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Review Article

Unmasking bacterial defenders: cutting-edge antimicrobial resistance detection innovations

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ABSTRACT

Antimicrobial resistance (AMR) records a resounding global health alarm, posturing ample challenges to the effectual treatment of bacterial infections. As the predominance of drug-resistant bacteria continues to rise, the improvement and implementation of advanced techniques for AMR detection have become essential. This review offers a broad overview of the up-to-date methodologies employed in the identification and classification of antimicrobial resistance in bacteria. In recent years, genomic approaches have revolutionized AMR detection, allowing for rapid and accurate evaluation of resistance genes and mutations. Whole genome sequencing, coupled with bioinformatics tools, has enabled the chasing of resistance evolution and transmission dynamics. Additionally, transcriptomics and proteomics have explained the underlying mechanisms of AMR, assisting in the development of targeted therapies. Furthermore, advanced phenotypic assays, such as microfluidic systems and mass spectrometry-based techniques, have improved our skill in assessing bacterial susceptibility to antibiotics. The addition of artificial intelligence and machine learning algorithms has simplified data analysis and expectation of resistance profiles with high precision. This review highlights the benefits and restraints of each technique and reflects their potential for clinical and public health applications. The continuing challenges in AMR detection, including the emergence of novel resistance mechanisms and the need for regulation, are also addressed. The review concludes that more accurate, precise, and economical methods are needed to understand the exact situation of resistance to adjust the management strategies accordingly.

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Introduction

When microorganisms, including bacteria, fungi, parasites, and viruses are involved, Antimicrobial Resistance (AMR) emerges which undergoes evolutionary changes that lead to their reduced susceptibility to the antimicrobial drugs, such as antibiotics, that are commonly employed for their treatment (Morrison and Zembower 2020). In the

21st century, AMR has elevated to become a paramount global apprehension, primarily due to the swift escalation of AMR infection occurrences and the scarcity of novel antimicrobial drugs being introduced to address this pressing challenge (Prestinaci et al. 2015). AMR is commonly dubbed the "Quiet Pandemic," demanding immediate and more efficient measures for management rather than being regarded as a concern that can be addressed in the future (Founou et al. 2021). In the absence of preventive actions, projections suggest that by 2050, the Antimicrobial Resistance (AMR) might emerge as the leading global contributor to mortality. In light of AMR, numerous international health organizations and governments have initiated measures to address this concern. The "One Health Approach" was established, necessitating a worldwide collaborative endeavor involving a diverse array of disciplines. This includes organizations like the World Organization of Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), which make sure that each organization operates within its area of competence while working with others to lessen the potential effect of AMR. The Ministry of National Health Services announced the release of Pakistan's National Action Plan (NAP) for AMR in May 2017**.** In Pakistan, national and provincial policies have been started based on the NAP (Saleem et al. 2022).

With the development of accessible whole genome sequencing (WGS) technology, the study of infectious illness is changing quickly, along with other medical and scientific disciplines (Didelot et al. 2012; Köser et al. 2012). These technologies are quickly becoming standard operating practices, which is changing how laboratories conduct their work. Tens of thousands of people die each year as a result of the worldwide health issue known as AMR. Historically, the detection of AMR has been used to gauge how a chemotherapeutic agent's growth-inhibitory actions have affected a bacterial population cultivated under certain laboratory conditions. Clinical laboratories still heavily rely on diffusion and dilution techniques, despite some auxiliary improvements, to direct clinical therapy and track AMR over time. The genetic sequence of many bacteria may be used to predict AMR with increasing accuracy, according to accumulating research. Strong bioinformatics tools are needed for the sequence-based approach to AMR detection to examine and visualize the genomic structure of the microbial "resistome," which is characterized by AMR genes and their predecessors (Cartwright et al. 2013). This paper provides a summary of the state-of-the art of employing new technologies to monitor AMR globally.

Significance of AMR

Major clinical implications of AMR

Antimicrobial resistance makes it difficult to effectively treat a variety of illnesses brought on by bacteria, fungi, and viruses. Without innovative medications, the effectiveness of cancer treatment, organ transplants, and even simple dentistry procedures would be threatened. Our capacity to cure common diseases like typhoid and the flu is threatened by the creation of novel resistance mechanisms inside the bacteria. As a result, patients will experience longer sickness and treatment, lifelong impairment, or even death. Antibiotic-resistant patients incur greater healthcare expenditures than non-resistant patients due to mandatory lengthy treatment and the requirement for pricey medications (Muhwezi et al. 2022).

Challenges

Effective surveillance and persistent study about the influence and worldwide inevitably epidemiology will exist given that AMR is still an issue on a worldwide scale that determines a therapeutic strategy that works. Recent research has mostly focused on assessing the prevalence of antibiotic resistance and measuring the burden of AMR. These studies show a significant vacuum in the information that is available on prevalence, failure of treatment, frequency, and kinds of opposition in various places, particularly in middle and developing nations. Effective worldwide policies have, however, been launched for the monitoring of the present scenario and line up new diagnostic techniques, developing vaccines and medications, and avoiding antibiotic resistance in the future (Väänänen et al. 2006; Wright, 2014; World Health, 2015). This may be facilitated by introducing an evidence-based approach, precise data-gathering techniques, educating foreign policymakers, creating better standards and procedures, sort out investment decisions and identifying significances.

