Chelonian Conservation And Biology

Vol. 17 No.2 (2022) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI:doi.org/10.18011/2022.04(1) 2667.2675

RAPID DIAGNOSTIC TESTS FOR IDENTIFYING ANTIBIOTIC-RESISTANT BACTERIA

Abdullah Nasser Alotaibi, Mansour Nasser Mansour, Ali Saeed Alshehri, Yahya Saad Alotaibi, Ahmed Mohammed Safhi, Abdullraouf Ahmed Abdullraouf Mohammed Saleh, Fahad Ahmed Bunayyan Alhejaili, Solaiman Rashed Alfaheed, Sattam Eid Motlq Alotaibi, Assaf Dawi Ghazi Alotibi, Salem Hamed Hamdi Al Mutiri, Bandar Abdalrahman Aldabasi, Hassan Mohammed Hazazi, Mousa Hamod Mohammed Hagawi, Adel Ahmed Yahya Harthi

Abstract

Antimicrobial resistance (AMR) poses a significant and difficult problem in public health. As a result, there is an increasing need for techniques and technologies that can quickly determine the susceptibility of microorganisms to antimicrobial agents (AST). The traditional approaches and technology used for AMR diagnosis and AST in clinical microbiology are laborious, have long turnaround times (TAT), and are often costly. Consequently, the prescription of empirical antimicrobial therapy contributes to the spread of antimicrobial resistance (AMR), resulting in greater death rates and increased healthcare expenses. This review provides an overview of the latest advancements in techniques and technology for combating antimicrobial resistance (AMR). The focus is on important research areas that enable the creation of cutting-edge diagnostic tools for AMR. Initially, we provide a concise overview of the traditional approaches used to tackle AMR detection, surveillance, and AST. Subsequently, we analyze the latest unconventional techniques and the progress made in each area, which include whole genome sequencing (WGS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry, Fourier transform infrared (FTIR) spectroscopy, and microfluidics technology. Here, we provide examples of commercially accessible diagnostic tools for AST. Ultimately, this article discusses viewpoints about the use of new ideas in order to create revolutionary technologies and procedures for AMR diagnostics.

Keywords: molecular diagnostics, antimicrobial resistance, antibiotic susceptibility testing, microfluidics, point-of-care, lab-on-a-chip, MALDI-TOF, FTIR, sequencing.

1. Introduction

Antimicrobial Resistance (AMR) has emerged as a prominent health issue in contemporary society. Antibiotic resistance is a result of the normal process of evolution in bacteria, although it may be sped up by many circumstances [1,2]. Specifically, the overuse and underuse of antibiotics in both people and animals result in the widespread dissemination of bacteria that are

resistant to antimicrobial drugs, together with their genes that confer resistance (ARGs) [3,4,5]. Antimicrobial resistance (AMR) has significant detrimental impacts on human health, healthcare systems, livestock, agriculture, environmental well-being, and subsequently, national economy [6].

Antimicrobial resistance (AMR) poses a formidable challenge to essential aspects of modern healthcare, resulting in significant patient deaths and illnesses, as well as substantial treatment expenditures [7,8]. The current antibiotic therapy techniques, which are widely accepted and used, contribute to their own decline by favoring the development of resistant strains. This emphasizes the need of continuously finding new medicines to stay ahead of the problem posed by antimicrobial resistance (AMR). Hence, it is essential to extend the duration of effectiveness of existing antibiotics while simultaneously pursuing the research and development of next-generation antibiotics. Furthermore, it is crucial to enforce effective control strategies for antibiotic use in order to decelerate the need for ongoing exploration of novel antibiotics [1].

If no action is done, the expenses associated with the increasing rates of AMR are projected to significantly increase [10]. The absence of potent antimicrobial agents is resulting in ordinary infections becoming potentially fatal, hence increasing the risk of life-threatening complications in treatments such as chemotherapy and surgical operations. Therefore, it is essential to restrict the improper and excessive use of antimicrobials in order to prevent the spread of antimicrobial resistance (AMR). Recent research [10,11,12,13] indicate that over 33,000 individuals perish annually in the European Union (EU) due to diseases caused by antibiotic-resistant bacteria. The EU has a significant yearly economic impact due to AMR, amounting to an estimated 1.5 billion euros. This includes healthcare expenses and losses in productivity [10]. Antibiotic resistance causes about 500,000 deaths annually worldwide, with infants accounting for more than 40% of these fatalities [14].

