American Journal of Biodiversity
https://biojournals.us/index.php/AJB
ISSN: 2997-3600

Genetic Detection of Antibiotic-Resistant Bacteria Using PCR and DNA Sequencing Techniques

Rasha Kadhim Hasson
Madenat Alelem University College Department Of Biology
Jeehan Muhammed Nasser Abood
University Of Baghdad Institute Of Genetic Engineering And Biotechnology Department Of Genetic Engineering And Biotechnology
Manar Ghalib Daebel Sirhan
University Of Kufa College Of Science Department Of Pathological Analyses
Yaseen Taha Rashid Majeed
University Of Kirkuk College Of Science Department Of Biology

Received: 2025 01, Oct
Accepted: 2025 10, Oct
Published: 2025 20, Oct

Abstract
Antibiotic resistance poses a global threat to human health and well-being. Conventional approaches to detecting resistance, such as culture and sensitivity testing, are time-consuming and do not always correlate with emerging resistance mechanisms. Molecular detection methods, including polymerase chain reaction (PCR) and DNA sequencing, have been integrated into routine diagnostic workflows for identifying clinically significant resistance and surveillance of outbreak situations. These approaches are complementary to culture-based methods and allow detection of undetectable resistance markers, confirming resistance predicted by phenotypic testing, and determination of variant alleles that modify resistance profiles. Detection workflows commence with sample processing and conventional or quantitative real-time PCR, followed by Sanger or next-generation sequencing. Variant-calling pipelines paired with publicly available resistance and annotation databases provide rapid information on known and novel mutations of clinical concern. Parallel phylogenetic analyses inform strain lineage

assignments and dissemination routes, supporting surveillance and outbreak investigation.

Introduction to Antibiotic Resistance and Molecular Detection

Antimicrobial resistance (AMR) now constitutes an urgent public health threat worldwide [1]. Resistance mechanisms pose serious challenges in treating bacterial infections, necessitating the development of new molecular detection methods. Specifically, polymerase chain reaction (PCR) and DNA sequencing techniques enable the rapid detection of antibiotic-resistant bacteria in clinical samples. Traditional culture-based approaches remain the gold standard in clinical diagnostics; however, they require several hours to days of pathogen isolation prior to making AMR-related decisions. These delays may adversely affect patient outcomes and increase the risk of AMR transmission [2]. The use of molecular methods can therefore help curb the AMR crisis.

Background on Antibiotic Resistance Mechanisms

Antibiotic resistance has become a significant public health threat throughout the world. Every year, millions of people die because of resistant strains of bacteria. Certain strains can bear more than one resistance determinant, raising the ominous possibility of an untreatable infection. The presence of a resistance gene does not guarantee its expression: additional factors such as the regulatory region, the presence of the right transcription factors, and environmental factors influence the level of expression. Monitoring antimicrobial resistance through surveillance of zoonotic and indicator bacteria is essential to assess its spread between animal and human populations.

Several mechanisms of drug resistance operate in bacteria, and a variety of molecular methods can help detect the presence of specific genetic determinants. Most antibiotics enter cells through porins or transporters, and mutations that alter the expression of the corresponding genes can affect susceptibility. Various efflux pumps, whose activity can be increased by specific mutations, target major antibiotic classes such as tetracyclines, fluoroquinolones, beta-lactams, and biocides. Enzymatic modification of the drug is another widespread mechanism, and resistance determinants can often be identified by their sequence similarity with proteins classified in the corresponding family; for this reason, traceback based on the enzyme's catalytic residues is frequently more robust than assessing amino acid identity. Finally, bacteria can modify the binding of a drug to its target, and allelic variation in the target itself—e.g., ribosomal RNA, RNA polymerase, penicillin-binding proteins—exposes a bacterium to resistance without compromising fitness. Monitoring antimicrobial resistance through surveillance of zoonotic and indicator bacteria from animals is essential, and emerging rapid resistance testing methods for clinical microbiology laboratories can impact patient management [2] [3].

Overview of PCR and DNA Sequencing in Diagnostics

The rapid emergence and global spread of multidrug-resistant (MDR) bacteria pose major clinical and public health threats. These organisms frequently exhibit resistance to critically important antibiotics, hampering effective therapy and augmenting the risk of mortality during routine procedures. Use of broadspectrum antibiotics further accelerates resistance development and transmission between strains and species, highlighting the urgent need for timely and precise detection of resistance determinants in clinical isolates. Traditional diagnostics rely on cultivation, biochemical testing, or mass spectrometry [1] , which require several hours or days before treatment decisions may be made. Nucleic-acid-based methods such as PCR and DNA sequencing represent complementary genetic approaches for rapid resistance

detection either in addition to or instead of culture; they provide direct, sensitive, and specific information on resistance mechanisms [2] and enable recoil from combinations of broad- and narrow-spectrum agents.

