramirez MV, Cowart KC, Campbell PJ, Morlock GP, Sikes D, Winchell JM and Posey JE, Journal of clinical microbiology, 2010, 48, 4003–4009. [PubMed: 20810777]

109. Shanmugakani RK, Akeda Y, Sugawara Y, Laolerd W, Chaihongsa N, Sirichot S, Yamamoto N, Hagiya H, Morii D, Fujiya Y, Nishi I, Yoshida H, Takeuchi D, Sakamoto N, Malathum K, Santanirand P, Tomono K and Hamada S, Frontiers in microbiology, 2019, 10, 149. [PubMed: 30800104]

110. Shanmugakani RK, Akeda Y, Yamamoto N, Sakamoto N, Hagiya H, Yoshida H, Takeuchi D, Sugawara Y, Kodera T, Kawase M, Laolerd W, Chaihongsa N, Santanirand P, Ishii Y, Hamada S and Tomono K, Antimicrobial agents and chemotherapy, 2017, 61.

111. Meaza A, Kebede A, Yaregal Z, Dagne Z, Moga S, Yenew B, Diriba G, Molalign H, Tadesse M, Adisse D, Getahun M and Desta K, BMC infectious diseases, 2017, 17, 280. [PubMed: 28415989]

112. Card R, Zhang J, Das P, Cook C, Woodford N and Anjum MF, Antimicrobial agents and chemotherapy, 2013, 57, 458–465. [PubMed: 23129055]

113. Perreten V, Vorlet-Fawer L, Slickers P, Ehricht R, Kuhnert P and Frey J, Journal of clinical microbiology, 2005, 43, 2291–2302. [PubMed: 15872258]

114. Card RM, Warburton PJ, MacLaren N, Mullany P, Allan E and Anjum MF, PloS one, 2014, 9, e86428. [PubMed: 24466089]

115. Yamamoto N, Hamaguchi S, Akeda Y, Santanirand P, Kerdsin A, Seki M, Ishii Y, Paveenkittiporn W, Bonomo RA, Oishi K, Malathum K and Tomono K, PloS one, 2015, 10, e0133204. [PubMed: 26218925]

116. Cao Y, Kim HJ, Li Y, Kong H and Lemieux B, Current protocols in molecular biology, 2013, 104, Unit 15 11.

117. Vincent M, Xu Y and Kong H, EMBO reports, 2004, 5, 795–800. [PubMed: 15247927]

118. Pasko C, Hicke B, Dunn J, Jaeckel H, Nieuwlandt D, Weed D, Woodruff E, Zheng X and Jenison R, Journal of clinical microbiology, 2012, 50, 810–817. [PubMed: 22170912]

119. Anderson SD and Gums JG, The Annals of pharmacotherapy, 2008, 42, 806–816. [PubMed: 18477729]

120. Garrison MW, Kawamura NM and Wen MM, Expert review of anti-infective therapy, 2012, 10, 1087–1103. [PubMed: 23167512]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

