Original Article

Antimicrobial Resistance: Mechanisms, Screening Techniques and Biosensors

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DOI: 10.47750/pnr.2022.13.S06.227

Abstract

Antimicrobial resistance is one of the greatest dangers to public health on a worldwide scale. Even though the evolution of resistant microbes is a naturally occurring phenomenon, the excessive or improper use of antimicrobial agents has significantly impacted resistance evolution. Effective treatment techniques can be guided by rapid diagnostic tests of these resistance to detect drug-resistant microorganisms quickly, assess antimicrobial susceptibility, and bacterial infections from differentiate viral. In addition, quick diagnostic test could assist epidemiological surveillance by monitoring developing resistance to pathogenic pathogens and their transmission. Methods for AMR detection, and surveillance, includes Microscopy, PCR, DNA microarray, matrix-assisted laser desorption,Pyrosequencing, NAAT, whole genome sequencing, Immuno detection, spectroscopy, FTIR, Biosensor, and microfluidics technology. The AMR turmoil necessitates a concerted effort from researchers to improve the current approaches for treatment and diagnosis by developing novel techniques that circumvent the limitations and drawbacks of the best alternatives and conventional AST methods. This review mainly summarizes the different emerging diagnostic techniques used to detect antibiotic resistance and the mechanism of drug resistance. These emerging diagnostic strategies might improve medication development strategies and the surveillance of resistance evolution.

Keywords: Antimicrobial resistance, Microscopy, PCR, DNA microarray, Pyrosequencing, NAAT, MALDI-TOF, FTIR, Biosensor.

1. INTRODUCTION

Microbial infections for ages have threatened humanity. Infection is the infiltration of disease-causing pathogens into an organism's bodily tissues, their multiplication, and the host tissues' reaction to the infectious agents and the toxins they create. Infectious diseases have always been a major global issue. Worldwide we suffer from several bacterial infectious diseases, including food poisoning, gastritis, ulcers, gonorrhoea, meningitis, strep throat, and otitis media. In some cases, these bacterial diseases pose life-threatening conditions. In the pre-antibiotic era (early 20th century), phage therapy was the most potent armament against infectious ailments of bacterial etiology. The advent of the antibiotic era (the 1950s-1970s) ensued in the loss of phage therapy, and antibiotics became widely available in the Western world. Anti-Microbial Resistance (AMR) crisis demands immediate action to control its spread.[1] It was highly noticeable that multi-drug-resistant microbial strains represent a significant threat.

Researchers were continuously working and exploring preventive measures to fight multidrug resistance, which needs robust diagnostic methods and surveillance. Several infections are treated with antibiotics that are likely to go away without any antibiotic treatment. Recently National Academy of Sciences 2018 stated that Antibiotic consumption is a primary driver for antibiotic resistance , so it should be used appropriately.[2] Limiting the use of antibiotics can be the first step against these superbugs. Hospitals are the spreading grounds for these multidrug-resistant microbes; thus, the situation goes uncontrollable. It's high time to understand this alarming situation where noble antibiotic production is less and bacterial resistance is high. Hence, a fast and accurate diagnostic approach for antimicrobial-resistant microbes is needed, which can be achieved by emphasizing the individualization of microbial strains and their mechanism of becoming resistant. So, an affordable, rapid and robust antimicrobial susceptibility technique is required, such as biosensors, Empirical therapy, Standard calibrated loop technique, Strokes method, E-test, Native spectroscopy, etc. Our review mainly demonstrates novel techniques to diagnose antibiotic-resistant microbes' sensitivity and specificity.

1.1. Antimicrobial resistance's history

Infectious diseases have always been an enigma to human health. Infectious diseases are supremely caused by microorganisms like bacteria, fungi, and algae. Antibiotics are a strategic means to stop infections caused by particular bacteria among people, animals, and crops. The spread of antibiotic resistance has been accelerated by globalization. Organisms can now spread further than ever before because of global trade and travel. In 2008, an Indian patient being treated in Sweden was found to have metallo-beta-lactamase 1, (an enzyme) that gives resistance to a wide range of antibiotics. These superbugs have developed resistance to antibiotics, and as a result, they have gained control of humans. Antimicrobial Resistance (AMR) is the ability of microbial and especially bacteria strains to show resistance to antibiotics while continuing to thrive.[3,4] Alexander Fleming's discovery of penicillin was a breakthrough in the fight against the pneumonia-causing bacteria Streptococcus pneumoniae. Furthermore, in 1945, he warned about the negative consequences of antibiotic abuse, which could lead to bacterial resistance. Currently, approx. Seven lakh people expire annually on account of drug-resistant bacteria. [5] The genetic basis is vital in recognizing the nature and type of resistance in bacteria. Further, Antibiotic resistance is categorized into two categories, i.e., extrinsic and intrinsic resistance. Extrinsic resistance, defined as antibiotic resistance, is acquired through a change in the organism's genetic makeup and occurs due to either mutation or acquisition.