Timely identification of pathogens is necessary for the most effective management of infectious illnesses. Despite significant advancements in medical technology, the time it takes to identify and characterize microbial infections, known as the turnaround time (TAT), may still range from several days [15,16,17]. Consequently, doctors are compelled to initiate empiric antibiotic treatments, usually with broad-spectrum medications, prior to establishing a definitive diagnosis. This approach may have harmful effects not only on the patient's health (specifically, causing an imbalance in the microbiome), but also on the worsening of the existing problem of antimicrobial resistance (AMR). Therefore, there is an urgent want for detection platforms in AMR diagnostics that are quick, highly sensitive, inexpensive, and cost-effective. The use of these platforms will greatly decrease the turnaround time (TAT) for determining antibiotic susceptibility, allowing for the selection of more effective, targeted medicines [15].

Diagnostic tests are regarded as a crucial tool in any approach to combat antimicrobial resistance (AMR). In the context of antimicrobial stewardship initiatives, rapid diagnostic tests (RDTs) pertaining to infectious illnesses are regarded as an essential and irreplaceable instrument. Rapid diagnostic tests (RDTs) have shown the ability to decrease death rates, shorten hospital stays, and cut healthcare expenses. Undoubtedly, these diagnostic tests have shown their

cost-effectiveness, not only via substantial cost reduction, but also by reducing the use of antibiotics [17,18,19,20].

The purpose of this study is to provide an overview of the techniques and technologies currently being used or developed to quickly identify antimicrobial resistance. Furthermore, a concise summary of the primary benefits and constraints of various methodologies and technologies is provided. Existing methodologies, including phenotypic and molecular-based techniques, as well as newer approaches like whole genome sequencing (WGS) and whole genome metasequencing (WGM), MALDI-TOF MS, and IR spectroscopy, are also critically evaluated. Emphasis is given on advanced techniques including microfluidics and lab-on-a-chip technologies, which show great promise in detecting antimicrobial resistance (AMR). Lastly, we provide a concise overview of the commercially accessible platforms specifically created for AST. Figure 1 illustrates a concise graphic that summarizes the approaches and technology examined in the current analysis.

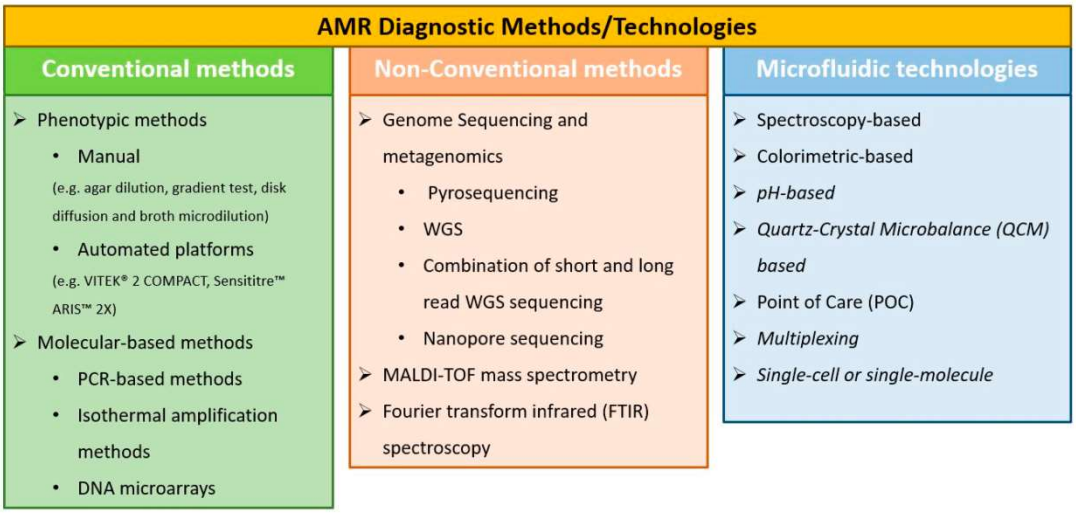

Figure 1. A concise graphic that summarizes the approaches and technology.

