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BEYOND SUSCEPTIBILITY TESTING: INNOVATIVE APPROACHES FOR MDR PATHOGEN DETECTION

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ABSTRACT The development of drug resistant bacteria remains a serious concern to global health issues. This review article presents new ways of screening that have been developed by many scientists. The introduction of molecular assays including polymerase chain reaction (PCR) and whole-genome sequencing (WGS) changed the landscape of currently available methods for detecting multidrug-resistant (MDR) pathogens which have the optimal accuracy and efficiency. However, phenotypic approaches such as antimicrobial susceptibility testing (AST), and MALDI-TOF MS are essential parts of the confirmation of the resistance mechanisms. CRISPR-Cas systems have initially been discovered as bacterial immune systems but in recent years they have become effective as well in the detection and treatment of MDR strains. Based on available literature, we identified the potential of these techniques for improving control and treatment of infections in clinical practice. Application of artificial intelligence in data analysis aimed at the rapid emergence of screening methods and novelties in the field of targeted antimicrobial therapy using the CRISPR-Cas systems. All such measures would in future enhance the fight against multidrug resistant pathogens and safety of the global population.

Keywords: *Bacteria; Approaches; Pathogen; Drug; Polymerase*

INTRODUCTION

The ongoing emergence and spread of multidrug-resistant (MDR) pathogens is a serious concern in terms of global health, as this undermines the effectiveness of standard antimicrobial therapies (Taj et al., 2024). As these pathogens become more common, it is imperative to make vigorous efforts to develop novel detection methods on time (Aljeldah., 2022). These methods would result in faster clinical diagnosis and appropriate therapeutic treatment of patients. As important as they are, traditional tests for susceptibility usually have some disadvantages including tedious processes and within them, erroneous results. On the other hand, other methods covering this or similar aspects have been further developed, as there is a constant search for improvements on what is currently further developed. In any case, this paper will explore and indicate these approaches towards the identification of the MDR pathogens. The utilization of molecular techniques appears to be the one of the most advantageous one. There are different types of polymerase chain reaction (PCR) that many microbiologists have utilized in amplification and diagnosis of genes that confer drug resistance (Kaprou, Bergšpica, Alexa, Alvarez-Ordóñez, & Prieto, 2021). Of late, however, whole genome sequencing can be mentioned as one more method, as it offers information about antibiotic resistance genes within a pathogen's genome. WGS coupled with other experimentation, makes it possible to determine the strains exhibiting multi-drug resistance because of the existence of ways for drug counter-actions (Köser, Ellington, & Peacock, 2014).

In addition to molecular methods, phenotypic testing remains essential to MDR pathogen detection (Muntean et al., 2022). Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has become a standard in protein composition-based rapid identification of microorganisms (Singhal, Kumar, Kanaujia, & Virdi, 2015). This technology is applicable to assess possible MDR strains through susceptibility phenotype initial screening. In addition to the established approaches, new technologies aimed at increasing the detection rates of MDR pathogens are also being developed. Diagnostic applications of the bacterial immune system CRISPR-Cas were also developed (Y. Wang et al., 2022). These can be rationally designed to detect specific DNA sequences of interest that confer drug-resistant phenotype and are quick and efficient. In addition to this, biosensing technologies and microfluidics are also advancing toward the development of point-of-care testing that will enable rapid testing in low-resource settings (Siavashy et al., 2024).MDR

pathogens detection and characterization studies incorporating classical as well as new screening methods have recently shown great success. In particular, one study focused on the use of CRISPR-Cas systems to target antibiotic resistance determinants within an MDR pathogen of global concern.

The study successfully employed the ATTACK system, which uses a combination of toxin-antitoxin modules and CRISPR-Cas to kill resistant bacteria. A further study was based on the clinical performance of WGS in Escherichia coli and Staphylococcus aureus isolates (Yehouenou et al., 2021), demonstrating that all known resistance genes were detected using this method with a very broad range profile indicating how helpful it might be for choosing appropriate treatment patterns. Cell composition This review will give an overview of these new approaches to MDR-pathogen detection and compare their strengths, weaknesses, and potential applications or use cases. Gaining knowledge of current advances may allow healthcare professionals and researchers to choose the most suitable approaches for detecting MDR pathogens, responding effectively (R. Wang et al., 2023).