In Intrinsic resistance, the organism's chromosomal genes are "born" with intrinsic antibiotic resistance and resistant to a specific class of antibiotics.

Resistance could be triggered by a variety of mechanisms, which may include: (i) the existence of an enzyme that inhibit the activity the antibiotics or antimicrobial agent; (ii) the existence of a substitute enzyme for the enzyme which is impeded by the antibiotics or antimicrobial agent; (iii) an alteration or mutation in the antibiotic/antimicrobial agent's target point, which diminishes the adhesion of the antimicrobial agent; and (iv) post-transcriptional or post-translational alteration of the antibiotics or antimicrobial agent's target, which minimise binding of Antimicrobial binding agent. (v) reduced up-taking of antimicrobial agent. (vi) strong active efflux system of the antimicrobial agent.(vii) overproduction or excessive supply of the targets of an antimicrobial agent. The below Figure1, illustrates the different mechanism that causes AMR in microbial strains.

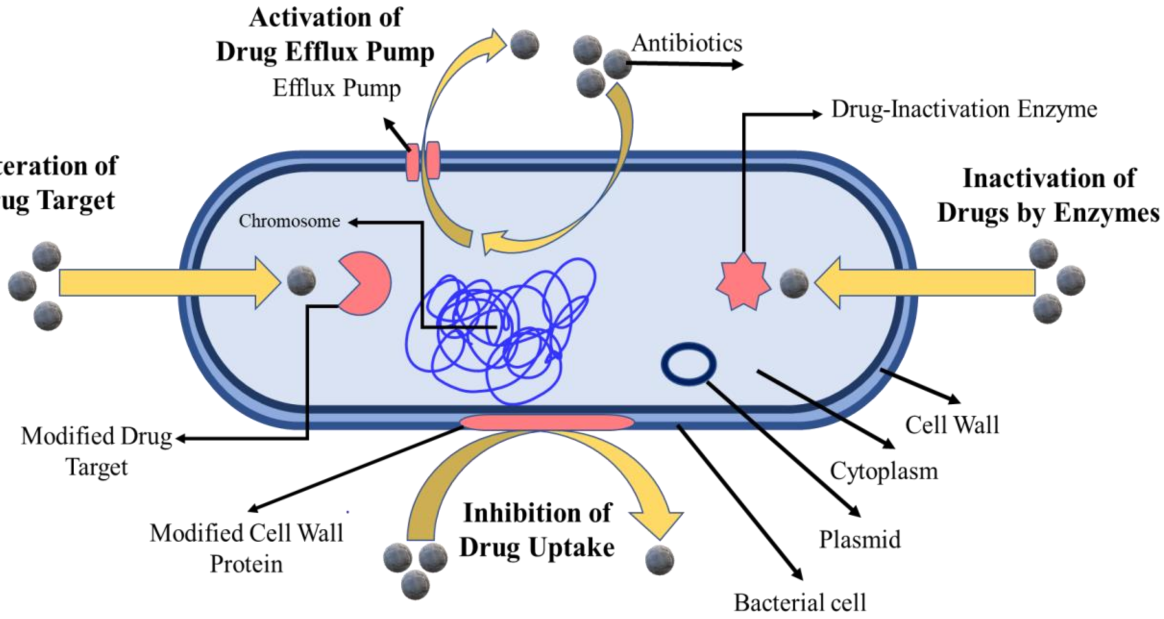

Figure1: Various mechanisms of Anti-Microbial Resistance (AMR)

1.2. Bacteria's resistance to antibiotics

Higher resistance against antibiotics often used to treat common bacterial diseases, such as urinary tract infections, sepsis, sexually transmitted infections, and various forms of diarrhea, have been documented worldwide, indicating a running out of effective antibiotics. For example, resistance to ciprofloxacin (a routinely used antibiotic) is rising. Drugs are inactivated by hydrolysis, such as –lactamase, or modification, as in the case of amino glycoside resistance. It could also manifest itself in other ways, such as the use of permeation barriers, active efflux, or blocking access to the target. It has been discovered that bacterial communities have a higher tolerance to environmental stress than any other living cells, and antibiotic resistance is an example of this tolerance. The increased resistance at the community level has a combinatorial influence on cellular resistance, resulting in a synergistic image of increased resistance. When bacteria are deprived of nutrients and under unfavorable living conditions, they become incredibly resistant. This could explain the greater resilience of cells in biofilms, as cells in the deeper layers of biofilms may face nutritional limitations. Aside from that, the development of cells known as emerger cells is another component that contributes to microbial resistance. Persister cells are slow-growing or non-growing phenotypes that are resistant to all stressors, including the antibiotic challenge. These persistor cells can survive even after most of the cells have died, resulting in reservoirs of surviving cells that could lead to an infectious relapse. Although the mechanisms and nature of community and cellular resistance differ, they have a synergistic effect. [6,7]

