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Review article

Integrative Multi-omics Approaches for Characterizing Antibiotic Resistance Mechanisms in Bacterial Pathogens: Challenges and Future Directions

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ABSTRACT

Antibiotic resistance (AMR) is no longer merely a genetic characteristic but a complex dynamic phenotype arising from interacting molecular networks. Conventional diagnostic methods such as minimum inhibitory concentration (MIC) test, PCR or even Whole-Genome Sequencing (WGS) have a limited understanding of resistance since they regard it as stable and unable to portray occurrence of phenomena like hetero-resistance, persistence and dissociation between genotype and phenotype. This review will highlight how integrative multi-omics (genomics, proteomics, metabolomics, transcriptomics, and metagenomics) is in a position to overcome these shortcomings by advancing the course of events starting from static gene catalogs to dynamic molecular networks. It also presents the idea of how integrative multi-omics can be employed to understand the mechanisms of antibiotic resistance in bacterial infections, with a focus on the existing issues and possible perspectives. This review derives from an elaborate literature analysis performed on online resources such as PubMed, Google Scholar, and Web of Science. This review highlights multi-omics approaches as suitable tools to counteract antibiotic resistance, illustrating the transition from static gene catalogs to dynamic molecular networks. Further, it addresses urgent issues in the field and considers possible advances in this context. The review establishes the rational system-based framework for the concept of antibiotic resistance as an emergent phenomenon of bacterial molecular networks. The work identifies critical bottlenecks in clinical translation and offers a clear roadmap, centered on AI integration, single-cell resolution, and in vivo relevance, for transforming multi-omics from a research tool into a frontline weapon against the AMR crisis.

Keywords: Antimicrobial resistance (AMR); Global threat; Horizontal gene transfer; MBLs production; Metagenomics; Metallo- β-lactamases; Multi-omics integration; Resistome.

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1. INTRODUCTION

Multi-omics employs an integrated analysis of diverse biological datasets, offering a transformative approach to understand, track and combat antimicrobial resistance (AMR) [1]. Integrative multi-omics delivers a holistic, systems-level perspective on how a bacterial cell adapts and survives under antibiotic stress, and does not rely on the detection of a known resistance marker or a single gene [2,3]. A multi-omics approach provides significant potential in understanding antibiotic resistance since genetic potential alone does not fully explain bacterial survival [4]. Accordingly, genomics typically identifies known resistance genes, mutations in drug targets, and possible regulatory regions via whole-genome sequencing (WGS) [5].

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Whereas transcriptomics (RNA-seq) shows which of these genes are highly expressed when bacteria face antibiotic stress or challenge. Proteomics confirms whether these transcripts are translated into functional proteins (e.g., efflux pumps, drug-degrading enzymes, or modified targets), which is an essential step, given that RNA levels do not always predict protein abundance. Finally, metabolomics uncovers key metabolic adaptations, like shifts in cell wall synthesis, energy pathways, or accumulation of protective compounds that ultimately enable bacteria to withstand treatment [3-8].

Unlike genetic resistance, which is heritable and results from chromosomal mutations or the acquisition of mobile genetic elements (MGEs) [9, 10], phenotypic resistance is generally transient and non-heritable, enabling decreased antibiotic susceptibility through mechanisms such as tolerance or persistence without altering the bacterial genome [10]. Hetero-resistance, in which subpopulations within a clonal population differ in susceptibility, is a distinct case: it is often genetically based but unstable (e.g., through gene amplification). Whenever exposed to antibiotics, bacteria employ two distinct survival strategies*: resistance* and *persistence*, each with unique mechanisms and clinical implications [11]. Antibiotic resistance is a *heritable genetic trait* that enables bacterial populations to grow in the presence of antibiotic concentrations that would *normally inhibit* them [12]. In contrast, antibiotic persistence is *a non-heritable, transient phenotypic state* in which a subpopulation enters *dormancy*, surviving lethal treatment without genetic change [13]. Horizontal acquisition *of resistance genes* via MGEs and chromosomal mutations that emerge during *bacterial replications are* the primary drivers of antibiotic resistance [14,15].