The burden of antimicrobial resistance

The healthcare system, global health, a country's GDP, and its population all experience major costs and health burdens as a result of antibiotic-resistant illnesses (Golkar et al. 2014). According to a recent UK study, AMR would allegedly cost the global economy $100 trillion annually. Antibiotic resistance is more deadly than cancer as it causes 10 million deaths annually (Azimi et al. 2019). AMR has potential effects on morbidity, mortality, and associated healthcare expenditures, in terms of finances, however, it can lead to yield loss, patient extra expenditures like the excess cost of hospital treatment owing to protracted treatment procedures, and declining GDP for the countries. This is because it places additional strain on resources like hospitals and primary care clinics, as well as insurance (Osgood-Zimmerman et al. 2018). Several methods, such as morbidity and death rates, treatment costs, and disability-

adjusted life years, can be used to calculate the burden (World Health, 2014). However, determining the true cost of AMR is filled with difficulties. Main causes are the lack of accurate data on the prevalence, incidence and division of AMR, which marks it difficult to estimate the impact (Woolhouse et al. 2016). As a result, we are less able to put AMR control techniques into practice. In addition, data from the public and private sectors are not gathered at the highest levels and provide insufficient details on specific patients' conditions and the results of their medical treatments. With regard to selection bias, there are further problems, the choice of a subject for AMR testing and the input of results into data systems, for instance (Thompson et al. 2016).

Forms of Resistance

1. Natural resistance (Intrinsic, Structural)

This type of resistance is not correlated with the use of antibiotics but rather results from the structural features of the bacteria (Kadhum and Hasan 2019). NR occurs as a consequence of inherent resistance, bacteria that do not stick to the target antibiotic's structure that are having to their properties, does not interact with their envisioned target (Waglechner and Wright, 2017). For example, vancomycin medicines, for instance, do not pass through the superficial membrane of Gram-negative bacteria, making them intrinsically resistant to vancomycin. (Antonoplis et al. 2019). Bacteria having no cell wall which include the bacterial species *Ureaplasma* and *Mycoplasma*, are inherently resistant to beta-lactam antibiotics. (Claessen and Errington, 2019).

2. Acquired resistance

Despite the fact that bacteria's genetic makeup has changed and resistance has developed as a result, acquired resistance still exists since the antibiotics that were formerly effective against the bacterium have no effect on it (Andersson et al. 2020). Plasmids and transposons are the cause of this kind of resistance (Aljanaby and Aljanaby, 2018). Bacterial chromosomes can alter randomly, leading to chromosomal resistance. Certain physical and chemical variables can cause these mutations to happen (Majeed and Aljanaby, 2019). This can be as a result of changes in the temperament of bacterial cells, which might be result in minimum bacterial drug permeability, or it might be because the target of medication inside the cell has changed (Al-Harmoosh et al. 2017). Antibiotic resistance (AR) can form to specific types of streptomycin, aminoglycosides, erythromycin, and lincomycin (Krause et al. 2016). DNA fragments known as plasmids can reproduce independently of chromosomal DNA (Thomas and Frost, 2014). Enzymes that are resistant to antibiotics are often produced by plasmids (Witwit et al. 2019). The four basic mechanisms by which bacterial cells retain hereditary material (resistance genes and plasmids), including transduction, transformation, conjugation, and process of transposition (Hasan and Al-Harmoosh, 2020). Antibiotic resistance genes are interweaved and placed at the beginning of several integrons on the chromosome or plasmid. In integrons, recombination is quite common (Adam et al. 2019).