Antibiotic Resistance Threats in the United States, 2019 (2019 AR Threats Report) by the Centres for Disease Control and Prevention (CDC) contains the most recent national death and infection estimates, highlighting the persistent threat of antibiotic resistance. As per one report, about 2.8 million antibiotic-resistant infections occur annually in the United States, resulting in over 35,000 deaths. Furthermore, in 2017, there were 223,900 cases of Clostridium difficile, with at least 12,800 deaths reported. The CDC is concerned about an increase in community-acquired resistant infections, which could put more people at risk, make detection and containment more complicated, and adversely affect the progress made in healthcare to protect patients. Furthermore, some examples are listed in table 1. New forms of resistance continue to emerge and spread, which is a cause for concern. In 2013, the Centres for Disease Control and Prevention (CDC) released the annual Antibiotic Resistance Threats Report, which raised awareness about the threat of antibiotic resistance.

Table1: List of case studies with names of antimicrobial resistant strains and threats caused by them.

Antibiotic resistant Bacteria	**Impact of Bacterial Infection**	**No of estimated cases in hospitalized patients**	**Estimated deaths**
Carbapenam-resistant Acinetobacter	Carbapenem-resistant Pneumonia, wound, bloodstream, and urinary tract infections are all caused by Acinetobacter. Almost 90% of these infections occur in people who have recently received treatment in a medical setting.	8,500 (2017)	700 (2017)
Drug-resistant *Campylobacter*	Campylobacter is a bacteria that causes diarrhoea (often bloody), fever, and abdominal pains in humans. It is transmitted from animals to humans by contaminated food, particularly raw or undercooked chicken.	448,400 cases per year	70 deaths per year
Vanomycin-resistant Enterococcus (VRE)	In healthcare settings, Enterococci can cause significant infections in patients, including infections of the bloodstream, surgical sites, and urinary tract.	54,500 (2017)	5,400 (2017)
ESBL-producing Enterobacterales	Extended-spectrum -lactamase is another name for it. Enterobacterales that produce ESBLs are a problem in hospitals and the general public. They can spread quickly and infect healthy people, causing or complicating illnesses. Extended-	197,400	9,100

	spectrum beta-lactamase (ESBL) is a type of beta-lactamase that may infect a wide range of bacteria. ESBLs are enzymes that degrade medicines like penicillins and cephalosporins, rendering them useless.		
Multidrug-resistant *Pseudomonas aeruginosa*	Infections with P. aeruginosa are more common in persons who have compromised immune systems, and they can be especially harmful for people who have chronic lung problems.	32,600 (2017)	2,700 (2017)
Drug-resistant nontyphoidal *Salmonella*	Salmonella nontyphoidal can be transmitted from animals to humans through food and causes diarrhoea, fever, and stomach pains. Some infections travel to the bloodstream, posing a life-threatening	212,500 cases per year	70 deaths per year
Drug-resistant Salmonella serotype Typhi	Typhoid fever is a dangerous disease caused by Salmonella Typhi that can be fatal. The majority of people in the United States become infected when visiting nations where the disease is frequent.	4,100 cases per year	Less than 5 deaths per year
Drug-resistant *Shigella*	Shigella enters the body through direct contact with faeces or through contaminated surfaces, food, or water. Diarrhea, fever, and stomach pains are the most common symptoms of Shigella infections.	77,000 cases per year	Less than 5 deaths per year
Methicillin-resistant *Staphylococcus aureus (MRSA)*	S. aureus is a common bacteria that can be found in hospitals and the general public. Due to its resistance to several drugs, MRSA can cause difficult-to-treat staph infections.	323,700 (2017)	10,600 (2017)
Drug-resistant *Streptococcus pneumoniae*	Pneumococcus is another name for Pneumococcus. Pneumococcal illness is caused by S. pneumoniae and can range from ear and sinus infections to pneumonia and bloodstream infections.	900,000 (2014)	3,600

Drug-resistant *M. Tuberculosis*	Mycobacterium tuberculosis (M. tuberculosis) TB is caused by the bacteria M. tuberculosis and is one of the most common infectious diseases and a leading cause of death worldwide.	847 (2017)	62 (2017)
Erythromycin-resistant Group A *Streptococcus* (GAS)	GAS, also known as resistant group A strep, can cause a variety of infections vary from mild illnesses to serious and fatal diseases such as strep throat, pneumonia, flesh-eating infections, and sepsis.	5,400 (2017)	450 (2017)
Clindamycin-resistant GroupB *Streptococcus* (GBS)	GBS, also known as resistant group B strep, is a serious infection that can affect people of all ages.	13,000 (2016)	720 (2017)

2. Techniques to diagnose Antimicrobial Resistance

Numerous of diagnosis techniques and hybrid technologies are explored by researchers. Few advantages and disadvantages of AMR (Anti-Microbial Resistance) diagnostic techniques are listed in table 3.