121. Ali MM, Li F, Zhang Z, Zhang K, Kang DK, Ankrum JA, Le XC and Zhao W, Chemical Society reviews, 2014, 43, 3324–3341. [PubMed: 24643375]
122. Chen X, Wang B, Yang W, Kong F, Li C, Sun Z, Jelfs P and Gilbert GL, Journal of clinical microbiology, 2014, 52, 1540–1548. [PubMed: 24574296]
123. Nelson MM, Waldron CL and Bracht JR, BMC infectious diseases, 2019, 19, 144. [PubMed: 30755177]
124. Wang Y, Yan W, Fu S, Hu S, Wang Y, Xu J and Ye C, Frontiers in microbiology, 2018, 9, 907. [PubMed: 29867818]
125. Koczula KM and Gallotta A, Essays in biochemistry, 2016, 60, 111–120. [PubMed: 27365041]
126. Glupczynski Y, Jousset A, Evrard S, Bonnin RA, Huang TD, Dortet L, Bogaerts P and Naas T, The Journal of antimicrobial chemotherapy, 2017, 72, 1955–1960. [PubMed: 28369469]
127. Trienski TL, Barrett HL, Pasquale TR, DiPersio JR and File TM Jr., American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists, 2013, 70, 1908–1912. [PubMed: 24128966]
128. Osterblad M, Hakanen AJ and Jalava J, Antimicrobial agents and chemotherapy, 2014, 58, 7553–7556. [PubMed: 25246404]
129. Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E and Harish BN, Journal of laboratory physicians, 2017, 9, 303–307. [PubMed: 28966495]
130. Tijet N, Boyd D, Patel SN, Mulvey MR and Melano RG, Antimicrobial agents and chemotherapy, 2013, 57, 4578–4580. [PubMed: 23817380]
131. Johnson G, Millar MR, Matthews S, Skyrme M, Marsh P, Barringer E, O'Hara S and Wilks M, BMC microbiology, 2006, 6, 83. [PubMed: 17010192]
132. van Griethuysen A, Pouw M, van Leeuwen N, Heck M, Willemse P, Buiting A and Kluytmans J, Journal of clinical microbiology, 1999, 37, 2789–2792. [PubMed: 10449453]
133. Whitesides GM, Nature, 2006, 442, 368–373. [PubMed: 16871203]
134. Streets AM and Huang Y, Biomicrofluidics, 2013, 7, 11302. [PubMed: 23460772]
135. Liu Z, Banaei N and Ren K, Trends in biotechnology, 2017, 35, 1129–1139. [PubMed: 29153761]
136. Yue M, Schmieder R, Edwards RA, Rankin SC and Schifferli DM, Applied and environmental microbiology, 2012, 78, 7480–7482. [PubMed: 22885744]
137. Keays MC, O'Brien M, Hussain A, Kiely PA and Dalton T, Bioengineered, 2016, 7, 79–87. [PubMed: 26942773]
138. Sandberg KD, Ishii S, LaPara T, Environ Sci Tech Let, 2017, 5, 20–25.
139. Walker GT, Rockweiler TJ, Kersey RK, Frye KL, Mitchner SR, Toal DR and Quan J, Clinical chemistry, 2016, 62, 353–359. [PubMed: 26637481]
140. Kalsi S, Valiadi M, Tsaloglou MN, Parry-Jones L, Jacobs A, Watson R, Turner C, Amos R, Hadwen B, Buse J, Brown C, Sutton M and Morgan H, Lab on a chip, 2015, 15, 3065–3075. [PubMed: 26086197]
141. Chang WH, Yu JC, Yang SY, Lin YC, Wang CH, You HL, Wu JJ, Lee MS and Lee GB, Biomicrofluidics, 2017, 11, 024101. [PubMed: 28798845]
142. Hardy V, Thompson M, Alto W, Keppel GA, Hornecker J, Linares A, Robitaille B and Baldwin LM, BMC family practice, 2016, 17, 149. [PubMed: 27809865]
143. Gregory K and Lewandrowski K, Clinics in laboratory medicine, 2009, 29, 433–448. [PubMed: 19840678]
144. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC and Dendukuri N, The Cochrane database of systematic reviews, 2014, DOI: 10.1002/14651858.CD009593.pub3, CD009593. [PubMed: 24448973]
145. Qin ZZ, Pai M, Van Gemert W, Sahu S, Ghiasi M and Creswell J, Eur Respir J, 2015, 45, 549–554. [PubMed: 25359338]
146. Pooran A, Theron G, Zijenah L, Chanda D, Clowes P, Mwenge L, Mutenherwa F, Lecesse P, Metcalfe J, Sohn H, Hoelscher M, Pym A, Peter J, Dowdy D and Dheda K, Lancet Glob Health, 2019, 7, e798–e807. [PubMed: 31097281]
147. Lanzas F, Ioerger TR, Shah H, Acosta W and Karakousis PC, Journal of clinical microbiology, 2016, 54, 2498–2502. [PubMed: 27440816]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

148. Grabsch EA, Xie S, Szczurek PB, Grayson ML and Howden BP, Pathology, 2013, 45, 612–614. [PubMed: 24018809]

149. Patel PA, Ledeboer NA, Ginocchio CC, Condon S, Bouchard S, Qin P, Karchmer T and Peterson LR, Journal of clinical microbiology, 2011, 49, 2266–2268. [PubMed: 21508148]

150. Kim JS, Kang GE, Kim HS, Kim HS, Song W and Lee KM, BioMed research international, 2016, 2016, 1081536. [PubMed: 26904669]

151. Wojewoda CM, Sercia L, Navas M, Tuohy M, Wilson D, Hall GS, Procop GW and Richter SS, Journal of clinical microbiology, 2013, 51, 2072–2076. [PubMed: 23596240]

152. Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, Robinson G, Carroll KC, Greer A, Morgan M, Chan R, Loeffelholz M, Valencia-Shelton F, Jenkins S, Schuetz AN, Daly JA, Barney T, Hemmert A and Kanack KJ, Journal of clinical microbiology, 2016, 54, 687–698. [PubMed: 26739158]

153. Ledeboer NA, Lopansri BK, Dhiman N, Cavagnolo R, Carroll KC, Granato P, Thomson R Jr., Butler-Wu SM, Berger H, Samuel L, Pancholi P, Swyers L, Hansen GT, Tran NK, Polage CR, Thomson KS, Hanson ND, Winegar R and Buchan BW, Journal of clinical microbiology, 2015, 53, 2460–2472. [PubMed: 25994165]

154. Huang TD, Melnik E, Bogaerts P, Evrard S and Glupczynski Y, Journal of clinical microbiology, 2019, 57.

155. Chen L, Shopsin B, Zhao Y, Smyth D, Wasserman GA, Fang C, Liu L and Kreiswirth BN, Journal of clinical microbiology, 2012, 50, 657–661. [PubMed: 22219302]

156. McEwan AS, Derome A, Meunier D, Burns PJ, Woodford N and Dodgson AR, Journal of clinical microbiology, 2013, 51, 1948–1950. [PubMed: 23554195]

157. Poirel L and Nordmann P, Journal of clinical microbiology, 2015, 53, 3003–3008. [PubMed: 26085619]

158. Hombach M, von Gunten B, Castelberg C and Bloemberg GV, Journal of clinical microbiology, 2015, 53, 3828–3833. [PubMed: 26424840]

159. Takissian J, Bonnin RA, Naas T and Dortet L, Antimicrobial agents and chemotherapy, 2019, 63.

160. Hopkins KL, Meunier D, Naas T, Volland H and Woodford N, The Journal of antimicrobial chemotherapy, 2018, 73, 3523–3526. [PubMed: 30189008]

161. Bianco G, Boattini M, Iannaccone M, Cavallo R and Costa C, The Journal of hospital infection, 2020, DOI: 10.1016/j.jhin.2020.02.009.

162. Tasse J, Dupieux C, Caillon J, Lanotte P, Lamy B, Aissa N, Bemer P, Mereghetti L, Michon AL, Lozniewski A, Bes M, Trouillet-Assant S and Laurent F, Diagnostic microbiology and infectious disease, 2016, 85, 419–421. [PubMed: 27216499]

163. Al-Zahrani IA, Saudi medical journal, 2018, 39, 861–872. [PubMed: 30251728]

164. Hrabak J, Chudackova E and Papagiannitsis CC, Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 2014, 20, 839–853.

165. Frye JG, Lindsey RL, Rondeau G, Porwollik S, Long F, McClelland M, Jackson CR, Englen MD, Meinersmann RJ, Berrang ME, Davis JA, Barrett JB, Turpin JB, Thitaram SN and Fedorka-Cray PJ, Microbial drug resistance, 2010, 16, 9–19. [PubMed: 19916789]

166. Chrystoja CC and Diamandis EP, Clinical chemistry, 2014, 60, 724–733. [PubMed: 24227285]

167. Lutgring JD and Limbago BM, Journal of clinical microbiology, 2016, 54, 529–534. [PubMed: 26739152]

168. Novelli-Rousseau A, Espagnon I, Filiputti D, Gal O, Douet A, Mallard F and Josso Q, Scientific reports, 2018, 8, 3957. [PubMed: 29500449]

169. Smith KP, Richmond DL, Brennan-Krohn T, Elliott HL and Kirby JE, SLAS technology, 2017, 22, 662–674. [PubMed: 28837780]

170. Vasoo S, Cunningham SA, Kohner PC, Mandrekar JN, Lolans K, Hayden MK and Patel R, Journal of clinical microbiology, 2013, 51, 3609–3615. [PubMed: 23966498]