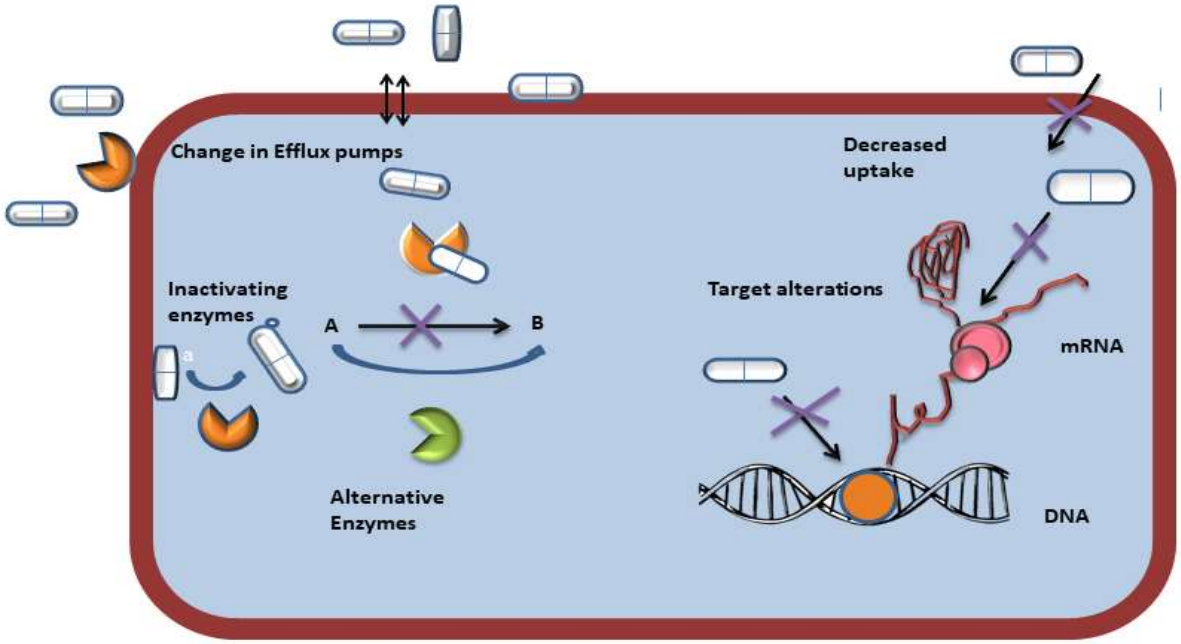
3. Cross-resistance

It speaks about a particular pathogen's resistance to one antibiotic when it also uses comparable mechanisms for resistance to other antibiotics (Etebu and Arikekpar, 2016). Antibiotics having similar chemical structures, such as erythromycin, neomycin, kanamycin, or cephalosporin and penicillin, are common examples of those that exhibit resistance (Jahne et al. 2015). Cross resistance may be chromosomal or non-chromosomal for example cross resistance between erythromycin and lincomycin (Ng, 2019).

4. Multi-drug and other types of resistance

Pathogens that has formed a resistance to their medications are often multidrug-resistant species, which makes it impossible for the germs to be controlled or eradicated by a single medication (Alanis, 2005). The development of multidrug resistant pathogenic bacteria was the result of improper use of antibiotics for therapy (Dheda et al. 2017). There are two basic processes used by bacteria to resist certain medications (Rello et al. 2016). Bacteria must first create a variety of genes, each of which codes for a certain type of antibiotic resistance (Tóth et al. 2020). Secondly, increased efflux pumps for gene expression, enzymatically inactivation of antibiotics, changes in target sites and other processes can also result in multidrug resistance (Salloum et al. 2020). Bacterial strains that are resistant to three or more antibiotic classes are referred to be MDR bacteria. If a species is thought to be extremely resistant to medications if it is resistant to all classes of useful antibiotics save one or two, is it considered to be pan-drug resistant if it is resistant to all types of usable antibiotics (Al-Harmoosh and Jarallah, 2015; Stanford et al. 2020). Multidrug resistance (MDR) is shown by *Acinetobactor* species for instance, are bacteria that are resistant to antibiotics from at least three different groups, such as quinolones, cephalosporins, aminoglycosides and all forms of penicillin (Escudero et al. 2018). In addition to polymyxin-resistant and tigecycline resistance, these bacteria may be highly drug resistant (XDR), become resistant to the three previously described antibiotic classes (MDR), and even be carbapenem resistant (Nikaido, 2009; Al-Harmoosh et al. 2016).

Fig. 1: Mechanism of antimicrobial drug resistance



Mechanisms of Antibiotics Resistance

A. The modifications

When it comes to the relationship between antibiotics and their target areas, which m complicated enzymes and ribosomes.ight include changes that occur in the drug-related receptor and their location are unique (Prashanth et al. 2012). The macrolide antibiotic class has the most often found resistance that corresponds to modifications in the ribosomal target (Shaikh et al. 2013). The most well-known examples here include strains of *Neisseria meningitides*, *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Enterococcus faecalis* that have evolved penicillin sensitivity as a result of changes in beta-lactamase enzymes and penicillin-binding proteins (Southon et al. 2020).

B. Enzymatic inactivation of antibiotics

Enzymatic inactivation is one of the main pathways of antibiotic resistance since the majority of bacteria produce enzymes that break down antibiotics (Pérez-Llarena and Bou, 2016). The most well-known representatives of this class comprise the modifying enzymes for beta-lactamases, amino glycosidase, chloramphenicol and erythromycin. (Sharkey and O'Neill, 2019).

C. Reduction of the inner and outer membrane permeability

This mechanism of rapid ejection and deviations from the pump systems a reduction in the permeability of the cell's internal and external membranes leads to a reduction in medication absorption (Santajit and Indrawattana, 2016). For instance, a mutation in a particular porin termed OprD might result in carbapenem resistance in a strain of *Pseudomonas aeruginosa* as a result of a reduction in membrane permeability caused by porin changes which might develop in the proteins of resistant strains (Nikaido and Pagès, 2012). Quinolone resistance and resistance to aminoglycosides can both be significantly impacted by a reduction in the permeability of the outer membrane (Li et al. 2012).

D. Active pumps system

The active pump systems are the primary mechanism through which resistance to the tetracycline class of antibiotics arises (Breidenstein et al. 2011). Tetracycline active pumping mechanism, which depends on energy, prevents them from concentrating inside and controlling chromosomal structures. For instance, active pumping systems can survive quinolones, beta-lactams, chloramphenicol, and 14-membered macrolides (Guo et al. 2020).

Commonly used techniques

A. Classical Methods:
1. Dilution Methods: Broth Dilution and Agar Dilution
2. Antimicrobial Gradient Method:

Despite the introduction of new technologies, conventional technologies are still widely used to gather data on bacterial sensitivity to antimicrobial drugs.