2.1. Microscopy

Bacteria upon agar media can be counted using microscopy methods long prior they reach the level that produces visible colonies. Drug vulnerability (MODS) for Mycobacterium tuberculosis can indeed be determined through microscopically analysing cell clusters (cords) in microtiter plates which are sealed. Rapid Micro Biosystems Inc.'s Growth Direct System uses digital imaging to detect micro colonies by enlightening microorganisms through blue light rays and transferring intracellular auto fluorescence directly onto the CCD (chip) deprived of magnifying. Automated microscopy methods may offer real-time growth curves, as well as quantified microbial enumeration have indeed been demonstrated. Accelerated Diagnostics (USA) recently commercialised MADM (multiplexed automated digital microscopy) along with FISH (fluorescent in situ hybridization) for rapid on-line AST. The Accelerate Pheno® technology can extract contaminants from clinical specimens (such as blood and urine) using rapid electrophoresis, that runs pollutants across a gel. Following this, a shift in the polarity of the electrical field resists the bacteria back into the fluid. Every 10 minutes, a fluorescence signal is observed from specimens collected from the microbial isolate proliferating in MH (Mueller-Hinton) media. This appears as the only United states food and drug growth-based fast diagnostic AST method at the present. Accelerate Phenoefficacy has been established in numerous clinical investigations, including those involving urinary tract infections and bloodstream infections.

2.2. PCR technique

PCR technique is among the widely used genomic amplification technology employed for the detecting AMR strains. Real-time quantitative digital and multiplex PCR technologies have lately increased clinical acceptability of genetic evaluation or testing. Changes in WGS have significantly influences the availability of ARG (antimicrobial resistant genes) targets, and causes opening of the way for (HT-qPCR) high throughput quantitative Polymerase Chain Reaction, which is actually comparably quick, easy, and performs simultaneous investigation of a huge massand quantity of ARGs. Furthermore, the HT-qPCR is also know as cost-effective method which was previously been utilized in many studies to analyze ARGs from a variety of clinical and sub clinical samples. For example, the Manifold RT-PCR was used to characterize AMR (anti-microbial resistance) Neisseria gonorrhoeae, which shows resistance to antibiotics such ascefixime,ciprofloxacin, spectinomycin, and azithromycin. However, this approach is well able to accurately detect mutations that causes antibiotic resistance in gonorrhoeal patients, but the due to limited assay sensitivity prohibits it from being employed for diagnostic testing in clinical specimens.

Furthermore, because it is quicker than conventional method such as culture-based AMR testing, it may be used as an AMR screening tool. For example, researchers also developed and stated about single-plex and multiplex RT-PCR based assays significantly employed for (MRSA) methicillin-resistant S. aureusin clinical samples from pediatric section. For quick AMR diagnosis in pathogens such as E. faecium, S. aureus, Kl. pneumoniae, A.baumannii, P.aeruginosa, Mycobacterium sp. and Enterobactersp. have been well classified by using this method.[8,9]

2.3. DNA Microarrays

Previously, DNA microarrays was created by using slides of glass dotted with a variety of distinct DNA probes which have specific reference genes existing in a microbial strain (specific) for which the WGS (whole-genome sequence) was available. DNA microarray technology has advanced dramatically during the previous two decades. For disposable microarrays, a quick and simple DNA-labelling approach, based on biotinylated primers, precise for the linkers has been documented. A melting curve test based on a rapid cartridge has been proposed by researchers to identify pyrazinamide-resistant Mycobacterium sp. As a point-of-care test, the assay may be done automatically in resource-constrained situations utilizing a closed cartridge AlereTM q analyzer.[10]

2.4. Pyrosequencing

Pyrosequencing was proposed in 2012 as a new, quick approach for detecting Yersinia pestis strains in fighting against bioterrorism. The kye mechanism of this method for detection and identification is focused on presence of virulence genes, which led to the development of a pyrosequencing-based assay for assessing ARG profiles .[11-14] Pyrosequencing was also tested to detect drug-resistant Mycobacteriumspp. in clinical settings. The pyrosequencing assay might reveal the drug resistance-allied mutations ofMycobacterium tuberculosis with an excellent specificity with 96-100%. Similarly, the quick identification of fluoroquinolone (FQ) resistance, kanamycin (KAN), rifampicin (RIF), and capreomycin (CAP) resistance in M. tuberculosisstrain was also utilized to evaluate the efficiency of pyrosequencing. The assay's sensitivity and specificity for detecting RIF, CAP, FQs, KAN etc., resistance were nearly 100%. This technique was thought to be one of the quickest and most successful methods for sleuthing resistance-based modifications in M. Tuberculosis and other similar species.[15]