171. Horii T, Monji A, Uemura K and Nagura O, Journal of microbiological methods, 2006, 65, 557–561. [PubMed: 16278026]

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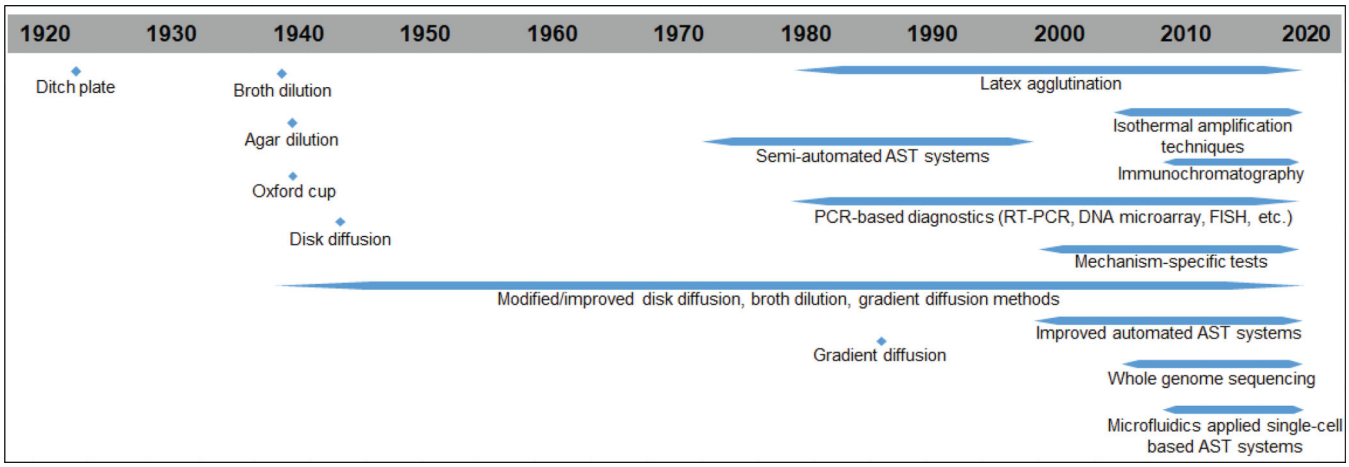

Fig. 1.

History of AMR diagnostics development. The timeline shows the development/ modification of different diagnostic techniques for AST and AMR since the inception of antimicrobial resistance to the currently existing rapid diagnostics.

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Table 1

Rapid single-cell-based AST systems

Principle of testing	Target pathogens	TAT	Equipment required	References
Growth rate - Microchannels & electrokinetics	UPEC	~ 1 h	Digital inverted epifluorescence microscope	85
Growth rate - Microfluidic chips & imaging	UPEC	~ 30 min.	Automated phase-contrast microscope	78
Morphological analysis -Microfluidic agarose channel & imaging	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Enterococcus* spp., *Staphylococcus aureus*	~ 4 h	Inverted optical microscope	82
Growth rate - Nanotube assisted microwave electroporation & imaging	*E. coli*, *P. aeruginosa*, *K. pneumoniae*	~ 90 min.	Fluorescence microscope	80
Growth rate - Raman spectra	*E. coli*	~ 4 h	Raman spectrometer	168
Morphological analysis - Microfluidics & imaging	*E. coli, K. pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*	~ 2 h	Cell observer microscope	169
Growth rate - Droplet microfluidics & fluorescence	*E. coli*	~ 1 h	Avalanche photodiode detector	83
Growth rate - Microfluidics & imaging	*E. coli*	~ 3 h	EMCCD camera & phase contrast microscope	81
Morphology -Microfluidics & imaging	*P. aeruginosa*	~ 3 h	Phase-contrast microscope	86
Viability - Plug-based microfluidics & fluorescence	*S. aureus*	~ 8 h	Epifluorescence microscope	79
Cell division - Microfluidics & dielectrophoretic force	*E. coli*	~ 5 h	Optical microscope	84

Abbreviations: TAT, turnaround time; UPEC, uropathogenic *E. coli*; EMCCD, electron multiplying charge-coupled device.