2. Advancements in microfluidics and lab-on-a-chip technologies

Lab-on-a-chip (LoC) devices using microfluidics are a very promising technology in several domains, including clinical diagnostics [21], food safety [22], and environmental monitoring [23]. LoC technology has recently been used for detecting antibiotic-resistant bacteria [3]. The LoC technology has many benefits over macro-scale approaches, including quick and high throughput analysis, precise fluid manipulation, low cost, low reagent and power consumption, lower sample volume, automation, integration, compactness, and portability [24-27]. Microfluidic-based detection techniques may be categorized into two primary groups: genotypic assays and phenotypic assays. Genotypic microfluidic assays such as PCR and LAMP specifically detect genetic markers, such as ARG, without the need for bacterial growth. This enables a reduced turnaround time (TAT) of only a few hours [28]. The integration of

microfluidics with isothermal DNA amplification techniques provides improved capabilities by eliminating the need for heat cycling.

This technique has great potential in the creation of cost-effective, user-friendly, and effective diagnostic instruments for ensuring food safety, clinical analysis, and environmental monitoring [29]. Phenotypic microfluidic assays, in contrast, observe the development of bacteria in the presence of antibiotics, providing precise data for antibiotic susceptibility testing (AST). Typically, bacteria are contained in tiny spaces such as chambers, channels, or droplets. This may be achieved by using antibodies on magnetic beads or membranes to catch the bacteria, or by encapsulating them in chambers with agarose and using hydrodynamic trapping. Hydrodynamic trapping is a technique used to immobilize bacteria, which may be effectively combined with microfluidics. This technology allows for the creation of densely packed trap arrays, seamless integration, scalability, and convenient biosensing.

However, it is worth noting that the trapping effectiveness is quite low. One disadvantage associated with the use of antibodies is the elevated expense, as well as the limited accessibility to certain strains. In regards to the droplet-based technology, it often necessitates costly and advanced readout techniques. The agarose-based approach may be used with normal multi-well plates, but the process of arraying is not simple. This complicates both the automatic detection and the data interpretation. Further research and advancements are necessary to overcome these constraints and make these systems economically viable. The next sections will explore many methodologies, including spectroscopy-based, colorimetric-based, pH-based, and quartz-crystal microbalance (QCM) based methods. Additionally, the discussion will include point-of-care (POC), multiplexing, single-cell, and single-molecule techniques.

3. Summary of AST Platforms Currently in the Market

The Adagio™ Antimicrobial Susceptibility Testing method, developed by Bio-Rad Laboratories, is an automated method that utilizes imaging equipment. It quantifies the diameter of the area where the growth of bacteria is inhibited by antibiotic discs. The system is equipped with advanced data management software that enables fast and precise creation of results and automatic interpretation of AST [30]. The Adagio system underwent evaluation for the automated analysis and interpretation of disk diffusion AST data in bacteria. The automated findings were visually validated and found to have good category agreement [31].

The VITEK® 2 COMPACT, developed by bioMérieux in Marcy l'Étoile, France, is a small, automated device designed to identify microorganisms and perform antimicrobial susceptibility testing (AST). It does this by minimizing the need for human intervention, therefore improving workflow efficiency and enabling faster reporting of results. The TAT (Turnaround Time) ranges from 2 to 18 hours, but it is necessary to isolate the source organism. The VITEK® 2 COMPACT is regarded as a cost-efficient and space-efficient solution. VITEK® 2 COMPACT utilizes a fluorogenic approach to identify organisms and a turbidimetric method for antimicrobial susceptibility testing (AST).