Dilution method comprises of agar dilution and broth dilution

1. **Dilution methods: broth dilution and agar dilution**

Agar dilution principle

In agar dilution method solid agar plates are prepared each containing different quantity of the antimicrobial agent. Microbial cultures are spread on the agar plates with different antimicrobial concentrations. The plate with the peak concentration that inhabits microbial growth represents MIC (Ma, 2006). The minimum inhibitory concentrations (MICs), or the lowest concentration at which an antimicrobial agent inhibits the growth of bacteria, may be found using the broth and agar dilution techniques (Golus et al. 2016; Vasilijević et al. 2019). The MIC value is used to determine the pathogen's susceptibility category to a particular antibiotic, as well as for organisms that produce unclear findings, particularly when there are no clinical breakpoints for disc diffusion.

Broth dilution principle

Microbial cultures are spread on the broth plates with different antimicrobial concentrations. The plate with peak antimicrobial concentration inhibits microbial growth, while the plate with the lowest concentration allows growth (Kowalska-Krochmal and Dudek-Wicher, 2021).

Advantages: Using broth dilution techniques has the advantage of enabling the measurement of MICs as well as the possibility of determining the minimum bactericidal concentration (MBC), which is the lowest concentration of an antimicrobial agent effective against 99.9% of bacteria (Balouiri et al. 2016). The in-tube dilution test, sometimes referred to as the macro dilution method, uses a matched media and repeated two-fold dilutions of antibacterial agents. The prepared tubes are filled with a known concentration of suspended bacteria as specified in (Balouiri et al. 2016). After 24 hours of incubation at 37 °C, the turbidity of the medium is utilized to measure bacterial growth, allowing for the visual computation of MIC values. The BMD technique is standardized, precise, and reasonably priced. Due to the use of 96-well micro titer plates, it is possible to test many antimicrobial compounds at once as well as a single plate; eight series of antimicrobial compounds were diluted two times. Following the creation of the dilutions, each well receives an injection of standardized bacterial inoculum, which is then incubated for at least 16 to 24 hours. Even though this procedure is thought of as conventional, adding a resazurin color redox indicator has improved it. Resazurin is a blue substance, when there are bacteria that are actively using their energy and transform into the pink, luminous substance resorufin. Fluorimetric measurements can be made to determine the decrease of resazurin to fluorescent resorufin (Jorgensen and Ferraro, 1998; Balouiri et al. 2016; Nikolić et al. 2016; Foerster et al. 2017; Spencer et al. 2020).

Before the non-selective medium solidifies, the agar dilution process includes adding various quantities of antimicrobial agents (Wiegand et al. 2008). The predefined inoculum of bacteria is then deposited on the surface of the agar in a specific location. After incubation whole night examined to see whether the infected areas have expanded. The minimum effective concentration (MIC) of an antibiotic is thought to be necessary to limit bacterial growth. This technique enables the examination of several bacterial strains simultaneously (Mirajkar and Gebhart, 2016).

Advantages and disadvantages

The gradient strip test combines the beneficial aspects of the disk-diffusion and dilution AST procedures. It makes the process of determining the minimum inhibitory concentration (MIC) straightforward and user-friendly. The process relies on a continual gradient-based diffusion of an antibiotic through agar. Gradient tests provide a number of benefits that are well recognized, including their ease of use, versatility in testing any antibiotic combination, and lack of need for particular knowledge or technology. Additionally, their use is appropriate when only a few antibiotics need to be evaluated. Gradient tests are sometimes limited to testing a few antibiotics per strain due to the high cost of each strip in comparison to the cost of discs. Given the availability of faster automated techniques with the accurate measurement of MIC, the gradient tests' incubation period of 16 to 24 hours can be considered a drawback.

Principle

In this method first of all prepare a culture plate of solid agar medium (eg. Mueller-Hinton agar for bacteria or Sabourad agar for fungi). In the culture plate place a gradient strip at specific temperature and time so that microorganisms grow on the agar surface. When the microorganism grows, they encounter different concentrations of antimicrobial agent from the strip. The concentration at which the growth of microorganism intersects with the strip corresponds to MIC (Humphries et al. 2018; Shields et al. 2018).

Different strips of antimicrobial gradient used for antibiotic resistance

For the identification of distinct AMR phenotypes, several gradient tests are available. Currently, there are tests (Epsilometer assays) for the phenotypic detection of enterobacterial ESBL generation, includes cefepime and clavulanic acid-containing strips, ceftazidime and clavulanic acid-containing strips (Pereckaite et al. 2018). Two-sided strips with antibacterial on one side and the other side, the same antibiotic combined with clavulanic acid of the gradient tests for ESBL finding. ESBL generation is indicated by an antibiotic and clavulanate combination that reduces the MIC by at least eight times (Garrec et al. 2011). Additionally, the zone beneath the clavulanic side implies a favorable outcome, like to the double-disk test. A gradient test can be used to identify bacteria that produce metallo-beta-lactamase (MBL). On one side

of the test strip for these tests is the antibiotic carbapenem, and on the other is the same antibiotic mixed with EDTA. It is possible to use imipenem with EDTA to detect MBL in *Acinetobacter* species and *Pseudomonas* species, albeit sensitivity and specificity may differ (Guzel et al. 2018). To find enterobacteria that generate ampC beta-lactamases, use an E test that is cefotetan-cloxacillin impregnated on one side and cefotetan on the other side (Peter-Getzlaff et al. 2011). *Staphylococcus aureus* glycopeptide resistance can be found using gradient assays, which employ a preset slope of teicoplanin and vancomycin on either side of the strip (Yusof et al. 2008). These tests may be carried out quickly, making them suitable for use as "screening" assays to find new resistance patterns in clinically important bacteria.