Methods for Screening MDR Pathogens:

1. Phenotypic Methods

Since the beginning of microbiology, phenotypic approaches that depend on the observable traits and actions of microorganisms have been essential. Traditional culturing methods, biochemical assays, antibiotic susceptibility testing (AST), and procedures based on microscopy are some of these techniques (Smith & Kirby, 2019). Phenotypic techniques continue to be essential in clinical diagnostics, microbial taxonomy, and environmental microbiology because of their affordability and usefulness, even if molecular technologies have largely replaced them in recent years. The capacity of phenotypic approaches to offer details on the physiology and metabolic capacities of microorganisms is one of its main benefits. Microbes can be identified using standard culture methods such as pigmentation, colony shape, and growth patterns, such as streaking on agar plates, broth enrichment, and differential media (Karnieli et al., 2017). Based on their enzymatic activity, biochemical assays such as urease, oxidase, catalase, and carbohydrate fermentation are utilized to further describe microbial species. Antibiotic susceptibility testing (AST), which includes techniques like broth dilution, E-test, and disk diffusion (Khan, Siddiqui, & Park, 2019), is still essential for directing clinical treatment decisions since it establishes a bacterial strain's antibiotic sensitivity.

A variety of microscopy techniques, including light and fluorescence microscopy, allows the viewing of cellular structures, biofilms, and microbial cells (Puri, Fang, & Allison, 2023). Gram staining, acid-fast staining, and spore staining, for instance, are useful for identifying different bacterial species according to the features of their cell walls, and advanced microscopy methods, such as confocal laser scanning microscopy (CLSM), have proven vital in the study of biofilms and the architecture of microbial populations. Phenotypic techniques have significant drawbacks despite their many advantages, including ease of use, low cost, and broad availability. Clinical decision-making can be delayed by the long processing times of traditional culture-based techniques, which can take up to 48 hours to produce results (Giuliano, Patel, & Kale-Pradhan, 2019). This is particularly true in situations of severe infections. Furthermore, under typical laboratory conditions, a large number of microorganisms are viable but non-culturable (VBNC) (Pazos-Rojas et al., 2023), which neglects the diversity and quantity of microbes in environmental samples and clinical situations. In many situations, precise identification and quantification is restricted by the incapacity to cultivate specific species (such as some diseases or extremophiles).

Additionally, because antibiotic susceptibility studies involve culturable bacteria and yield results that are not always representative of in vivo circumstances, they have certain limitations. For example, the effects of biofilm development and intracellular persistence, which frequently need greater antibiotic doses for successful therapy, cannot be taken into account by AST. The interpretation of results from culture and biochemical assays can also be complicated by phenotypic plasticity, the ability of microbes to modify their characteristics in response to their surroundings. For instance, under specific conditions, microorganisms may display alternative metabolic pathways or resistance mechanisms, resulting in variations in test results (Reygaert, 2018). Furthermore, despite being helpful, microscopy-based approaches can be subjective and require specific training for suitable interpretation. Confocal microscopy accessibility in smaller labs may be restricted due to the expense of equipment and expertise in the field.

2. Molecular Methods*:*

Molecular techniques have revolutionized microbiology by offering instruments for efficient microbe identification, characterization, and functional study. Microbiologists may now examine microbial populations at the genetic level thanks to techniques like metagenomics, next-generation sequencing (NGS), and quantitative real-time PCR (qPCR) (Dreier et al., 2022), which reveal diversity and roles that previous methods frequently neglect (see Table 1). These developments, which provide quicker, more precise, and sensitive microbe detection and quantification, are essential for environmental microbiology, clinical diagnostics, and food safety. Since quantitative PCR has a high sensitivity and specificity, it is still frequently utilized to detect pathogens. Whole-genome sequencing (WGS), made possible by NGS, has completely changed the field of microbiological study by providing an extensive understanding of microbial genomes, their evolution, and their function in illness (Hilt & Ferrieri, 2022). Studies on pathogen evolution, monitoring of antibiotic resistance, and epidemic tracking have all expanded with the use of WGS in pathogen surveillance. Studies of complex environments, such as the human microbiome, soil ecosystems, and water bodies, have benefited greatly from the application of metagenomics, which enables researchers to examine complete microbial populations