2.5. MALDI-TOF Mass Spectrometry

To identify Anti-Microbial-Resistance in microbial strains, (matrix-assisted laser desorption ionization time-of-flight) MALDI-TOF can be employed instead of normal phenotypic or genotypic bacterial characterisation. MALDI-TOF MS profiles protein (mostly ribosomal, 2-30kD) from entire bacterial cell genome extract to generate a spectral fingerprint. During the preparation of test, the specimen is varied with the milieu, an energy-absorbent solution then the entrapped material in the matrix become crystallized (when it dries). When a laser beam contacts a material, it ionizes it, creating protonated ions that may be separated depending on their mass-to-charge (m/z) ratio. This ratio is mainly calculated by the time it takes in each protonated ion to transit the extent of the tube. The TOF data is used to generate a (PMF) "Peptide Mass Fingerprint", which is a one-of-a-kind mass spectrum. The PMF peaks are further matched with database of reference peaks which is unique to every genera and species microorganisms, and this further help inidentifying specimen. MALDI-TOF-MS has also been used to discover antibiotics or drug resistance mechanisms (e.g., carbapenemases). MALDI-TOF MS is a technology that is consistent, fast (within minutes), specific, accurate, simple, cost-effective, and ecofriendly.[16] Few application of MALDI-TOF MS for AMR (antimicrobial resistance) detection is enlisted in Table 2:

Table 2: MALDI-TOF MS applications for particular (AMR) antimicrobial resistance detection.

Microorganism	Antibiotic
E. coli	Colistin
S. aureus	Methicillin
Candida auris	Echinocandins
Enterobacteriaceae	Carbapenem
K. pneumoniae	Carbapenems (carbapenemase-producing isolates)
Enterococcus faecium	Vancomycin

2.6. Hybridization-Based Systems

FISH is really among the specialised approach for quantitatively visualising the presence of the target organism. PNA-FISH uses peptide nucleic probes, which allowing for faster and more specific binding than RNA or DNA based probes. It is used in commercialized Quick Fish technique (OpGen, USA) which enables the identification of specific genes by targeting 16S rRNA.[17] Xpress Fish selectively identifies the mecA gene in Staphylococcus, allowing for the detection of resistant bacteria just in 2 hours (once the blood agar appears positive) when it is used conjunction with Quick Fish-based diagnosis. PNA-FISH techniques are not suitable for on-line growth management or monitoring because target cell permeabilization, fixation, and hybridization require a temperature around 55°C. Clinical labs using mass spectrometry are unlikely to necessitate PNA-FISH technology; for example, the Bruker MALDI Septityper kit PBP2A can detect the mecA-encoded PBP2A-protein in within one hour and at a reasonable cost.

2.7. Nucleic Acid Amplification Technology (NAAT)

Once paired with a syndromic method, NAAT is an exceptionally strong technique for detection of pathogens. Many screening panels, such as those offered by Elitech, Eplex, Becton Dickinson, etc., feature AMR-gene detection. They can offer therapeutically useful data, particularly when a thorough antibiogram is really not required. Pathogens such as Legionella, Neisseria gonorrhoea, Chlamydia trachomatis, Mycoplasma, etc., are well known for, possessing relatively high drug resistances. However, the identification of individual AMR-genes does not provide conclusive evidence for antibiotic resistance. The detected AMR genes may not be related to the microbial pathogen which was causing the sickness, or the identified resistance gene’s inactivation. NAAT neither defines Minimum Inhibitory Concentrations nor directly recommends which antibiotics to use. NAAT has the benefit of being reasonably quick to update for emerging new microorganisms and its resistance variables. First-generation "molecular assays" such virulence genotyping, RFLP (Restriction-Fragment-Length-Polymorphism), multi locus sequencing, PFE (pulsed-field electrophoresis), and MLTRA (multiple locus tandem repeat analysis), were better suited than typing and outbreak analysis of AMR. These technologies do not allow for quick diagnosis as they necessitate a large volume of pure nucleic acid. Hybridization-based techniques and molecular marker system, on the other hand, have persevered and are being creatively integrated with NAAT technology for the detection of Antimicrobial resistant strains and its phenomena.

2.8. Immunodetection

Immunodetection is a sensitive and specific approach for identifying pathogenic microorganisms, toxin (proteins), and viruses. Although immunodetection does not necessitate the destruction of the microbial species, it may be possible to identify pathogens and track their progress in a sequential manner. It's also appropriate for lateral flow (LF) tests and could be implemented into biosensor technologies, microfluidics, and even in DNA/RNA-based assessment. For example, numerous industries, including Beckton Dickinson, Mizuho Medy etc., have introduced easy to perform strip tests fordiagnosis of viral and bacterial strains that cause venereal illnesses. Typically, the specific antibodies in immobilised form coated on strips, or on plates of biosensor surfaces for efficient and selective target binding. To produce a quantifiable signals, the detecting immunoglobulins (antibody) can be tagged with redox enzymes or fluorescent dyes. For instance, the researcher's developed LF-test which detects chloramphenicol resistance in P. aeruginosafrom clinical samples. Similarly, by using a PBP2a-specific chicken IgY (immunoglobulin), researchers have developed a similar immunochromatography assay for the identification of MRSA.[18]

2.9. Mass spectrometry

Mass spectrometry (MS) is one of the techniques which is also integrated with other AMR technologies, notably for the diagnosis of septicaemia. In this sample droplets are easily deposited onto disposable MS-target plates of the MALDI-TOF or in Direct-On-Target Microdroplet Growth Assay (DOT-MGA), further incubated for 3-4 hours, and then analyzed using MS. This method is one of the quick and sensitive.