The Accelerate Pheno™ system, developed by Accelerated Diagnostics in the United States, is a completely automated system that can quickly conduct identification and antimicrobial

susceptibility testing (AST) on samples without the need for culture. It can identify samples in about 2 hours and perform AST in about 7 hours [32]. Gel electrofiltration is used to clean the samples. Fluorescence in situ hybridization is used to rapidly and completely automate the process of pathogen detection, species identification, and quantification. Additionally, it includes an automated digital microscope for morphokinetic cellular analysis (MCA), which enables the monitoring of phenotypic characteristics such as size, shape, and division rate of individual living cells. This analysis may be conducted while the cells are exposed to antibiotics, and it can also be used to estimate MIC values. The primary benefit of this system is its user-friendliness, while its major drawbacks are the limited ability for any kind of intervention and the need to exclusively process freshly obtained blood cultures.

The Alfred 60AST system, developed by Alifax, S.r.l. in Italy, is a highly advanced automated system that can do several tasks such as bacterial culture, residual antimicrobial activity (RAA) testing, and susceptibility testing. These tasks include sample inoculation, reading, and result transmission. This device utilizes light scattering to identify living bacteria, giving real-time information on growth curves and bacterial counts. Additionally, it can determine the medication resistance of the bacteria within a few hours (4-6 h) with high accuracy and precision. The combination of the Alfred 60AST system with MALDI-TOF MS for direct identification is regarded as a fast antimicrobial susceptibility testing (AST) method. The primary benefit of this system is its adaptability, since it permits user interventions. However, this flexibility may also be seen as its primary downside, as it necessitates the presence of experienced staff capable of interpreting the outcomes, such as development curves.

The MicroScan WalkAway + System, manufactured by Beckman Coulter, Inc., is capable of identifying microorganisms and providing AST results in an efficient and automated manner. It can process up to 40 or 96 panels at a time. The system requires minimum work and may provide results within 4 hours, however certain samples may need overnight incubation. BD Phoenix™, developed by Becton, Dickinson, and Company, is an antimicrobial susceptibility testing (AST) system that delivers fast, dependable, and precise outcomes by analyzing colony inoculums. The system utilizes an oxidation/reduction indicator and a turbidimetric growth detector. In addition, it is possible to process 200 identifications (ID)/AST sets in under 4.5 hours. The Sensititre™ ARIS™ 2X (Thermo Fisher) system utilizes broth microdilution, a well-accepted method, to identify bacterial pathogens and detect growing antibiotic resistance. This system incorporates automation, which saves time and improves efficiency in the laboratory, ultimately leading to better patient care. The growth measurements and endpoint MIC estimations rely on the bacterial isolates hydrolyzing a fluorogenic substrate.

GeneFluidics, Inc. provides automated systems for research purposes that may be used for both identification and antimicrobial susceptibility testing (AST). The ProMax®, UtiMax®, and BsiMax® platforms may provide identification and AST results from isolates, urine, and whole blood samples. The TAT for identification is not applicable for ProMax®, 1 hour for UtiMax®, and 6 hours for BsiMax®. The TAT for AST is 3 hours for ProMax®, 2 hours for UtiMax®, and 3.5 hours for BsiMax®. GeneFluidics' products use molecular-based identification of species-

specific phenotypic indicators to identify resistance and susceptibility. This is achieved by analyzing the change in 16S rRNA content of each target pathogen under different antibiotic settings, without the need for PCR. The detection method is dependent on an array of electrochemical sensors.

4. Summary

The emergence of antimicrobial resistance (AMR) has prompted a collaborative effort among academia, risk managers, risk assessors, government, and industry to improve the current methods for diagnosing and treating AMR. This involves the development of innovative tools that overcome the limitations of existing diagnostic and treatment approaches, including the gold standard methods and existing antimicrobial susceptibility testing (AST) methods. The primary constraints of the already accessible tools include: the need for preliminary sample processing procedures; their limited sensitivity; the inability to identify microorganisms in some instances; and the absence of integration, automation, and portability. To identify certain infections, it is necessary to follow extensive biological methods including culture, isolation, and identification. It is crucial to prioritize and work towards significant progress in developing new testing platforms that have higher performance characteristics. This will enable their timely approval and commercialization. Devoting time and exertion to enhancing current techniques, technologies, and platforms is also feasible.

References

1. McAdams, D.; Wollein Waldetoft, K.; Tedijanto, C.; Lipsitch, M.; Brown, S.P. Resistance diagnostics as a public health tool to combat antibiotic resistance: A model-based evaluation. *PLoS Biol.* **2019**, *17*, e3000250.