Disk diffusion test

Principle

On the surface of solid agar medium, bacteria are equally spread. After that small antimicrobial disk place on the medium. The culture plate is incubated at specific temperature and time so that bacteria grow on the medium. The diffusion of the antimicrobial disks occurs when the antimicrobial agents from the paper disks diffuse into the surrounding agar medium. When the antimicrobial agent diffuses outward, it creates a concentration gradient. If the microbes are predisposed to antimicrobial agent, then there should be the formation of radius called as Zone of inhibition. The zones of inhibition are formed following a day of 35°C incubation, and the dimensions of every examined antibiotic's zones are then noticed either manually or mechanically (Le Page et al. 2016). The disc diffusion (DD) test, created in 1940, continues to be the most widely used antimicrobial susceptibility testing (AST) technique in clinical microbiology laboratories (Heatley, 1944). It is now normal to consider the therapeutic relevance of the most prevalent microorganism that cause human illnesses (Fr, 2010). The method involves placing a number of antibiotic-impregnated discs on agar that has already been injected with a bacterial solution. As the antibiotic spreads radially over the agar medium, it produces a gradient in antibiotic concentration.

Advantages and disadvantages

Due to its inexpensive cost, simplicity of usage, and capacity to administer a range of bacterial species and medications, disc diffusion is the AST technique that is employed the most frequently in microbiology labs (Balouiri et al. 2016). Depending on the type of sample the isolate was taken from and the species of bacteria it contains, the clinical laboratory can mix antibiotic discs in a number of ways because there are numerous alternatives available (Hombach et al. 2013). The discovery of unusual phenotypes and the visibility of contamination are made possible by straightforward interpretation. The major disadvantages, however, are the difficulty to determine the MIC and the length of the waiting period for results. A prompt diagnosis and course of therapy are essential for people who are very unwell. Furthermore, it is crucial to consider how the biological properties of the lag and log stages of bacterial growth impact the usage of antibiotics (Idelevich et al. 2017). However, methods to speed up the DD incubation period have been offered for decades (Liberman and Robertson, 1975; Coyle et al. 1984; Jorgensen et al. 2000). The AST is automated by DD, which reduces the time needed to get findings and create the final report (Hombach et al. 2017). RAST employs positive blood culture bottles directly and contains breakpoints for 4, 6, and 8-hour short incubations. Disc diffusion RAST uses positive blood culture bottles directly, it has been specified by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Jonasson et al. 2020). Without significant expenditures, RAST may be adopted in ordinary laboratories. So yet, just a few bacterial species and medications have been confirmed to work with the approach. Additionally, the turnaround time for reporting AST results has been significantly decreased compared to the standard AST techniques, such as DD, and the 24-48 h needed for culture by utilising a MALDI-TOF MS in conjunction with RAST directly from positive blood bottles (Idelevich et al. 2014). According to studies, AMS is significantly impacted by the direct DD testing's predictive effectiveness from positive blood cultures (Savage et al. 2021).

Comparison between DD and MIC on the basis of performance

Quality control testing of the medium and antibiotics is essential to ensure that disc diffusion is delivering accurate and trustworthy results (Åhman et al. 2019). In present scenario, MIC determination is more accurate than the DD method. For instance, the most accurate and precise phenotypic method for combining the EUCAST-based zone edge test with the inhibitory zone diameter will allow you to identify penicillinase-producing S. aureus strains (Aubry et al. 2020). The DD technique may be used to test for antibiotic sensitivity to other drugs or an entire class of antibiotics. It can also be used to identify the presence of a population of bacteria that are resistant to multiple antibiotics other phenotypic AST techniques are unable to detect some types of resistance to macrolides in a sample, such as ESBL, carbapenemases, inducible resistance to macrolides, or induced resistance to macrolides. The information presented above implies that the DD approach will continue to be a popular AST technique in the future.

Antibiotic-resistant bacteria detection using chromogenic agar media

Principle

Chromogenic agar media is designed with specific chromogenic substrates that change its colour to specific enzymatic activities. To detect AMR, these agar media are often modified to identify bacteria with a specific resistance mechanism. When we streak the bacteria on the color plate, they will produce enzymes that will interact with the chromogenic substrate to produce a specific color. According to the color manual then we decide which bacterial colonies show resistance (Ledeboer et al. 2007; Gordon and Wareham, 2009). The primary goal of developing a chromogenic medium was to make it possible to identify and detect resistant bacteria more quickly. The substrates are metabolized by certain enzyme systems in the target species to release the chromogen. An obvious color shift in the medium may then be further examined to determine the chromogen. Therefore, target diseases can develop as colored colonies thanks to these selective and differential media. Many media have been developed since the initial chromogenic medium for the identification of microorganisms resistant to antibiotics was introduced 20 years ago, including those for the detection of clinically significant resistant pathogens like MRSA, Vancomycin-resistant Enterococcus (VRE), and Gram-negative bacteria that produce ESBL, carbapenemases, or are colistin-resistant.