Amplicon-based PCR analysis offers a straightforward strategy for detecting specific resistance genes and variants explicitly linked to particular antibiotics. Analysis of 16S rRNA enables direct determination of resistance to aminoglycosides and certain polypeptides; characterization of 23S rRNA targeting clarithromycin; and amidase detection, notably those conferring extended-spectrum β-lactam resistance. Amplification and sequencing of the appropriate genes not only facilitate accurate variant screening but also yield generic information on additional resistance groups. Flow cytometry provides an alternative approach for quantifying bacterial loads and the evaluation of resistance to β-lactams [4].

PCR-Based Approaches for Resistance Gene Detection

Antibiotic resistance poses a global health threat and has motivated the development of methods to detect antibiotic-resistant microorganisms and their resistance genes rapidly. Traditional culture-based methods enable indirect resistance gene detection by assessing antimicrobial susceptibility but are time-consuming and limited to culturable microorganisms. PCR-based approaches have emerged as rapid methods to detect resistance genes directly from bacterial cultures, support timely clinical management, and remain compatible with culture-based diagnostics [2]. Several PCR-based approaches are available for resistance gene detection: Conventional PCR followed by gel electrophoresis enables the identification of resistance markers by expected amplicon size based on the genes' presence regardless of copy number. Real-Time PCR (qPCR) quantifies the number of copies of a target resistance gene in an initial nucleic acid extract, whereas standard curves allow the extrapolation of the resistance gene load. Multiplex PCR allows multiple resistance markers to be amplified in a single reaction; multiple amplicon sizes guide the interpretation of results. Conventional and multiplex PCR may enable timely confirmation of resistance genes detected by direct sequencing, while results across different targets indicate the involvement of common or distinct resistance mechanisms.

Conventional PCR and Gel Electrophoresis

PCR (Polymerase Chain Reaction) enables the amplification of specific nucleic acid sequences from various types of biological samples in a laboratory setting. It has grown into an essential tool for molecular diagnostics, quantitative genome analysis of different pathogens, and the investigation of antimicrobial resistance. Conventional and reliable PCR methods have been developed for the identification of Staphylococcus aureus and the detection of various drug-resistance genes such as mecA, blaZ, ermA, tetK, and tetM. These approaches are therefore widely adopted for the clinical detection of such priority micro-organisms. The resultant PCR amplicons of the corresponding resistance genes can be directly visualized on agarose gel electrophoresis or quantified through real-time PCR amplification.

The diagnostic performance and clinical significance of conventional PCR techniques targeting drug-resistance genes such as mecA have been evaluated to further enhance the method. The capability of a diagnostic approach to detecting a particular resistance determinant in pure culture of an organism at clinician-relevant levels, alongside the availability of add-on tests for characterizing co-resistance mechanism to other antibiotics, determines its potential clinical utility [4]. Guidance on the interpretation of S. aureus PCR amplification products has been established to support the global effort to curb antibiotic resistance in the clinical setting. The emergence of antibiotic-resistance mechanisms among different genera and species of micro-organisms, including Acinetobacter, Enterobacter, Escherichia coli, Klebsiella, Pseudomonas, Salmonella, and Serratia, has continued to be a major public health threat.

Quantitative Real-Time PCR (qPCR) for Gene Quantification

This section describes qPCR methods to quantify antibiotic-resistance genes. With standard curves derived from known targets, sample-cDNA copy numbers can be estimated, facilitating monitoring of bacterial loads and resistance-gene persistence under selective pressure [4]. ΔCt thresholds in multiplex assays convey clinical significance based on sequences present and DNA concentrations, while

quantification of components from biochemical pathways directs pathogen identification and therapy selection.