2. Collignon, P.C.; Conly, J.M.; Andremont, A.; McEwen, S.A.; Aidara-Kane, A.; for the World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR); Agerso, Y.; Andremont, A.; Collignon, P.; Conly, J.; et al. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies to Control Antimicrobial Resistance From Food Animal Production. *Clin. Infect. Dis.* **2016**, *63*, 1087–1093.

3. Leonard, H.; Colodner, R.; Halachmi, S.; Segal, E. Recent Advances in the Race to Design a Rapid Diagnostic Test for Antimicrobial Resistance. *Acs Sens.* **2018**, *3*, 2202–2217.

4. Ferri, M.; Ranucci, E.; Romagnoli, P.; Giaccone, V. Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2857–2876.

5. Hashempour-Baltork, F.; Hosseini, H.; Shojaee-Aliabadi, S.; Torbati, M.; Alizadeh, A.M.; Alizadeh, M. Drug Resistance and the Prevention Strategies in Food Borne Bacteria: An Update Review. *Adv. Pharm. Bull.* **2019**, *9*, 335–347.

6. Friedman, N.D.; Temkin, E.; Carmeli, Y. The negative impact of antibiotic resistance. *Clin. Microbiol. Infect.* **2016**, *22*, 416–422.

7. Roope, L.S.J.; Smith, R.D.; Pouwels, K.B.; Buchanan, J.; Abel, L.; Eibich, P.; Butler, C.C.; Tan, P.S.; Walker, A.S.; Robotham, J.V.; et al. The challenge of antimicrobial resistance: What economics can contribute. *Science* **2019**, *364*, eaau4679.

8. O'Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*; HM Government and Welcome Trust: London, UK, 2016.

9. Bell, G.; MacLean, C. The Search for 'Evolution-Proof' Antibiotics. *Trends Microbiol.* **2018**, *26*, 471–483.

10. Anderson, M.; Clift, C.; Schulze, K.; Sagan, A.; Nahrgang, S.; Ouakrim, D.A.; Mossialos, E. *Averting the AMR Crisis: What Are the Avenues for Policy Action for Countries in Europe?* European Observatory on Health Systems and Policies: Copenhagen, Denmark, 2019.

11. Raoult, D.; Leone, M.; Roussel, Y.; Rolain, J.-M. Attributable deaths caused by infections with antibiotic-resistant bacteria in France. *Lancet Infect. Dis.* **2019**, *19*, 128–129.

12. Dadgostar, P. Antimicrobial Resistance: Implications and Costs. *Infect. Drug Resist.* **2019**, *12*, 3903–3910.

13. Ben, Y.; Fu, C.; Hu, M.; Liu, L.; Wong, M.H.; Zheng, C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ. Res.* **2019**, *169*, 483–493.

14. Rafiqi, F. *Antimicrobial Resistance Benchmark 2020*; Access to Medicine Foundation: Amsterdam, The Netherlands, 2020.

15. Cansizoglu, M.F.; Tamer, Y.T.; Farid, M.; Koh, A.Y.; Toprak, E. Rapid ultrasensitive detection platform for antimicrobial susceptibility testing. *PLoS Biol.* **2019**, *17*, e3000291.

16. Dubourg, G.; Raoult, D. Emerging methodologies for pathogen identification in positive blood culture testing. *Expert Rev. Mol. Diagn.* **2016**, *16*, 97–111.

17. Giordano, C.; Piccoli, E.; Brucculeri, V.; Barnini, S. A Prospective Evaluation of Two Rapid Phenotypical Antimicrobial Susceptibility Technologies for the Diagnostic Stewardship of Sepsis. *Biomed Res. Int.* **2018**, *2018*, 6976923.

18. Cals, J.W.L.; Ament, A.J.H.A.; Hood, K.; Butler, C.C.; Hopstaken, R.M.; Wassink, G.F.; Dinant, G.-J. C-reactive protein point of care testing and physician communication skills training for lower respiratory tract infections in general practice: Economic evaluation of a cluster randomized trial. *J. Eval. Clin. Pract.* **2011**, *17*, 1059–1069.

