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Review

# Evaluation of Detection Techniques for Antimicrobial Resistance

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## Abstract

Antimicrobial resistance is globally known term in this 21<sup>st</sup> century. When antibiotic does not work against microbes, then antimicrobial resistance occurs. Many people have become resistant to antibiotic because of their haphazard use of antibiotic. People does not maintain the proper use of antibiotic and resistance is developed. Antimicrobial Resistance (AMR) is a broad term, has now become a global concern day by day. Antimicrobial agents are utilized to treat different microbiological infection in human and animals. Antimicrobial resistance refers to the resistance of antibiotics against specific microorganisms. However, there are few methods for detecting antibiotic resistance in the laboratory. Among of them conventional and molecular techniques are popular. This review article outlines a clear description of various techniques of antimicrobial resistance detection. Previous technology and innovative future technology have been moderately described in this article.

**Keywords:** antimicrobial resistance; antibiotic; resistant; AMR techniques; conventional method; molecular method

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## 1. Introduction

Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, fungi, parasites, and viruses go changes that help them to withstand the effects of antimicrobial drugs, including antibiotics, that are intended to eliminate or control them (WHO, 2021). Antimicrobial Resistance is the most pressing global challenges of the 21st century, focused by the rapid rise in resistant infections and the limited growth of antimicrobial drugs which are new in nature to address the problem (Prestinaci *et al.* 2015). The major drivers of the present problem are the excessive or inappropriate utilize of antibiotics in multiple settings, particularly in clinical treatments, agriculture, animal health, food industry etc. (Llor and Bjerrum, 2014). Often described as the "Silent Pandemic," AMR demands urgent and effective action now, rather than being treated as an issue are considered in the future (Founou *et al.*, 2021). Previously, antibiotic was life savings drug to treat against microbes. But haphazard use of antibiotics, now-a-days, antimicrobial resistance has been developed. Misuse of drugs, overdose of medicine and frequent uses of specific antibiotic, antimicrobial resistance has become a global health concern.

AMR test is done due to determine whether microorganisms are resistant, intermediate or susceptible to various antimicrobial compounds. There are few techniques for determining antimicrobial resistance against microbes. These include phenotypic and molecular methods. Phenotypic method is a conventional method and molecular method is an advance technology. Different traditional techniques are helpful for routine lab examination but molecular methods are used in reference laboratory.

This paper will cover the phenotypic and molecular techniques of AMR, their advantages, disadvantages, limitations and future techniques for antimicrobial resistance (AMR).

## 2. Background

Some of the researchers conducted their research on antimicrobial resistance, its techniques, advanced technology, advantages, disadvantages, limitations and future technologies.

Among of them some are being described in the below:

**Yamin (2023)** wrote a review article on “Current and Future Technologies for the Detection of Antibiotic-Resistant Bacteria”. This article described about the conventional, molecular, advanced and prospective technologies of AMR test. Also stated advantages & constraints of AMR techniques. **Vasala (2020)** also wrote a review article on “Modern Tools for Rapid Diagnostics of Antimicrobial Resistance”. The aim of this article is to highlight the requirements & driving factors behind Antimicrobial Susceptibility Test (AST) development, outline the advantages and constraints of existing Antimicrobial Susceptibility Test (AST) methods, bring forth prospective new technologies suitable for point-of-care testing (POCT), and discuss the AST approaches most probable to succeed in the future. **Tang (2023)** wrote a review article on “Antimicrobial Resistance (AMR)”. This review offers an updated overview of the strategies employed by international governmental organizations, including the United Nations’ 17 Sustainable Development Goals (SDGs), to handle the obstacle of antibiotic resistance. It also examines the “One Health Approach,” a multidisciplinary framework that recognizes the interconnection between humans, animals, and the surroundings in order to achieve optimal health outcomes. **Dadgostar (2019)** wrote a review article on “Antimicrobial Resistance: Implications and Costs”. He put a note that antimicrobial resistance (AMR) is the most pressing threats to worldwide human health and has significant challenges to the proper prevention and care of infectious diseases. Despite numerous efforts over recent decades to address this issue, the global trend of AMR continues to rise without indication of decline. The abuse and overutilize of antimicrobial agents in both healthcare and agricultural settings are regarded as the primary drivers of resistance development. **Karp (2017)** had written a review article on “National Antimicrobial Resistance Monitoring System: Two Decades of Advancing Public Health Through Integrated Surveillance of Antimicrobial Resistance”. In this review article it has been described the function of the National Antimicrobial Resistance Monitoring System (NARMS) in generating data that support efforts to combat resistance and demonstrate how such programs can yield broad benefits for public health. **Acar (2002)** wrote a article on “Antimicrobial resistance: an overview”. In this article, he described that the rising antimicrobial resistance within bacterial germs of clinical importance, along with the spread of tolerant strains from hospital settings into community environments, is increasingly recognized as a serious public health threat. The use of antimicrobials—whether in humans, animals, plants, or food production can drive the progress of bacterial resistance. In particular, the utilize of these agents in livestock production is considered a major contributing factor, especially in bacterial species shared between humans and animals. **Burch (2022)** wrote an article on “Targeting current and future threats: recent methodological trends in environmental antimicrobial resistance research and their relationships to risk assessment”. He stated that antimicrobial resistance (AMR) represents an escalating threat to public health. Strengthening surveillance of genetic markers of AMR in environmental reservoirs could provide a more comprehensive understanding of its global dynamics, similar to the monitoring of SARS-CoV-2 through wastewater analysis. The objective of this study was to analyze the methods used in highly cited research on AMR within environmental engineering and agricultural systems, thereby offering insights into current practices and emerging trends for monitoring antimicrobial resistance genes (ARGs). **Kaprou (2021)** stated an article on “Rapid Methods for Antimicrobial Resistance Diagnostics”. This paper is related to recent advances in cutting-edge methods and technologies, organized across key research domains, aimed at addressing the growing challenge of AMR through the development of innovative diagnostic tools.

### 3. Antimicrobial Resistance Status in Worldwide

As far as know, WHO declared that, antimicrobial resistance (AMR) is one of the largest global health problems in the 21st century and requires immediate solutions. Infections which are common have become incurable due to the development of AMR. About much than 700,000 people kill of antimicrobial drug-resistant infections every year, and this number is likely to reach ten million by

2050 (United Nations meeting on antimicrobial resistance, 2016). According to present understanding, in EU and EEA countries about more than 33000 people are killed every year due to antibiotic-resistant bacteria. They also cause close to 900000 disability-adjusted years (Cassini *et al.*, 2019). If supportive measures are not discovered, AMR is likely to become the leading cause of death worldwide by 2050 (O'Neill, 2012). Global estimates indicate that in 2019, over 1.2 million human deaths were straightly related to AMR, and this number could rise to nearly 10 million annually by 2050 if proactive manage approaches are not adopted (O'Neill, 2012).

#### 4. Complications Associated with AMR:

AMR is recognized as a main problem to human health systems worldwide which affect not only growing nations but also developed ones (Founou *et al.*, 2017) (Prestinaci *et al.* 2015). The growing inability to care for contagious diseases with antibiotics signals an uncertain future for healthcare (Chokshi *et al.*, 2019). Contagion caused by resistant organisms result in severe illnesses, prolonged hospital stays, growing healthcare expenses, greater reliance on costly second-line treatments, and higher rates of treatment failure (Prestinaci *et al.*, 2015) (ECDC, 2017) (Shrestha *et al.*, 2018). Antibiotic resistance weakens the capability of the human immune system to combat infectious diseases and creates serious complications for vulnerable patients undergoing treatments such as chemotherapy, dialysis, surgeries, or joint replacements (Antibiotic resistance threats in the United States; 2013, 2019). In addition, man with prolonged diseases like diabetes, asthma, and rheumatoid arthritis are expected to be disproportionately affected by antibiotic resistance (CDC, 2019). The most recognized examples of AMR is methicillin-resistant *Staphylococcus aureus* (MRSA), which is linked to high mortality rates worldwide each year (Founou *et al.*, 2017). Similarly, multidrug resistant gram-negative bacteria (MDR-GNB) have made the management of infections such as pneumonia and urinary tract infections increasingly difficult (Bassetti *et al.*, 2019) (Castillo *et al.*, 2018) (Annavaiahala *et al.*, 2019). Rising resistance in diseases like tuberculosis, gonorrhoea, and typhoid fever further adds to the growing health and economic burden globally, with developing nations being particularly affected (Llor and Bjerrum, 2014). At present, about 4.1% of new tuberculosis cases are classified as multi-drug resistant (Chatterjee *et al.*, 2018). In countries such as India, the Philippines, Russia, and South Africa – where TB prevalence has historically been high – the incidence of multidrug resistant TB is projected to rise sharply by 2040 (Friedrich, 2017).