Multiplex PCR for Simultaneous Resistance Markers

For simultaneous detection of multiple resistance markers in a single assay, multiplex PCR is a well-established, cost-effective option [5]. To achieve reasonable amplicon sizes across diverse target genes, compatibility of oligonucleotide primers—especially their melting temperatures—becomes crucial in assay design. In clinically relevant contexts, multiplexing of colorimetric and quantitative polymerase-chain-reaction (PCR) marker screens [6] enables simultaneous analysis of up to five markers relevant to colistin resistance, extended-spectrum β-lactam content, and/or specific β-lactamase genes. Confirmation of resistance-gene detection via DNA sequencing subsequently facilitates assessment of allelic variation.

DNA Sequencing Techniques in Resistance Genomics

Antimicrobial resistance (AMR) threatens effective treatment of infectious diseases, driving an urgent need for timely and accurate diagnostics. Genetic methods for locating AMR genes fulfill this need, forming a complimentary role in relation to conventional culture techniques [2]. Polymerase chain reaction (PCR) and DNA sequencing techniques offer rapid, specific detection of well-characterised resistance genes, quantification of AMR gene copy numbers, and extensive profiling of resistance determinants on plasmids or chromosomal locations. In infection control, they help assess infection versus colonisation status, understand routes of pathogen spread, and discover variants with uncharacterised phenotypic effects [7].

Table 1 summarises typical diagnostic workflows and common approaches to specimen preparation. Key concepts covered in this section include expected amplicon patterns, analytical sensitivities, and interpretation strategies linked to various PCR methods; requirements for confirmed detection of known resistance genes enabling clinical action; parameters for characterising novel resistance genes and variants discovered during outbreak investigations; and indications for each sequencing technology alongside its impact on analysis design.

DNA Sequencing Techniques in Resistance Genomics

Sanger Sequencing for Known Resistance Genes

Sanger sequencing is suitable for investigating known resistance genes exhibited by the organism, particularly when multiple different AMR gene PCRs have already ruled out common determinants. Select PCRs also target specific variants linked to observed phenotypes. High-quality sequence reads can verify identity and confirm presence of specific alleles such as mutations, insertions, duplications, or deletions. [8][9][10]

Next-Generation Sequencing (NGS) for Comprehensive Profiling

NGS offers a broad-spectrum approach well-suited to simultaneous characterisation of plasmid and chromosomal AMR determinants during prospective outbreak analysis. Sequencing libraries can be prepared from metagenomic and amplicon-enriched templates; additional read enrichment strategies include target-capture and, particular to bacterial genomes, single-colony isolation. For strains with previously sequenced genomes, improved quality and completeness enable characterisation of AMR genotypes acquired through plasmids. [11][12]

Sanger Sequencing for Known Resistance Genes

Sanger sequencing complements other assays by confirming known resistance genes in clinical samples. It is usually applied when one or two specific resistance alleles are required. The peak quality of Sanger traces from Illumina and other platforms often exceeds Phred score 40 (99.99% accuracy), permitting unambiguous resolution of single-nucleotide polymorphisms (SNPs), insertions, and deletions on amplicons below 600 bp.

A diagnostic workflow for resistance-gene detection may begin with conventional PCR and gel electrophoresis. DNA sequences from amplicons showing the expected size can then be submitted for

Sanger sequencing to interrogate single-target, high-confidence variants that accumulate during clonal evolution [2].

Next-Generation Sequencing (NGS) for Comprehensive Profiling

Next-generation sequencing (NGS) technologies have revolutionized the genomic exploration of drug-resistant pathogens, providing invaluable insights into antimicrobial resistance mechanisms [13]. Notably, NGS enables the comprehensive identification of resistance genes, variants, and plasmid characterization from a single clonal isolate in a single run. The ability to precisely characterize both acquired and intrinsic resistance genes represents a significant advancement in surveillance and understanding of the wider resistance landscape.

A variety of NGS platforms and methods are now available, and the choice of workflow depends on the clinical question and the available sample type. Options include either short-read or long-read sequencing of total genomic DNA, in addition to the sequencing of purified plasmid DNA. The desired coverage and the nature of resistance-related sequences influence these decisions. For plasmid-transmitted resistance genes, the characterization of plasmids via plasmid-sequencing strategies—including the separation of plasmid and chromosomal DNA or the direct sequencing of plasmid DNA from a total extract—provides critical information on resistance gene mobility.

Bioinformatics and Data Interpretation

Antibiotic-resistant bacteria pose a significant threat to global public health, necessitating urgent surveillance, diagnostics, and control measures. The widespread misuse of antibiotics has accelerated the emergence and spread of such pathogens [14]. Consequently, monitoring antimicrobial resistance (AMR), providing timely alerts, and informing intervention measures have become pressing necessities for healthcare facilities.