19. Holmes, E.A.F.; Harris, S.D.; Hughes, A.; Craine, N.; Hughes, D.A. Cost-Effectiveness Analysis of the Use of Point-of-Care C-Reactive Protein Testing to Reduce Antibiotic Prescribing in Primary Care. *Antibiotics* **2018**, *7*, 106.

20. Hunter, R. Cost-effectiveness of point-of-care C-reactive protein tests for respiratory tract infection in primary care in England. *Adv. Ther.* **2015**, *32*, 69–85.

21. Papadakis, G.; Pantazis, A.K.; Ntogka, M.; Parasyris, K.; Theodosi, G.-I.; Kaprou, G.; Gizeli, E. 3D-printed Point-of-Care Platform for Genetic Testing of Infectious Diseases Directly in Human Samples Using Acoustic Sensors and a Smartphone. *ACS Sens.* **2019**, *4*, 1329–1336.
22. Tsougeni, K.; Kastania, A.S.; Kaprou, G.D.; Eck, M.; Jobst, G.; Petrou, P.S.; Kakabakos, S.E.; Mastellos, D.; Gogolides, E.; Tserepi, A. A modular integrated lab-on-a-chip platform for fast and highly efficient sample preparation for foodborne pathogen screening. *Sens. Actuators B Chem.* **2019**, *288*, 171–179.
23. Fernández-Gavela, A.; Herranz, S.; Chocarro, B.; Falke, F.; Schreuder, E.; Leeuwis, H.; Heideman, R.G.; Lechuga, L.M. Full integration of photonic nanoimmunosensors in portable platforms for on-line monitoring of ocean pollutants. *Sens. Actuators B Chem.* **2019**, *297*, 126758.
24. Yılmaz, B.; Yılmaz, F. Chapter 8—Lab-on-a-Chip Technology and Its Applications. In *Omics Technologies and Bio-Engineering*; Barh, D., Azevedo, V., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 145–153.
25. Jung, W.; Han, J.; Choi, J.-W.; Ahn, C.H. Point-of-care testing (POCT) diagnostic systems using microfluidic lab-on-a-chip technologies. *Microelectron. Eng.* **2015**, *132*, 46–57.
26. Sackmann, E.K.; Fulton, A.L.; Beebe, D.J. The present and future role of microfluidics in biomedical research. *Nature* **2014**, *507*, 181–189.
27. Kaprou, G.D.; Papadopoulos, V.; Papageorgiou, D.P.; Kefala, I.; Papadakis, G.; Gizeli, E.; Chatzandroulis, S.; Kokkoris, G.; Tserepi, A. Ultrafast, low-power, PCB manufacturable, continuous-flow microdevice for DNA amplification. *Anal. Bioanal. Chem.* **2019**, *411*, 5297–5307.

28. Zhang, G.; Zheng, G.; Zhang, Y.; Ma, R.; Kang, X. Evaluation of a micro/nanofluidic chip platform for the high-throughput detection of bacteria and their antibiotic resistance genes in post-neurosurgical meningitis. *Int. J. Infect. Dis.* **2018**, *70*, 115–120.
29. Giuffrida, M.C.; Spoto, G. Integration of isothermal amplification methods in microfluidic devices: Recent advances. *Biosens. Bioelectron.* **2017**, *90*, 174–186.
30. Strauss, M.; Zoabi, K.; Sagas, D.; Reznik-Gitlitz, B.; Colodner, R. Evaluation of Bio-Rad® discs for antimicrobial susceptibility testing by disc diffusion and the ADAGIO™ system for the automatic reading and interpretation of results. *Eur. J. Clin. Microbiol. Infect. Dis.* **2020**, *39*, 375–384.
31. Idelevich, E.A.; Becker, K.; Schmitz, J.; Knaack, D.; Peters, G.; Köck, R. Evaluation of an Automated System for Reading and Interpreting Disk Diffusion Antimicrobial Susceptibility Testing of Fastidious Bacteria. *PLoS ONE* **2016**, *11*, e0159183.
32. Humphries, R.; Di Martino, T. Effective implementation of the Accelerate Pheno™ system for positive blood cultures. *J. Antimicrob. Chemother.* **2019**, *74*, i40–i43.