#### 5. Conventional and Molecular Techniques of AMR:

##### 5.1. Phenotypic Techniques (conventional methods)

There are several techniques of conventional methods. These directly measure the impact of antibiotics on bacterial growth. Disk Diffusion Method (Kirby–Bauer test) is a unique protocol of conventional techniques (Yamin *et al.*, 2023). Antibiotic-mixed paper disks are placed on Muller-Hinton agar plate inoculated with specific bacteria. Then after incubation for 18-24 hours of agar plate zone of inhibition is measured and interpreted as resistant, intermediate and sensitive (Yamin *et al.*, 2023). Broth Dilution Method is another type of AMR test (Yamin *et al.*, 2023). There are types of broth dilution method which are two in number. One is Macro dilution and another is Micro dilution. Macro dilution is a serial dilution of antibiotics performed in test tubes. And Micro dilution is performed on microtiter plate. Both of them determine Minimum Inhibitory Concentration (MIC). Another test is known as Ep silo meter test or E-test (Yamin *et al.*, 2023). A strip which contains antibiotic concentrations of gradient and placed on inoculated agar. Where bacterial growth intersects the strip, it is pointed and MIC is measured (Yamin *et al.*, 2023). One more popular method is agar dilution method (Yamin *et al.*, 2023). In case of agar dilution method, agar plates are used. Bacteria are injected onto agar plates which contain different antibiotic concentrations and utilized to detect MIC. Some ready instruments are utilized for AMR test such as VITEK 2, Phoenix and Micro Scan (Yamin *et al.*, 2023) They quickly detect resistance and MIC.

## 5.2. Molecular Techniques

There are few nanoscopic techniques for AMR test and these are also known as genotypic methods. These methods directly detect resistant genes. Among of them PCR is one which is a widely populated technique which identifies genes like *tetA*, *tetB*, *mecA* (MRSA), *blaCTX-M* (ESBL), *blaNDM* (carbapenemase) etc (Sales *et al.*, 2021). Another one is Multiplex PCR which detects multiple resistant genes at a time (Yamin *et al.*, 2023). Real time PCR (qPCR) is also known as quantitative PCR. Real time PCR is used for rapid detection and quantification of resistant genes (Yamin *et al.*, 2023). DNA Microarrays are utilized for screening of many resistant genes simultaneously (Yamin *et al.*, 2023). Whole genome sequencing is used for comprehensive possible of all AMR genes (Yamin *et al.*, 2023).

## 6. Advantages and Limitations of Traditional Techniques of AMR

The advantages of traditional culture-based methods for microbial detection lie in their well-established protocols, structured approach, and widespread familiarity. These techniques provide a consistent and routine framework for laboratory work, creating a predictable environment for both teaching and research. Culture methods are particularly effective in transmitting foundational knowledge, as they are well-aligned with standardized practices and clearly defined learning objectives. Moreover, direct, hands-on interaction between instructors and students enhances understanding, fosters interpersonal connections, and allows educators to better address individual learning (What are the advantages and disadvantages of traditional teaching methods, 2023).

Despite these advantages, culture-based methods also present notable limitations. They are often time-consuming and labor-intensive, as microbial growth and isolation can require extended incubation under tightly controlled conditions such as specific temperatures, oxygen levels, and selective media. Furthermore, these techniques are prone to contamination, which may compromise accuracy. Compared to modern molecular approaches—such as next-generation sequencing (NGS) and whole-genome sequencing (WGS)—traditional culture techniques are less precise and less efficient. Advanced genomic tools provide faster, more accurate, and more comprehensive insights into microbial communities and antimicrobial resistance (AMR), surpassing the capabilities of conventional methods (Strengths and weaknesses of Indian culture, 2021) (Figdor D *et al.*, 2008).