Amplification of specific resistance genes via polymerase chain reaction (PCR), followed by conventional sequencing or next-generation sequencing (NGS), offers a molecular approach that complements culture-based methods. The ultimate objective of this method is to facilitate the comprehensive characterization of AMR genes in both known and unidentified pathogens, supporting elucidation of resistance mechanisms as well as tracking of their dissemination [7]. Resistance monitors, such as the Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae, and Staphylococcus aureus, form the focus of initial laboratory studies aimed at optimizing integration of these molecular detection techniques into conventional workflows.

Resistance Gene Databases and Annotation

The widespread use of antibiotics in medicine and agriculture has contributed to the emergence of resistant pathogenic bacteria, leading to antibiotic treatment failure and increased morbidity and mortality [2]. Therefore, rapid detection of antibiotic-resistant pathogens in clinical samples is necessary to guide appropriate therapy. However, conventional culture-based susceptibility testing requires a few hours to several days. Genes associated with antibiotic resistance, such as acquisition of antibiotic-degrading enzymes or modification of antibiotic targets, are frequently encoded in the bacterial genome [15]. The direct measurement of antibiotic-resistance genes in infected clinical specimens using molecular methods provides a powerful alternative to conventional culture-based methods. Because the presence of resistance genes indicates the possible existence of corresponding resistance mechanisms, the detection of resistance genes becomes a practical strategy to assess antimicrobial resistance.

Several different sequences of resistance genes have been widely adopted as a robust detection mechanism for the identification of antibiotic-resistant bacteria. There are also several public databases listing a complete catalogue of bacterial resistance genes, variants, and their resistance mechanisms. Once the sequence of the amplified resistance genes is obtained, the sample can be compared to the extensive catalogue provided in the databases for bacterial resistance identification. Genomic information generated

by various sequencing platforms combined with bioinformatic tools has allowed the identification of resistance genes and variants in complex samples.

Antimicrobial Resistance (AMR) constitutes a major global health threat; therefore, many countries worldwide have decided to take actions throughout the next few decades to mitigate and eliminate the rise of AMR. All countries have activated an AMR plan highlighting the importance of resistance-gene detection.

Variant Calling and Mutation Impact Assessment

Genotyping enables the identification of both known and novel mutations within resistance genes of interest, directly linking observed variants to functional and evolutionary characteristics of the pathogen population. Bioinformatics workflows for variant-location detection typically involve mapping high-throughput sequence reads to a reference genome, followed by variant calling and impact evaluation [16]. The completeness of the reference assembly influences the soundness of these methods; therefore, assemblies representing the full gene content, in addition to the intact chromosome, are recommended for capture–seq studies targeting mobile genetic elements [17]. Automated pipelines will facilitate routine installation, ultimately providing robust annotation for clinically and epidemiologically relevant bacterial pathogen datasets. Support for *Snippy* and *Nanonemo* ensures that common *Bacteroides* taxonomy trees, AMR gene sets, and *Bacteroides* plasmid identifiers remain available for *Bacteroides* datasets. By integrating extensive resistance variant databases into an automated annotation framework, the pipeline contributes to proactive surveillance of emerging genetic threats.

Phylogenetics and Strain Typing in Resistance Surveillance

Microbial genotyping supports epidemiological resistance surveillance by determining strain lineages, tracking dissemination pathways, and refining outbreak frameworks [2]. Genomic data provides the phylogenetic context—needed to interpret the significance of sequence-based resistance information and grasp strain mobility within health institutions [17].

Clinical and Public Health Applications

Preventing antibiotic resistance development and dissemination not only preserves effective therapy options, but also alleviates pressure on antimicrobials used in livestock [7]. ANC Resident Provides accurate data on diagnosis, prevention and control of agricultural animal diseases, safeguarding public health and preserving trade. The implementation of data- and information-sharing networks and the coordination of “one health” approach is a prerequisite for sustainable development. Safe trade of live animals, animal products, inputs and equipment is vital for regional animal health, food safety and humane treatment. Trade relationships are complemented by joint training and technical exchanges, and by capacity development in workplace health and safety and biosecurity [2].