Advantages and disadvantages: Chromogenic agar is frequently less expensive and time-consuming to utilize than traditional culture medium (Perry, 2017). They are useful in infection prevention since their main function is screening people who have been exposed to different microorganisms (Kekic et al. 2021) and infection prevention in hospitals. It is occasionally necessary to confirm the identification of the resistant bacteria by further identification since chromogenic media's sensitivity and specificity vary based on the producer and the type of microorganism found. New chromogenic mediums are being developed because of their broad application (Tierney et al. 2016; Abdul Momin et al. 2017).

2. Antibiotic-Resistant Bacteria Colorimetric Tests for Detection

Principle

This test based on the colour change principle. There are few substrates which bind with the bacteria cell wall giving a specific colour which are detected by the spectrophotometer. The key principle is that sensitive bacteria do not produce same enzymatic or metabolic changes, resulting in different colour pattern or no colour (Nordmann et al. 2012). The phenotypic tests used to identify AMR are known as colorimetric assays. Based on a bacterial enzyme that hydrolyzes a test component that may be identified by alterations in pH levels and chromogenic material color. A detection solution comprising antibiotics and dyes such as phenol red are pH indicator is briefly added to a suspension of bacteria or bacterial lysate, and the mixture is incubated for a short period of time. The pH of the detection solution is changed by the development of antibiotic-resistant bacteria or by the activity of bacterial enzymes, which results in a color shift that may be visually observed.

Advantages: These examinations have shown themselves to be incredibly sensitive and exact, rapid, easy to conduct and interpret, and quick. Carba NP (bioMérieux, Marcy-l'Étoile, France) is the colorimetric test looking for bacteria that produce carbapenemase, is an excellent demonstration of this kind. The test provides accurate findings in 30 min to 2 hours, making it the fast and simple method of limiting carbapenemase production.

Modern AST technologies include:

1. Micro dilution susceptibility testing-based automated and semi-automated devices:

Principle

In this method a specific amount of solutions are inserted into the wells of a microplate. After that bacterial inoculations which are prepared from patient sample are inserted into the wells. Then incubate the sample at specific temperature and time. After incubation read the sample and analyze the MIC results which show that either bacteria is resistant, susceptible or intermediate (Fredborg et al. 2013).

Advantages: Clinical microbiology laboratories are under greater pressure to give rapid AST results and reliable microbe identification (ID) results (Benkova et al. 2020). The efficiency of laboratories has substantially increased because of semi-automatic and automatic tools for bacterial ID and AST. Automation has successfully been used in the majority of clinical microbiology labs today to save costs, accelerate turnaround times, and boost output (Sader et al. 2006; Benkova et al. 2020). These equipment can deliver findings faster (6–12 h) than traditional human evaluation methods by employing optical systems for sensing small changes to evaluate bacterial growth and antibiotic susceptibility (Jorgensen and Ferraro, 1998). The Vitek 2 system with software main advantage is its ability to detect clinically important resistant bacteria sensitivity to an additional four to ten antibiotics, including *Staphylococcus aureus* and *Enterococcus faecalis* (Ligozzi et al. 2002; Spanu et al. 2003; Benkova et al. 2020). Phoenix combination panels with ID and AST (51 for ID and 85 for AST) can be used in the Phoenix instrument to perform up to 100 tests. The device automatically reads at intervals of 20 minutes throughout incubation for up to 18 hours, providing reliable and quick susceptibility data with a simple

workflow for the lab technician. The Phoenix system's released a new panel to assess the susceptibility of gram-negative bacteria. in 2014, and it works in conjunction using the MALDI-TOF from BD Bruker (Evangelista and Karlowsky, 2016).

VITEK 2 Systems

For the counting and identifying of bacteria and yeasts, the first repeat of the VITEK system was developed in 1973, with a turnaround time of 13 hours. The 64-well plastic cards used by using 17–20 antimicrobial agents, the VITEK 2 System is an AST system based on BMD are the next-generation instrument. One card is used to identify the bacteria (an ID card), and the other is used to determine how susceptible the bacteria to various antibiotics (AST card), if the bacterial isolate has not already been recognized. There are two Vitek 2 instruments with capabilities for 60 and 120 test cards (ID and AST), respectively. While the Advanced Expert System (AES) facilitates the identification of AMR, results are delivered in 4–18 hours and include the MIC and type of susceptibility. The 15, 30, and 60 cards are now available for use with Vitek 2 Compact instruments.

Phoenix System

Identification testing (ID) and antimicrobial susceptibility testing (AST) are performed while utilizing the Phoenix System in clinical microbiology laboratories, which is extensively used and recognized.