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TABLE 2

Technologies applied for the rapid diagnosis of AMR

Technology	Examples of target pathogens	AMR target	TAT	Multiplexity	Specimen-direct (specimen)	Result interpretation	References
PCR-based Genotypic Techniques							
PCR-dipstick	CPE & MCRPEn	6 carbapenemase genes, *mcr-1*	~2 h	Yes	Yes (stool/ rectal swab)	Naked eye	109
Real-time PCR	CPE	bla_{NDM}, bla_{KPC}	~1.5 h	yes	Yes (stool/ rectal/ perirectal swab)	Fluorescence	170
Line probe assay	MDR-TB	*rpoB*, *katG*, *inhA*	~5 h	Yes	Yes (sputum)	Naked eye	111
Microarray	Gram-negative	Varied	~5 h	Yes	No (NA)	Fluorescence	112
Isothermal Amplification-based Genotypic Techniques							
LAMP	CRAb	bla_{OXA-23}	~40 min.	No	Yes (sputum)	Naked eye	115
HDA	MRSA	*mecA*	~2 h	Yes	No (NA)	CCD image	118
RPA	Macrolide-resistant	*mef(A)*	~1 h	Yes	No (NA)	Fluorescence	123
RCA	RIF-resistant Mtb	*rpoB*	~3 h	Yes	Yes (sputum)	Fluorescence	122
MCDA	MRSA	*mecA*	~1 h	Yes	No (NA)	Visual	124
ICAN	*Neisseria gonorrhoeae*	*gyrA* mutation	~2 h	No	No (NA)	Naked eye	171
Phenotypic techniques							
IC	CPE	NDM, KPC, OXA-48	~45 min	Yes	No (NA)	Visual	55
Latex agglutination	MRSA	PBP2a	~10 min	No	No (NA)	Visual	54
Carba NP	CPE	Carbapenemase	~2 h	No	No (NA)	Visual	56
AK bioluminescence	MRSA	Methicillin resistance	~5 h	No	Yes (nasal swab)	Bioluminescence	131

Abbreviations: TAT, turnaround time; CPE, carbapenemase-producing *Enterobacteriaceae*; MCRPEn, *mcr-1*-positive *Enterobacteriaceae*; MRSA, methicillin-resistant *Staphylococcus aureus*; MDR-TB, multidrug-resistant tuberculosis; LAMP, loop-mediated isothermal amplification; CRAb, carbapenem-resistant *Acinetobacter baumannii*; HDA, helicase-dependent amplification; CCD, charge-coupled-device; RPA, recombinase polymerase amplification; RCA, rolling circle amplification; Mtb, *Mycobacterium tuberculosis*; MCDA, multiple cross displacement amplification; ICAN, isothermal chimeric primer-initiated amplification; IC, immunochromatography; CPE, carbapenemase-producing *Enterobacteriaceae*; AK, adenylate kinase; NA, not applicable.

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TABLE 3

Commercially available rapid diagnostics for AMR

Commercial product	Company	Technology	Targets	Specimen	Sensitivity/ specificity	TAT	POC applicability	FDA clearance/ CE-IVD marked
Genotypic diagnostics								
GenoType MTBDR*plus* VER 2.0	Hain Lifescience GmbH	DNA-STRIP	MDR-TB	Pulmonary	~96–100/ 87–92	~5 h	Requires thermal cycler and long TAT	Yes/Yes
Anyplex™ vanR Real-time Detection	Seegene	RT-PCR	VRE	Bacterial culture	Unknown	~2 h	Requires RT-PCR equipment, long TAT, not specimen-direct	No/No
Xpert® MTB/RIF	Cepheid	RT-PCR	RIF-resistant TB	Respiratory	Varied	~2 h	Expensive	Yes/Yes
BD GeneOhm™ MRSA ACP	BD Diagnostics	RT-PCR	MRSA	Nasal swab	92/94	~3 h	Requires RT-PCR equipment	Yes/Yes
GenoType MRSA	Hain Lifescience GmbH	DNA-STRIP	MRSA	Bacterial culture	Unknown	~4 h	Requires thermal cycler	No/Yes
Xpert® MRSA NxG	Cepheid	RT-PCR	MRSA	Nasal	Varied	~1 h	Expensive equipment	Yes/Yes
Xpert® Carba-R	Cepheid	RT-PCR	*bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{OXA}$, *bla*$_{VIM}$	Rectal/ perirectal swabs	Varied	~1 h	Expensive equipment	Yes/Yes
BD GeneOhm™ VanR	BD Diagnostics	RT-PCR	*vanA*, *vanB*	Rectal/ anal swab	~86–100/ 82–96	~2 h	Requires RT-PCR equipment	Yes/Yes
VERIGENE® BC-GP	Luminex	Hybridization	*mecA*, *vanA*, *vanB*	Blood culture	~92–100/ 81–100	~3 h	Requires VERIGENE® System and blood culture	Yes/Yes
VERIGENE® BC-GN	Luminex	Hybridization	*bla*$_{CTX-M}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{OXA}$, *bla*$_{VIM}$	Blood culture	~95–100/ 99–100	~3 h	Requires VERIGENE®System and blood culture	Yes/Yes
FilmArray® BCID	BioFire Diagnostics	RT-PCR	*mecA*, *vanA*, *vanB*, *bla*$_{KPC}$	Blood culture	~98–100/ 98–100	~2 h	Requires BioFire® FilmArray® System	Yes/Yes
FilmArray®Pneumonia Panel *Plus*	BioFire Diagnostics	RT-PCR	*mecA mecC*, *bla*$_{CTX-M}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, bla$_{VIM}$, *bla*$_{OXA}$	BAL/ sputum	~96/97–98	~1 h	Requires BioFire®FilmArray® System	Yes/Yes
ePlex® BCID-GP	GenMark Diagnostics	RT-PCR	*mecA*, *mecC*, *vanA*, *vanB*	Blood culture	100/100	~1.5 h	Requires ePlex®System	Yes/Yes
ePlex® BCID-GN	GenMark Diagnostics	RT-PCR	*bla*$_{CTX-M}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{OXA}$	Blood culture	100/100	~1.5 h	Requires ePlex® System	Yes/Yes
Unyvero LRT	Curetis AG	RT-PCR	*bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{OXA}$, *mecA*	Tracheal aspirate	~93/97	~4.5 h	Long TAT and requires Unyvero System	Yes/Yes