Diagnostic Workflow in Clinical Microbiology

Stepwise integration of PCR and sequencing workflows into routine diagnostic approaches enables genetic detection of antibiotic-resistance determinants in bacteria, enhancing clinical microbiology laboratory capacity to monitor resistance mechanisms and inform public health responses [2]. Initial sample handling and preparation should follow standard protocols. The appropriate application of both technology platforms depends on existing knowledge of the organism and associated resistance markers, sample type, and end-user preferences. PCR assays can target well-characterized resistance genes or genetic neighbourhoods in common pathogens, with multiplexing capability extending the upper limit to six simultaneous PCR targets with dedicated amplicon sequencing for confirmation.

Surveillance and Outbreak Investigation

The emergence of antibiotic resistance is jeopardizing the treatment of even common infections. Sequence-based surveillance of resistant clinical isolates enables the monitoring of resistance mechanisms, supports the tracking of their spread, and fosters a better understanding of epidemic dynamics in the

context of epidemiology and outbreak investigation [18]. Resistance genes conferring classical phenotypic resistance can be detected even when novel phenotypes arise, thus guiding affected patients and informing public-health measures [19].

Next-generation sequencing (NGS) facilitates the characterisation of resistance genes alongside species identification and strain typing, complementing other markers such as multi-locus sequence typing (MLST) [4]. The combination of polymerase chain reaction (PCR) and sequence-based detection of antibiotic-resistance mechanisms advances diagnostics, surveillance, and responses to the growing threat of resistant bacteria, which can also be encountered in aquiculture, agriculture, and food due to environmental dissemination.

Technical Considerations and Quality Assurance

Antibacterial prevention for diagnostic pipeline of clinical microbiology is not only important for individual patients but also for public health, as resistant strains can spread and reach some risk groups—even in non-clinical settings [20]. Cross-species detection and typing have been achieved to follow strains from environment to humans [17]. High-resolution monitoring of resistance genes is complementary in outbreaks involving resistant strains or in epidemiology to understand evolving public-health threats. Among diverse genomic-based techniques that detect resistance, amplification and sequencing of specific marker genes have gained visibility within diagnostic laboratories. Enrichment of the classical culture method with PCR/sequencing hold promise for resistance discovery and extensive data collection is already generated for custom bioinformatics approaches.

Starting microbial focus on resistance groups and making choice between uncomplicated workflow with dedicated targeted amplification/sequencing approaches and wide-scale exhaustive profiling were operational concerns among selected laboratories. Understanding of sample-independent pre-conditions controls dominate as major technical parameters determined diligence of proceeding application and compatibility with routine service for resistance genotyping on direct clinical samples was examined on designed test-beds. Sample pre-treatment protocols help to universally extend service over alternatively-sampled biological matrices already in practice. Sample processing validation of alternate extraction kits, nucleate-number estimation via dye-binding assays, colourimetric analysis for DNA-contamination residues detection covering corresponding analytical tools is basis of compatibility rationale integrated to posterior amplicon-size control, positive-negative insert check on sequencing data, and in situ coliphage-analogue test towards environmental reach security.

Sample Preparation and DNA Quality

PCR amplification and sequencing of resistant mechanisms are informative; successful amplification and high-quality sequencing requirements guide preliminary sample preparation. DNA must remain intact at 30–1000 bp, with a threshold size of 150 bp [21] ; DNA degradation below this limit becomes detectable and signals control detachment from the reaction mix. Potential PCR and sequencing inhibitors (e.g., humic acids, polyphenols, quinones) plus sample-specific extraction protocols drive method choice and compatibility with downstream procedures. Chemical contaminants (e.g., EDTA, protein, phenol, xylene) exert strong influence on enzyme activity, while mechanical factors (e.g., pH, UV, freezing) affect polymerase selectivity. DNA extraction methods (e.g., boiling, organic separation, silica-based) solidify downstream procedures, retain viable bacteria, and discard co-extracted inhibitors at single-cell resolution [4].

Controls, Validation, and Reproducibility

Antimicrobial resistance remains a global health concern, highlighted by WHO's report (2014) indicating that E. coli and Klebsiella spp. are among the most prevalent resistant pathogens. In Sweden, >90% of prescriptions are observational, a method advertised to combat the occurrence of resistant bacteria. Genetic detection is a method that allows the rapid identification of bacteria responsible for disease, as well as the specific mechanism that contributes to resistance. Molecular techniques such as PCR and DNA

sequencing have provided rapid identification of microorganisms and detection of resistance mechanisms at a comparatively low cost [17]. This section provides an overview of controls, validation and reproducibility.