MicroScan WalkAway plus System

A wide variety of Gram-positive and Gram-negative aerobic bacteria may be accurately and quickly identified, and their susceptibility can be determined using the MicroScan WalkAway + System. Three different panel configurations are used by the instrument: MIC panels, combination panels, and breakpoint combo panels. Two different types of systems exist: versions with 40 and 96 panels. The panels are manually infected, then automatically read after being rehydrated by the RENOK inoculator. After 4.5–18 hours, the findings are read using rapid panels (Evangelista and Karlowsky, 2016).

MicroScan AutoScan 4

A semi-automated device called the AutoScan 4 is typically employed for the testing of new antimicrobial medicines or in smaller labs. Equipment offer streamlined ID/AST testing in a very dependable and reasonably priced package. The technology makes use of traditional MicroScan AST panels that are incubated offline. The panels are either manually infected or automatically read using the MicroScan Renok device (Evangelista and Karlowsky, 2016).

MicroScan WalkAway System

The initial version of the MicroScan WalkAway System is called the AutoSCAN-3 that is now in the market. The instruments Auto-SCAN-4 and Auto-SCAN-WalkAway have been updated, and the new models feature dry panels that don't require refrigeration. The Auto-SCAN-WalkAway technology can process 96 panels simultaneously and can identify bacterial enzymatic activity (Benkova et al. 2020).

However, it's noteworthy to note that automated algorithms were able to identify inducible clindamycin resistance in 524 isolates of *Staphylococcus* species that have specificity and sensitivity for Phoenix of 99.6% and 100%, and for Vitek 2 of respectively 91.1% and 99.8% (Buchan et al. 2012). The multicenter evaluation revealed that, except viridans group *streptococci* and penicillin, where categorical agreement between a BMD reference method and the Phoenix system ranged from 87% to 92% for *streptococcal* isolates of beta-hemolytic *streptococci*, viridans group *streptococci*, and *S. pneumoniae* in 2013 *Streptococcus* groups A, B, C, and G (Richter et al. 2007). However, caution should be used when employing an AST of *Stenotrophomonas maltophilia* since a significant outcomes of AST analysis utilising Vitek 2, Phoenix, and MicroScan, a rate of errors may be seen (Khan et al. 2021).

2. Molecular-Based Techniques for Resistance Detection

Principle

In this a clinical sample is taken from bacterial culture or patient specimen, has been collected and prepared for analysis. Genetic material is extracted from the bacterial cells in the sample. Then apply molecular techniques such as Polymerase Chain Reaction for amplifying specific target regions of the genetic material. Then the amplified targets are interacted with specific markers to identify the resistance using techniques such as gel electrophoresis, DNA sequencing or fluorescent probes (Zhou et al. 2018). Specific resistance genes, their mutations, and their expression are all immediately detected by molecular AST. As a complement to or replacement for (AST), these molecular methods have been developed and evaluated. Results of the test are frequently available in one to several hours (Schumacher et al. 2018). Three categories: hybridization, sequence and amplification-based comprise the majority of molecular AST techniques. In methods based on amplification, the sequence of target gene is selected for recognition; in methods based on hybridization detection, hybridized nucleic acid probes focus on the target gene sequences; and genome alignments are examined to look for changes that confer resistance or resistance genes.

Polymerase Chain Reaction

Principle

PCR principle involves a repetitive cycle of denaturation, annealing, and extension. In this method, DNA sample is heated to separate the double-stranded DNA into single strands. After that short DNA patterns called primers bind to complementary sequences on the target DNA. DNA polymerase enzyme synthesizes new DNA strands by extending from the primers. Then, in this way, amplify the DNA to check the resistance genes and mutations. Polymerase chain reaction (PCR) is the most used nucleic acid amplification-based technique for identifying certain resistance genes. Both real-time and traditional PCR need the amplification of antibiotic resistance-encoding nucleic acid sequences. As we understand more about the genetic basis of antibiotic resistance, fresh PCR-based methods are being created to detect the genetic determinants of resistance to various antibiotics for diverse bacterial species (Sekyere and Asante, 2018). The Acuitas® AMR Gene Panel, a multiplex system which was recently launched by OpGen, Inc. (Rockville, MD, USA), identifies 28 genetic AMR indicators, covering specific medications from nine distinct antibiotic classes, from 26 different infections (Simner et al. 2022).

Advantages and disadvantages: This test has an advantage over other molecular tests that are commercially accessible in that it also identifies genes for resistance to "last-resort antibiotics," such as colistin, as well as non-beta-lactams. Quantitative PCR, sometimes referred to as real-time PCR or qPCR, is one method used most commonly in clinical microbiology. Even though the technique is more expensive, qPCR has several benefits over traditional PCR, including the ability to assess real-time data, higher sensitivity, and a lower chance of improved multiplexing abilities and carryover contamination. Several systems, including the user-friendly GeneXpert® Instrument Systems and BD MAX System platform (both from Cepheid Corp., Sunnyvale, CA, USA) are available and may be employed for the identification of MRSA, VRE, ESBLs, carbapenemases etc., are partially or even entirely automated (Findlay et al. 2015). Because qPCR can detect the number of copies of the bacterial genome during growth in the presence of antibiotics, it may also be used to distinguish between resistant and susceptible strains based on their phenotypic characteristics (Matsuda, 2017). The system's key drawback is that it cannot explain the resistance mechanism because it needs the previous culture, which prevents it from using the original clinical samples.