2.10. Fourier Transform Infrared (FTIR) Spectroscopy

Consequently, optical technologies and their usage in the medical and microbiological disciplines have made significant strides forward. Infrared (IR) spectroscopy as well as microscopy facilitate the accumulation of biochemical data at the molecular scale for microbes by providing greater spectral and spatial resolution. Fourier transform infrared (FTIR) spectroscopy is a descriptive technique which has arisen as an intriguing and contemporary tool enhancing the techniques used for bioassay due to the molecular-level chemical information it may deliver. FTIR spectroscopy permits the determination of the Infrared light absorption by substances such as lipids, lipo-polysaccharides, polysaccharides, peptides, and nucleic material, yielding a distinctive FTIR spectrum that depicts the sample's full composition. These distinctive spectrum of cell molecules provide an abundance of structural and functional information. Fourier Transform Infrared spectroscopy has been employed to distinguish the biomolecular alterations related with the emergence of antimicrobial resistance in prokaryotes.[19]

2.11. Microfluidics

In several sectors, such as food safety, clinical diagnostics procedures,and environmental pollutant monitoring, lab-on-a-chip (LoC) systems employing microfluidics are an intriguing approach. Current applications of LoC technology include the detection of antibiotic-resistant bacteria. In comparison to macro-scale approaches, the LoC technology provides the several benefits such as rapid and high productivity analysis, precise fluid manipulation, cheap, minimal reagent and energy consumption, automated, a small amount of sample, compact, and portable. The two kye categories of microfluidic-based detection technologies are genomic-genotypic as well as phenotypic tastings. Genotypic microfluidic analyses (such as PCR) address genetic markers (such as, ARG), thereby avoiding bacterial growth as well as enabling a shorter turnaround time (few hours). Due to the absence of thermal cycling, the integration of microfluidics with isothermal DNA amplification techniques offers better features. This method is extremely promising for development of inexpensive and practically effective diagnostic approach for clinical, and ecological applications. Alternatively, phenotypic microfluidic assays generally monitors the bacterial growth with antibiotics, hence providing correct antimicrobial susceptibility test’s results. In this, bacterial cells are typically restricted in small volumes (e.g., droplets or chambers) collected with both the assistance using antibody on magnetic beads or membranes, orby encapsulation in agarose-coated compartments or hydrodynamically trapped. Hydrodynamic trapping, for instance, is a technology for immobilising microorganisms that is compatible to microfluidics and offers extremely dense trapping arrays, high scalability, simple integration, and simple biosensing; nevertheless, the trapping effectiveness is moderately poor. The high cost and limited access to certain genotypes are disadvantages associated with this technique.[20]

Table 3: Advantages and disadvantages of AMR (Anti-Microbial Resistance) diagnostic techniques

Method	Advantages	Disadvantages
WGS, WMS	• Enough for selective, non-culturable microorganism. • For long-read sequencing technologies, mobility and cost, minimal infrastructure for laboratory, and on-site sequencing are important considerations. • AMR's genetic basis has been established. Novel resistance mechanisms can be identified. • Multiple AMR factors are being studied concurrently (for WMS, from different hosts)	• Expensive equipment • Methodology is difficult and time-consuming. • Trained staff are required. • MIC cannot be defined. • There is no complete association with phenotype.
DNA Microarrays	• Sample purification is no longer required. • Polymicrobial samples were examined. • Multiplexed AMR 2 determinant targeting. • Adaptation to newly added resistance factors is rather rapid.	• Personnel with training are required. • Expensive laboratory equipment • MIC cannot be defined. There is no complete

		association with phenotype.
MALDI-TOF MS	• Rapid examination and automated • Low operating expenses and requires small sample size	• Expensive equipment with no portability • Individual, pure strain testing. Prior cultivation is required. • A mathematical sorting process is required.
FT-IR Spectroscopy	• Rapid examination and maximum throughput • Low operating expenses and requires small sample size	• Expensive equipment • Culture conditions alter IR spectra • MIC cannot be defined.
Microfluidics and Lab-on-a-chip	• Analysis with high throughput and speed and Fluid manipulation with precision • Minimal reagent usage and low sample size and simple sample modification • Procedure that is automated • Integrity, compactness, and portability	• MIC cannot be defined. • Scalability concerns • Issues with manufacturing reproducibility • A high surface-to-volume ratio • Surface preparation
Biosensors	• Minimal reagent usage and requires very low sample size. Have simple sample modification. • Procedure that is automated • Integrity, compactness, and portability	• Scalability concerns • Expensive equipment