Limitations, Sensitivity, and Specificity

Clinicians and paramedics often face a dilemma: how to diagnose and treat infections accurately and quickly. Rapid and accurate diagnosis of antibiotic-resistant organisms is crucial for timely and precise antimicrobial treatments [2]. Consequently, incorporation of polymerase chain reaction (PCR) and sequencing approaches at different steps in the diagnostic pipeline extends their potential applications. Such applications include screening, establishing thresholds for clinical significance, and confirming and comprehensively characterizing various antibiotic-resistance genes [22].

Conventional methods, such as culture-based approaches and minimum inhibitory concentration determinations, remain the gold standard. Their inherent time lag can exacerbate an ongoing gap in antibiotic-resistance monitoring. To this end, these methods recognize that resistance is only of concern if the organism is present within the patient sample, thus providing no information on whether an antibiotic should be administered for treatment. The amount of amplified DNA detectable through gel electrophoresis is limited by the sample and assay employed.

Ethical, Legal, and Social Implications

The growing threat of antibiotic resistance has emerged as a global public health challenge [16]. Asymptomatic carriage of resistant bacteria, influencing both individual patient management and population-level transmission dynamics, raises complex ethical questions regarding the use of genotypic testing relative to conventional laboratory cultures [23]. The increasing demand for genotypic resistance-testing strategies has contributed to growing ethical interest in the field [17]. Bacterial resistance has historically been linked to the inappropriate or suboptimal use of antimicrobials, yet current technologies allow the deployment of genotypic resistance determinants as a universal control measure across all pathogens.

Privacy and Data Security in Genomic Diagnostics

Clinical laboratories and public health agencies routinely generate genomic data on pathogen isolates to monitor and mitigate the threat of antibiotic resistance. This information can help identify the presence of resistance genes or mutations in clinical specimens and supports surveillance, outbreak investigation, and intervention planning. Because sensitive human information can be inferred from genomic data, however, data protection is essential [24]. A responsible stewardship strategy incorporates data-de-identification techniques prior to data sharing, limitations on access, and specific non-reproductive usage prohibitions [17]. The use of open-source software, repository deposits, and uncontrolled distribution channels with sensitive data should also be avoided. A genomic diagnostic report with interpretations and recommendations for follow-up actions, including relevant public health connections, is another means of supporting responsible communication [4].

Responsible Reporting and Clinical Actionability

The Convention on Biological Diversity (CBD) recognises the need to maintain the integrity of biomes and ecosystems, and the Global Biodiversity Strategy proposes actions to conserve the diversity of all ecosystems. Nature's ecological additives, valuated and rediscovered by generalist ecologists, have the ability to renew themselves, be regenerated, and be preserved without depleting ever-thinned stocks. Ultimately, basic local ecological knowledge forms the foundation for the emergence of new disciplines: the ecology of the local biomes, or local expressive ecologies to indicate the nature of the aural waveforms in a local environment.

Precise determination of resistancenot only enables responsible public reporting but also supports actionable delivery and tracking of multistate resistance across different inclusion scenarios [2] ; [17].

Future Directions and Emerging Technologies

Widespread adoption of molecular detection has stimulated interest in testing alternative platforms, which could translate to various settings and applications. One example is the emerging use of CRISPR-based systems for diagnostics, combining specificity with isothermal amplification and signal reporting. A number of prototype devices have shown performance in bacteriophage detection, HIV monitoring, and antibiotic resistance screening. Significant engineering and validation remain necessary to achieve broader implementation across resistance targets and contexts. Construction of multi-target assays would further enhance utility [2].

The continued escalation of antibiotic resistance and the demand for rapid-response diagnostics have motivated the development of portable, battery-operated, and integrated sequencing devices. Several prototypes enabling the sequencing of entire genomes have appeared. Systems promising targeted screening of antibiotic resistance genes, libary preparation in under ten minutes, and seamless integration with PCR amplification and reverse transcription constitute active research directions. Such capabilities could expedite workflows, simplify sample-preparation steps, enable milieu-independent applications, and facilitate dissemination to settings with limited resources; interoperability with other diagnostic techniques would further enhance value [1].