Mass Spectrometry

Principle

Mass spectrophotometry is used to analyze and detect the molecular composition of bacteria. In this method, we measure the mass-to-charge ratio of resulting ions after ionization. Resistance mechanism involves changes in bacterial proteins or metabolites which can be detected by mass spectrophotometry. By comparing the mass spectra of resistant and susceptible strains mass spectrophotometry using specific biomarkers (Sparbier et al. 2013; Jung et al. 2014).

The technique known as matrix-assisted laser desorption ionization-time of flight mass spectrometry, or MALDI-TOF MS, was first used to identify bacteria and yeast around 15 years ago. It was first developed in the 1980s. Due to its quick turnaround time, low sample number requirements, and affordable per-sample costs, it has been used to categorize the various bacterial protein compositions and their corresponding protein biomarkers (Ho and Reddy, 2011). A number of MALDI-TOF MS-based techniques, such as monitoring bacterial culture-induced antibiotic alterations (such as beta-lactam hydrolysis), have been proposed for the quick identification of antimicrobial resistance (Coyle et al. 1984; Oviaño et al. 2017a), acetylation of fluoroquinolones (Oviaño et al. 2017b), direct detection of proteins involved in specific resistance mechanisms (Camara and Hays, 2007; Griffin et al. 2012), and detection of stable isotope labeling that requires expensive, isotopically labeled media. Using MALDI-TOF MS, it is possible to identify bacteria that produce beta-lactamases by measuring the hydrolysis of the target beta-lactam antibiotic as indicated by peak disappearance. The test for identifying carbapenemase synthesis as a result (Oviaño et al. 2016) based on the level of antibiotic hydrolysis, automatically assesses sensitivity or resistance. After 30 minutes of exposing bacteria to the antibiotic, the method's sensitivity and specificity were 98% and 100%, respectively. After 60 minutes, both values were 100% (Akyar et al. 2019; Oviaño et al. 2020). Detonation nanodiamonds (DNDs) are a quick and innovative approach for concentration and extraction of *A. baumannii* carbapenemase-associated proteins prior to MALDI-TOF MS analysis, which is another test for the detection of carbapenemases (Chang et al. 2018). When compared to conventional imipenem susceptibility testing, the suggested platform's sensitivity and specificity might be 96% and 73%, respectively, and 100% when compared to results from PCR. In contrast to previous mass spectrometric approaches, this one does not call for the inclusion of a carbapenemase substrate and can identify the carbapenemases made by *A. baumannii* in as little as 90 minutes. Other bacteria that produce carbapenemase can be found using it effectively.

An alternative semi-quantitative MALDI-TOF MS technique for AST known as the MALDI Biotyper-Antibiotic Susceptibility Test Rapid Assay (MBT-ASTRA) measures the relative growth rates of bacterial isolates exposed to antibiotics in comparison to untreated controls during a short

incubation stage. Software is used to calculate and compare the areas under the curves (AUCs) of the spectra of bacteria treated to antibiotics or not (Sparbier et al. 2016). The MBT-ASTRA assay's disadvantage is that each species and antibiotic combination must have its own optimal concentration of antibiotics and incubation period (Sparbier et al. 2016).

A unique method for identifying antibiotic resistance when bacteria have treated with breakpoint doses on the mark plate of MALDI-TOF MS is used in the DOT-MGA, or direct-on-target micro droplet growth assay (Idelevich et al. 2018). A 96.3% validity, 91.7% sensitivity, and 100% specificity were obtained under these conditions. ESBL and AmpC beta-lactamase activities were recently detected using a screening panel (Correa-Martínez et al. 2019). According to Yoon et al. endemic AMR clinical strains, such as *Enterobacterales* that produce ESBL, AmpC, and carbapenemase, as well as MRSA, VRE, CRAB, CRPA, would be best detected using MALDI-TOF MS due to its high speed and straightforward application. (Yoon and Jeong, 2021).

Conclusions

A key component of the treatment of infectious illnesses is the prompt provision of suitable antibiotic medication based on precise AST. The use of standard AST techniques, like DD and BMD, is important in laboratory practice despite their disadvantages to acquire the proper findings in compliance with the established EUCAST and CLSI procedures or for assessment using the results of creative strategies. Molecular based approaches are quick, reliable, and have a high level of sensitivity and specialty. NGS technology has to be more fully incorporated into conventional microbiology, though. A highly selective system with decreased time and cheap consumable costs, MALDI-TOF MS seems to hold promise. However, the system's purchase and upkeep costs are considerable, and this technology has not yet been verified for use with all species and antimicrobials. To accelerate the assessment of antimicrobial effectiveness in clinical microbiology laboratories and it will soon be important to continue developing the cutting-edge AST approaches and tools that are already in use.

Ethical Statement

This review was unbiased and contained no ethical concerns, so no ethical concerns were required.

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Consent to Publish

All the authors consent to publish this article.

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Competing Interest

The authors declare they have no relevant financial or non-financial interests to disclose.

Author Contribution

MA, QUH, SG, AU, and GM wrote the manuscript. SG, MAFK, and AN managed figures and tables. FD and AS revised the manuscript.

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