## 7. Challenges in Traditional Techniques of AMR:

Traditional antimicrobial susceptibility testing (AST) techniques including agar disc diffusion and broth microdilution, typically require 18–24 hours or longer to generate results. Such delays are often unsuitable for timely clinical decision-making, where rapid treatment is critical (Ramzan *et al.*, 2024). These techniques require mainly large sample volumes, which is difficult to obtain for certain clinical specimens (Ramzan *et al.*, 2024). Another drawback is that culture-based techniques are limited to microorganisms that can develop under laboratory conditions. As a result, non-cultivable or slow-growing bacteria carrying antimicrobial resistance (AMR) genes may go undetected (Galhano *et al.*, 2021).

While traditional phenotypic approaches can confirm the presence of resistance, they generally do not identify the underlying resistance mechanisms or pinpoint the specific genes involved (Galhano *et al.*, 2021). Food samples pose additional challenges, as inhibitory compounds may interfere with polymerase chain reaction (PCR)-based detection of AMR genes when traditional techniques are utilized (Galhano *et al.*, 2021). Furthermore, standard Polymerase Chain Reaction cannot differ between viable and non-viable cells, leading to detection of resistance genes in both. This limitation complicates accurate assessment of the actual resistance profile present in a sample (Galhano *et al.*, 2021).

## 8. Innovative Technology of AMR:

There are few innovative techniques for AMR in microbial detection. Among of them, some of technologies are described below:

- **PCR:** Molecular-based techniques, particularly Polymerase Chain Reaction (PCR), have transformed the molecular biology field by allowing fast and accurate amplification of specific DNA regions (PCR, 2023) (PCR, 2024). In this technique, short synthetic DNA fragments known as primers are designed to recognize and bind to a target region of the genome. Through a series of repeated thermal cycles, the DNA segment is exponentially amplified (PCR, 2023). PCR has a variety range of applications including the detection of genetic mutations, monitoring of gene expression, identification of disease-related genes, and genetic fingerprinting (PCR, 2023). It also has an important role in gene mapping, phylogenetic studies, and molecular diagnostics (PCR, 2023).

- **NGS:** Next-Generation Sequencing (NGS) is a revolutionary technology that has redefined genetic analysis by allowing fast and highly accurate sequencing of DNA and RNA. Dissimilar traditional Sanger sequencing, NGS can simultaneously sequence hundreds to thousands of genes or even entire genomes within a short time, providing a much higher throughput and efficiency (Qin D, 2019) (What is Next Generation Sequencing, 2023). NGS has extensive medical utilization including disease diagnosis, prognosis, therapeutic guidance, and patient monitoring, establishing it as a cornerstone of personalized precision medicine (Qin, 2019). Its scalability, high speed, and capability to detect genetic variants at very low allele frequencies have transformed genetic analysis, paving the way for advancements in genomic research, clinical diagnostics, reproductive health, and various other scientific disciplines (What is Next Generation Sequencing, 2023).

- **Microarray Technology:** Microarray technology is an advanced molecular technique that allows comprehensive genetic analysis across different organisms, facilitating the detection and diagnosis of bacterial, viral, fungal, and parasitic diseases at both the genus and species levels. This technology performs an important part in analyzing gene expression, identifying specific DNA sequences such as single-nucleotide polymorphisms (SNPs), and performing genome-wide association studies (GWASs) (Microarray Technology. 2024) (Technologies in Molecular Diagnostics, 2019). Microarrays are highly effective in detecting large genetic defects, copy number variations (CNVs), and SNPs, forming them vital instruments for gene expression profiling, cancer diagnostics, investigations of Mendelian disorders, and assessments of reproductive health (Technologies in Molecular Diagnostics, 2019).