3. Biosensors

Clark and Lyons initially addressed the biosensor concept in 1962 when they designed an oxidase enzyme-based electrode for the glucose detection. Since then, advancements in nanotechnology have assisted in the growth and specialization of biosensors for various applications. Biosensors are rapidly emerging as sensitive, selective, and cost-effective analysis techniques for early-stage diagnosis, essential for individualized health care management. Nanotechnology has improved and strengthened the sensing area through efficient device integration, sensing unit fabrication, interface, packaging, and performance, allowing biosensors to be adapted to the needs of illness management and patient disease profiles, i.e., in a rationalized manner. Biosensors are devices that use biological and biochemical reactions to detect numerous analytes in industrial, medical, and environmental applications. A biosensor comprises a biocatalyst (bioreceptor), which can be a cell, enzyme, tissues, oligonucleotide, etc., as well as a transducer (amperometric, semiconductors, potentiometric, thermometric, piezoelectric, photometric, etc.). Biosensors are the right approach for point-of-care devices, with the potential to reduce as they can reduce pathogen multidrug resistance and improve antibiotic stewardship. Nano-carbon-based sensing tools have recently been reported to provide fast and efficient real-time detection of bacterial cells, and the integration of their derivatives can lead to next-generation sensing devices. Biosensors are an analytical device that combines biological detecting elements known as bio-receptors with a physical transducer and is a primary method for measuring microbial cell reporters, which can be used with High-Throughput Screening (HTS) approaches. Figure 2 shows the basic components of biosensors used for detection of AMR strains.[21]

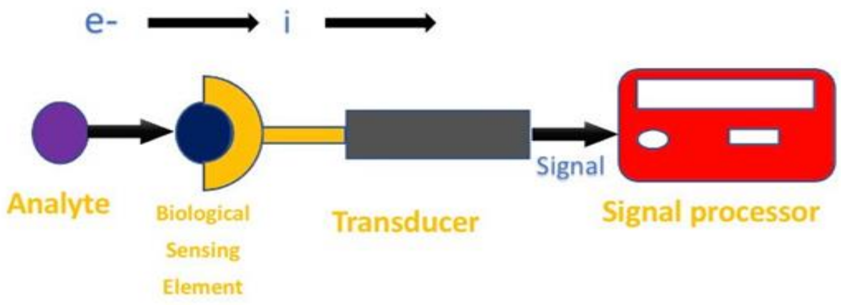

Figure 2. Basic mechanism and components of Biosensor

Biosensors are often used to detect biomolecules associated with illnesses, such as DNA or RNA (nucleic acids), proteins, and cells. This is achievable because of their three primary components: The reader device which includes the physiologically sensitive element, the detector element, and the signal device. Biomolecules are detected using enzymes, bacteria, organelles, antibodies, and nucleic acids. For the quick in-situ detection of AMR strains, the biosensor is among the best choice as it is a low-cost, portable microfluidic chemiresistive.

3.1 Types of biosensors

3.1.1 Electrochemical Biosensor- Electrochemical sensors monitor changes in electrical characteristics due to chemical property changes. Essentially, a chemical reaction causes a change in current, potential, or conductivity at the electrode, which is recorded by the transducer. Enzymes, nucleic acids, antibodies, entire cells, and receptors can all be detected with these biosensors.

3.1.2. Magneto-elasticBiosensor- The utilization of beads and nanoparticles with surfaces functionalized for biomedical interactions in magnetic sensors for bioassays makes them an invaluable tool for developing quantitative tests.

3.1.3. Acoustic wave Biosensor- Mechanical acoustic waves are used to transmit signals in acoustic wave biosensors. Currently, there are three types of sensors: bulk acoustic wave (BAW), surface acoustic wave (SAW), and acoustic plate mode (APM).

3.1.4. Immunosensors- Immunosensors are based on the interaction between antibodies and antigens and are utilized in developing immunoassays with high specificity and sensitivity. They use polyclonal, monoclonal, and recombinant antibodies to sense foreign molecules.

3.1.5. Whole-cell Biosensor - A typically utilized enzyme for whole-cell biosensors that can detect bacterial contamination is luciferase, which causes a light-emitting response. Antimicrobials that affect the transcriptional/translational machinery have been detected using bacteria that express the luciferase operon.

3.1.6. Biofilm Biosensor- Biofilm biosensors are a type of whole-cell live biosensor that can be used to develop bioreporters for environmental monitoring and drug discovery. Biofilm biosensors with oxygen electrodes were developed in this order of ideas to assess the respiration rate of microorganisms in a water purification system. However, it is vital to note that these biosensors necessitate continual cell care and the expenditure of nutrients if long-term storage is required.