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Commercial product	Company	Technology	Targets	Specimen	Sensitivity/ specificity	TAT	POC applicability	FDA clearance/ CE-IVD marked
Magicplex™Sepsis Real-time Test	Seegene	RT-PCR	*mecA*, *vanA*, *vanB*	Whole blood	Unknown	~6 h	Long TAT and requires real-time PCR system	No/Yes
BD MAX™ MRSA XT	BD Diagnostics	RT-PCR	MREJ, *mecA*, *mecC*	Nasal swab	~93/97	~2 h	Requires BD MAX System	Yes/Yes
NUCLISENS® EASYQ® MRSA	bioMérieux	NASBA	MRSA	Nasal swab	~94/96	~3 h	No multiplex detection	Yes/Yes
NUCLISENS® EASYQ® KPC	bioMérieux	NASBA	bla_{KPC}	Stool & rectal swab	~93/99	~3 h	No multiplex detection	No/No
Phenotypic diagnostics								
RAPIDEC® CARBA NP	bioMérieux	Enzymatic hydrolysis	Carbapenemase producers	Bacterial culture	~ 98/98	~2 h	Not specimen-direct	Yes/Yes
NG-test® CARBA 5	NG Biotech	IC	Carbapenemase producers	Bacterial/ blood culture	100/100	~15 min.	Not specimen-direct	Yes/Yes
NG-Test® CTX-M MULTI	NG Biotech	IC	ESBL producers	Blood culture	100/100	~15 min.	Not specimen-direct	No/Yes
NG-Test® MCR-1	NG Biotech	IC	MCR-1 producers	Bacterial culture	~ 100/98	~15 min.	Not specimen-direct	No/Yes
Alere™ PBP2a SA	Abbott	IC	MRSA	Bacterial/ blood culture	~98/99	~6 min.	Not specimen-direct	Yes/Yes
SLIDEX MRSA	bioMérieux	Latex agglutination	MRSA	Bacterial culture	~99/100	~30 min.	Not specimen-direct	No/No
3M™ Baclite™ Rapid MRSA Test	3M	AK bioluminescence	MRSA	Nasal swab	~94/96	~5 h	Long TAT and requires 3M BacLite system	No/No

Abbreviations: TAT, turnaround time; MDR-TB, multidrug-resistant tuberculosis; RT-PCR, real-time PCR; VRE, vancomycin-resistant *Enterococci*; RIF, rifampin; TB, tuberculosis; MRSA, methicillin-resistant *Staphylococcus aureus*; NASBA, nucleic acid sequence-based amplification; IC, immunochromatography; ESBL, extended-spectrum β-lactamase; MCR, modern colistin resistance; AK, adenylate kinase; BAL, bronchoalveolar lavage.