- **Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF):** Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a cutting-edge systematic method that electrifies samples into charged particles, enabling precise measurement of their mass-to-charge ( $m/z$ ) ratios (MALDI-TOF Mass Spectrometry, 2023) (Nenoff, *et al.*, 2013). This innovation provides notable benefits for analyzing biomolecules such as peptides, lipids, saccharides, and other complex organic macromolecules without causing fragmentation or degradation. It is particularly effective for studying large biomolecules that are prone to breakdown under conventional analytical methods (MALDI-TOF Mass Spectrometry, 2023). MALDI-TOF MS plays a crucial role in the rapid detection of oligonucleotide fragments, allowing verification of synthesis efficiency and sequence accuracy. It is globally acknowledged for its clarity, rapidness, precision, and high sensitivity in determining oligonucleotide sequences (MALDI-TOF Mass Spectrometry, 2023). Moreover, its application in MALDI Imaging Mass Spectrometry (MALDI-IMS) enables direct profiling and visualization of proteins within tissue sections, offering detailed insights into the molecular composition, relative abundance, and spatial distribution of peptides and proteins (MALDI-TOF Mass Spectrometry, 2023). Overall, this versatile technology is capable of analyzing a wide variety of biomolecules, generating low-energy ions with minimal fragmentation, and providing quick information procurement with least maintenance requirements (MALDI-TOF Mass Spectrometry, 2023).

- **Electrospray ionization MS (ESI-MS):** Mass spectrometry (MS)-based techniques, especially Electrospray Ionization Mass Spectrometry (ESI-MS), have revolutionized analytical chemistry by offering a highly effective means to study molecular species and their interactions, including association and dissociation processes (Banerjee and Mozumder, 2012) (Ho *et al.*, 2003).

Electrospray Ionization Mass Spectrometry (ESI-MS) is a gas-phase analytical technique that employs a “soft” ionization process to generate ions while conserving the anatomical integrity of macromolecules, enabling the determination of intact molecular species (Siuzdak, 2004). This method is mainly beneficial for studying bio macromolecules such as proteins, peptides, and oligosaccharides, as it minimizes fragmentation that typically occurs during ionization (Electrospray Ionization Mass Spectrometry, 2018)

- **Biosensor Technology:** Biosensors are essential analytical tools that combine a biological recognition element with a physical transducer to detect specific chemical substances. They are globally utilized in diverse areas as for example hospital diagnostics, surroundings monitoring, food safety, and pharmaceutical research. In a biosensor, the biological component interacts with the target analyte, generating a signal that the transducer converts into a measurable and quantifiable output (Mehrota, 2016). Advances in nanotechnology have led to the advancement of highly sensitive and compact biological detectors which are important for rapid, cost-effective analyses in disease diagnosis, healthcare, and environmental monitoring. Ongoing innovations in biosensor design focus on combining different sensor types and transduction methods to improve detection performance and meet emerging analytical challenges (Vigneshvar *et al.*, 2016).

## 9. Conclusions

Antimicrobial resistance has become one of the most serious threats to global public health. The rapid rise of resistant microorganisms, combined with the slow development of new antibiotics, has made accurate and timely detection of resistance essential for effective treatment and control. This review highlights that conventional phenotypic methods such as disk diffusion, broth dilution, and E-test remain important for routine antimicrobial susceptibility testing because they are standardized, cost-effective, and widely used. However, these techniques are time-consuming and may fail to detect specific resistance mechanisms at the genetic level. Molecular and advanced technologies, including PCR, multiplex PCR, real-time PCR, microarray, whole genome sequencing, MALDI-TOF MS, ESI-MS, and biosensor-based approaches, provide faster, more sensitive, and more precise detection of antimicrobial resistance genes. These techniques not only identify resistance mechanisms but also support surveillance, epidemiological tracking, and informed therapeutic decisions.

Overall, both conventional and molecular methods have distinct strengths and limitations. An integrated approach that combines phenotypic testing with advanced molecular diagnostics offers the most reliable strategy for accurate detection and effective management of antimicrobial resistance in clinical, veterinary, food, and environmental settings.

## 10. Future Scope

The future of AMR detection lies in combining rapid phenotypic testing, advanced molecular diagnostics, real-time data analytics, and global surveillance systems. Continued innovation, interdisciplinary collaboration, and integration of technology into routine healthcare practice will be essential to effectively manage and contain antimicrobial resistance worldwide.

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