3.1.7. Fluorescent Biosensor -Green Fluorescent Protein (GFP) most often utilizes fluorescent microbial biosensor protein due to its stability. In this case, recombinant E. coli expressing GFP was utilized as a screening platform to assess the antibacterial efficacy of silver nanoparticles (AgNPs), with increased GFP fluorescence indicating cell lysis in AgNP-treated pathogens.

3.1.8. Nano sensors- The development of biosensors is an exciting use of nanotechnology; nano sensors are an essential tool for obtaining information from nanoparticles, and they are categorized as physical, chemical, and biological nano sensors. These nano sensors can be put into nanowires, which are nanostructures with key features (mechanical, electrical, thermal, and multifunctional), resulting in an electrochemical sensor with significantly higher sensitivity and specificity. [22-26]

3.2. Vitality of Biosensors

Antibiotics are widely used in animals for both prevention and treatment. Their accumulation in the human body due to the food chain may pose significant health risks. As a result, developing sensitive and specific technologies for easy and rapid antibiotic screening in animal-derived foods is extremely important. The most widely utilized detection methods are chromatographic techniques such as HPLC and LC-MS/MS, as well as immunological approaches such as enzyme-linked immunosorbent assays (ELISA). These approaches are sensitive enough but time-consuming and expensive to implement. They also demand expert workers and expensive equipment. Biosensors are new analytical tools that can be used to perform easy, on-site, low-cost, and specific tests.[27]

Furthermore, multiple biosensor types can be used with antimicrobial resistance detection. Each kind of biosensor has its benefits and drawbacks. In quantitative and semiquantitative recognition, fluorescent biosensors can be a good choice. With the usage of nanomaterials like UCNPs, their affectability has recently been increased to pg mL1. Nonetheless, a fluorophotometer is required to examine the results in this type of biosensor. Low detection limits, an extensive linear response range, and the capacity to evaluate small amounts of samples are all characteristics of electrochemical biosensors. Low reproducibility, extensive sample pre-treatments, and the need for equipment to read data are all drawbacks. The miniaturization of electrochemical biosensors to create portable and reusable devices could be a viable technology in antibiotic detection. In electrochemical biosensors, choosing effective anode materials enhances electrical conductivity and reactant flow while reducing biorecognition time and boosting affectability. When used appropriately, nanomaterials such as metal, many oxide nanoparticles, and carbon nanostructures can be used to build electrochemical biosensors with a fast reaction time and great affectability. Nanomaterials can improve optical and magnetic qualities in optical biosensors, improving affectability and specificity in recognition. Accordingly, nanotechnology proposes new apparatuses to fail existing difficulties and limitations of biosensors to increase from the research center to their business application in the place of care. Among the unique sorts of nanomaterials, AuNPs have been utilized in the creation of numerous business antimicrobial packs and biosensors like BiooAuroFlow™ parallel stream test strips (PerkinElmer Co.), Charm ROSA tests (CHARM Science Inc) and IDEXX SNAP tests (ThermoFisher SCIENTIFIC). Even though regardless of many advances in this field, a fantastic innovation that can produce monstrous measures of nanomaterial-based biosensors with a superior grade, high accuracy, diminutive size, the compact and minimal expense is still profoundly wanted. Future endeavours should zero in on further developing biorecognition components to build their soundness against genuine natural conditions. Test conditions, including pH, temperature, consistency, and ionic strength, can influence the restricting action of a wide range of biorecognition components. Finally, revamped transducers with improved execution will be built with better nanomaterials and biorecognition components. Another critical issue in developing anti-toxin biosensors is the design of display frameworks based on the immobilization of many receptors to detect multiple anti-infection chemicals simultaneously, which can save investigation time and cost.[28-30]

4. Conclusion

In the past decade, molecular-level investigation of microbial resistance has yielded a plethora of information. With the use of several techniques such as FTIR, Biosensors, Microscopy, Immuno detection, spectroscopy, microfluidics technology, and molecular amplification techniques (PCR, DNA microarray, Pyrosequencing, NAAT, WGS (complete genome sequencing), (matrix-assisted laser desorption/ionization time-of-flight) MALD-TOF, our understanding of the transmission and distribution of resistance markers amongst microbial species has advanced significantly. In routine diagnostic susceptibility-bioassay may also help in the elimination of this problem. The variety of point mutations or genes leading to resistance and the labour-intensive aspect of existing amplification techniques remain obstacles. The combination of biosensor and molecular technology has the potential to address these issues. Molecular assays for many resistance biomarkers (e.g., mecA in resistant staphylococcus aureus and vanA in Enterococci) are robust and trustworthy; however, assays for other resistance markers are often unidentified or require practical evaluation. Furthermore, phenotypic (MIC) screening will remain crucial for the foreseeable future to discover emerging novel resistance mechanisms.

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