CRISPR-Cas systems have become a potent tool in molecular microbiology in recent years. CRISPR-Cas9 and related systems, which were first developed as a bacterial immune defense

mechanism (Loureiro & da Silva, 2019), have been used to modify specific genes in bacteria and other animals. The technique gives scientists previously unattainable control over gene function by enabling them to delete, alter, or regulate particular genes in the genomes of microorganisms. CRISPR-based methods are being utilized in microbiological research to investigate the roles of genes, modify microbial strains for biotechnology, and create CRISPR-based diagnostics that can quickly and very specifically diagnose diseases (Puig-Serra et al., 2022). As mentioned in Table 1 CRISPR-Cas diagnostics, like SHERLOCK and DETECTOR, are becoming more and more renowned because they are incredibly sensitive instruments that can identify viral and bacterial DNA with little to no equipment, which makes them perfect for point-of-care applications. Even with these advances, there are still a few restrictions. For example, NGS and WGS platform prices can be unaffordable, particularly in environments with restricted resources. Furthermore, accurate analysis and interpretation of the enormous amount of data generated by these technologies require the use of sophisticated bioinformatics tools and knowledge (Pereira, Oliveira, & Sousa, 2020). Several molecular approaches are similarly limited in their ability to distinguish between living and dead cells, which can be challenging when attempting to examine microbial viability in biofilms or clinical samples. Furthermore, even though CRISPR-Cas technologies are precise, more development and regulatory monitoring are needed because of off-target consequences and ethical concerns with gene editing.

A comparison of multiple screening methods demonstrates the trade-offs between resource requirements, accuracy, and speed. Although many clinical laboratories still use phenotypic methods as the gold standard, molecular tests, and CRISPR-based techniques provide quicker and more accurate alternatives, especially for identifying new or emerging resistance mechanisms. There is great potential for the CRISPR-Cas system in terms of screening and therapeutic uses in the future. However, sustaining CRISPR-Cas system stability in a variety of bacterial populations and preventing the emergence of CRISPR-based antibiotic resistance remain difficult tasks. Using CRISPR in conjunction with traditional methods may improve the management of multiresistant bacteria.

Table 1: Overview of Molecular Methods in Microbiology

Methods	Application	Benefits	Challenges
qPCR	Pathogen detection, gene quantification	Rapid, sensitive, and quantitative analysis	Requires high-quality DNA and expensive equipment
NGS (Next-Generation Sequencing)	Genome sequencing, microbial diversity studies	High-throughput, detects unculturable microbes, comprehensive	Expensive, requires complex bioinformatics analysis
WGS (Whole Genome Sequencing)	Complete genome analysis, epidemiology	Provides full genetic information, tracks evolution	High cost, time-consuming data analysis
Metagenomics	Environmental microbiology, microbiome studies	Studies whole communities without culturing	Difficulty in interpreting mixed microbial community data
CRISPR-Cas	Gene editing, pathogen detection	Highly specific, versatile for diagnostics and gene function	Off-target effects, delivery challenges in live microbial systems

CONCLUSION

It is essential to screen for multidrug-resistant bacteria to effectively control and manage infections. Clinical decision-making and a thorough understanding of resistance mechanisms require a combination of phenotypic, molecular, genomic, and fast diagnostic techniques. Even if every approach has advantages and disadvantages, technological developments keep improving our capacity to identify and categorize MDR pathogens. Future efforts to solve the problems caused by antibiotic resistance are expected to heavily rely on integrated strategies that fuse genetic insights with quick diagnostics. To make these strategies more accessible and optimized for a range of healthcare environments especially those most impacted by the escalation of multidrug resistance further research is required. Public health officials, physicians, and microbiologists should work together more closely.

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