CRISPR-Based Diagnostics

Bacterial infections caused by antibiotic-resistant (ABR) pathogens are difficult or impossible to treat, posing an urgent threat to global public health [25]. Such infections lead to increased risk of treatment failure, prolonged illness, more expensive care, and the need for alternative treatment that may be less effective and more toxic. The hospitals cannot guarantee treatment success if a neonate is exposed to ESBL-producing Enterobacterales. The rapid identification and control of these pathogens are therefore critically important in hospitals. Conventional methods for detecting ABR pathogens rely on culturing suspected samples from patients infected or colonised with ABR pathogens. The advantage is that these methods can identify pathogens and ABR genes, but the process is slow. Polymerase chain reaction (PCR) along with DNA sequencing can be used instead of cultivation-based methods for detecting ABR pathogens.

The CRISPR/Cas system, a defence mechanism for bacteria, where Cas proteins pair with RNA from the organisation of the invading nucleic acid, has been engineered into a powerful tool for detecting nucleic acids and controlling the infection of plasmids carrying ABR genes. The diagnostic system based on the CRISPR/Cas systems can detect plasmid-like sequences rather than a specific gene and is able to identify the spread of both the complete plasmid and the resistance gene during a plasmid-based epidemic.

Integrated Point-of-Care Genomic Platforms

Emerging pathogens with antibiotic resistance are a major concern for health systems worldwide. In collaborative research involving the School of Public Health and. the Faculty of Medicine, work demonstrates how clinical microbiology laboratories may implement a point-of-care antibiotic resistance surveillance strategy to respond to these threats using routine diagnostics. A combination of polymerase chain reaction (PCR) and DNA sequencing methods detect and characterize antibiotic-resistant genes and their variants after simple sample extraction. This approach integrates with existing workflows and uses a bioinformatics pipeline to inform bacteriology of changes before and during sample culture. It ensures a highly relevant period of resistance monitoring, an increase in the number of sequenced resistomes, and an opportunity to study variant activity. All steps have been well characterized in both the literature and ongoing public-health programs. Routine testing for transposable resistance genes in Escherichia coli and Klebsiella pneumoniae is recommended [2].

Conclusion

Antimicrobial resistance (AMR) poses a serious and rapidly growing threat to public health that affects both developed and developing countries. AMR is often associated with no effective treatment,

ineffective treatment, lengthened hospitalization, high health costs, and increased mortality rates. AMR remains a growing global concern despite advances in the pharmaceutical and healthcare sectors. AMR continues to evolve through the transmission of resistant microorganisms (often referred to as "superbugs"), which are defined as pathogens capable of overcoming multiple antibiotic agents. Among various diagnostic methods, Polymerase Chain Reaction (PCR) and sequencing have garnered significant attention for AMR bacteria detection and profiling. Different approaches have been developed with PCR and (next-generation) sequencing as complementary tools for various AMR diagnostic applications.

The overall detection method is highly dependent on AMR laboratory capability, while clinical samples, environment samples, food samples, and veterinary samples can all be considered to guide the workflow design. When applying PCR/sequencing techniques to AMR detection, the capability of an institution across clinical/industrial/food applications enables better downstream pathway selection. The amplification and sequencing of the 23S rRNA region can reveal whether one is profiling an E. coli, Salmonella Typhimurium, or any other samples. In an environment possessing a wider spectrum of plasmids, utilizing a corresponding approach to capture AMR-associating plasmids can provide additional insights beyond the limited core genomes. Antibiotic resistance (ABR) continues to threaten human health globally, especially in Africa. The celebration of eliminating one ABR determines the viabilities and capacities for emerging ABR from circulating pathogens.

References:

1. K. Alby and M. B. Miller, "Bacterial Infections," 2015. ncbi.nlm.nih.gov
2. H. Frickmann, W. Omurwa Masanta, and A. E. Zautner, "Emerging Rapid Resistance Testing Methods for Clinical Microbiology Laboratories and Their Potential Impact on Patient Management," 2014. ncbi.nlm.nih.gov
3. A. Rohde, J. Andre Hammerl, and S. Al Dahouk, "Rapid screening for antibiotic resistance elements on the RNA transcript, protein and enzymatic activity level," 2016. [PDF]
4. J. R. Waldeisen, T. Wang, D. Mitra, and L. P. Lee, "A Real-Time PCR Antibiogram for Drug-Resistant Sepsis," 2011. ncbi.nlm.nih.gov
5. A. Rita Rebelo, V. Bortolaia, J. S Kjeldgaard, S. K Pedersen et al., "Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes," 2018. ncbi.nlm.nih.gov
6. R. Hatrongjit, A. Kerdsin, Y. Akeda, and S. Hamada, "Detection of plasmid-mediated colistin-resistant and carbapenem-resistant genes by multiplex PCR," 2018. ncbi.nlm.nih.gov
7. S. M. Diene, C. Bertelli, T. Pillonel, J. Schrenzel et al., "Génomique et métagénomique bactériennes: applications cliniques et importance médicale [Bacterial genomics and metagenomics: clinical applications and medical relevance]," 2014. [PDF]
8. E. A. Mesfin, M. Merker, D. Beyene, A. Tesfaye, Y. A. Shuaib, "Prediction of drug resistance by Sanger sequencing of Mycobacterium tuberculosis complex strains isolated from multidrug resistant tuberculosis suspect patients in …," *Plos One*, 2022. plos.org
9. P. Drevinek, R. Hollweck, M. G. Lorenz, et al., "Direct 16S/18S rRNA gene PCR followed by Sanger sequencing as a clinical diagnostic tool for detection of bacterial and fungal infections: a systematic review and …," Journal of Clinical …, 2023. asm.org
10. A. M. Nafea, Y. Wang, D. Wang, A. M. Salama, "Application of next-generation sequencing to identify different pathogens," *Frontiers in...*, 2024. frontiersin.org
11. C. S. C. Liu and R. Pandey, "Integrative genomics would strengthen AMR understanding through ONE health approach," Heliyon, 2024. cell.com

12. N. S. Olsen and L. Riber, "Metagenomics as a transformative tool for antibiotic resistance surveillance: highlighting the impact of mobile genetic elements with a focus on the complex …," Antibiotics, 2025. mdpi.com
13. R. Guillen, "Next-generation sequencing technology in relation to our understanding and tackling of Antimicrobial Resistance [Tecnología de secuenciación de próxima generación en relación con nuestra comprensión y lucha contra la resistencia antimicrobial]," 2019. [PDF]
14. P. Bradley, N. Claire Gordon, T. M. Walker, L. Dunn et al., "Rapid antibiotic-resistance predictions from genome sequence data for Staphylococcus aureus and Mycobacterium tuberculosis," 2015. ncbi.nlm.nih.gov
15. H. Dong and D. Ming, "A Comprehensive Self-Resistance Gene Database for Natural-Product Discovery with an Application to Marine Bacterial Genome Mining," 2023. ncbi.nlm.nih.gov
16. G. Maboni, R. de Paula Baptista, J. Wireman, I. Framst et al., "Three Distinct Annotation Platforms Differ in Detection of Antimicrobial Resistance Genes in Long-Read, Short-Read, and Hybrid Sequences Derived from Total Genomic DNA or from Purified Plasmid DNA," 2022. ncbi.nlm.nih.gov
17. S. Pholwat, J. Liu, M. Taniuchi, R. Chinli et al., "Genotypic antimicrobial resistance assays for use on E. coli isolates and stool specimens," 2019. ncbi.nlm.nih.gov
18. E. A. Oniciuc, E. Likotrafiti, A. Alvarez-Molina, M. Prieto et al., "The Present and Future of Whole Genome Sequencing (WGS) and Whole Metagenome Sequencing (WMS) for Surveillance of Antimicrobial Resistant Microorganisms and Antimicrobial Resistance Genes across the Food Chain," 2018. ncbi.nlm.nih.gov
19. M. Otto, "Next-generation sequencing to monitor the spread of antimicrobial resistance," 2017. ncbi.nlm.nih.gov
20. I. Carter, C. Halliday, T. P. Sloots, T. M. Pryce et al., "PCR Methodology," 2010. ncbi.nlm.nih.gov
21. A. Flint, A. Laidlaw, L. Li, C. Raitt et al., "Choice of DNA extraction method affects detection of bacterial taxa from retail chicken breast," 2022. ncbi.nlm.nih.gov
22. I. Katharina Sigmund, N. Renz, S. Feihl, C. Morgenstern et al., "Value of multiplex PCR for detection of antimicrobial resistance in samples retrieved from patients with orthopaedic infections," 2020. ncbi.nlm.nih.gov
23. E. Jamrozik and G. S Heriot, "Ethics and antibiotic resistance," 2022. ncbi.nlm.nih.gov
24. A. E., "Cryptographic solutions for genomic privacy," 2016. [PDF]
25. Y. Wu, D. Battalapalli, M. J. Hakeem, V. Selamneni et al., "Engineered CRISPR-Cas systems for the detection and control of antibiotic-resistant infections," 2021. ncbi.nlm.nih.gov.