Horizontal gene transfer (HGT) spreads resistance genes via conjugation, which is direct cell-to-cell contact via a pilus that is primarily mediated by plasmids. Transformation, which is the uptake of free deoxyribonucleic acid (DNA) released from dead bacteria, is another resistance gene spreading mechanism for HGT [15,16]. A third route, transduction, occurs when bacteriophages mistakenly package a fragment of bacterial DNA, including resistance genes, and deliver it to a new host cell during a later infection [16].

The rapid spread of antibiotic resistance genes being driven by MGEs both within and across bacterial species significantly complicate diagnosis and clinical treatment [15,17,18]. Among these, plasmids, which are extrachromosomal, circular DNA molecules capable of independent replication, act as primary vehicles for HGT [19]. Through conjugation, plasmids efficiently shuttle critical resistance genes encoding carbapenemases, metallo-β-lactamases (MBLs), and extended-spectrum β-lactamases (ESBLs), between diverse bacterial populations [20]. Transposons rapidly spread multi-drug resistance (MDR) genes by "jumping" between chromosomes and plasmids [20] carrying a wide array of resistance determinants for beta (ß)-lactams, aminoglycosides, and colistin [15,21]. Integrons act as stationary gene-capture systems, assembling these resistance genes into cassettes that are then mobilized by transposons and plasmids [22,23]. This hierarchical network, where plasmids carry transposons, which in turn carry integrons packed with resistance genes, creates a highly efficient and stable dissemination platform [24,25]. This shows the use of multi-layered mobility for the rapid global spread of resistance genes, not only resistance mechanisms [26].

Antibiotic resistance involves six main mechanisms: (1) enzymatic inactivation, either degradation or modification of the antibiotic; (2) target site alteration, mutations or enzymatic changes reducing antibiotic binding; (3) target bypass, a new protein performs the original function unaffected by the antibiotic; (4) decreased influx, porin downregulation limits antibiotic entry; (5) active efflux, transmembrane pumps export antibiotics; and (6) target protection, a protein shields the target from antibiotic inhibition (Fig. 1). Together, these strategies enable bacteria to survive antibiotic exposure through diverse molecular pathways [27]. Existing antibiotic resistance testing methods treat resistance as static rather than dynamic, creating a critical knowledge gap in understanding its true mechanistic basis. For instance, minimum inhibitory concentration (MIC) tests show only growth-inhibition endpoints without revealing underlying mechanisms, thereby missing phenomena like hetero-resistance and persister cells [28–32]. The techniques of genotyping that are based on PCR are able to find known genes involved in resistance. However, they fail to detect new mechanisms, unexpressed genes, and complex interactions between different genes [33-35]. Furthermore, whole-genome sequencing is incapable of revealing whether or not gene expression is active, as well as any related post-translational changes. As a result, a large discrepancy exists between resistance determination and understanding based on biological systems that function in a complex, dynamic manner [36].

The aim of multi-omics strategies is to provide solutions to the limitations of various methodologies. As an interdisciplinary approach, this method integrates the ample information obtained from many large-scale biological research works in genomics, transcriptomics, proteomics, and metabolomics to build a comprehensive systems-level understanding of complicated biological phenomena [37,38]. Through analyzing all of these layers of molecules together, this method makes the regulatory pathways connecting genotype to phenotype clear, thus serving as a link between the genetic design and functioning or disease.

Consequently, multi-omics research interprets antibiotic resistance as a multidimensional system-level phenotype arising from a network of genomic, regulatory, and metabolic processes. The research calls for an analysis of antibiotic resistance using not only transcriptomics, proteomics, and metabolomics but also integrated approaches rather than examining isolated components.

2. REVIEW METHODOLOGY

This review was developed through a comprehensive literature search conducted across PubMed, Google Scholar, and Web of Science. The following key terms were used: antibiotic resistance mechanism, multi-omics, multi-omics integration, proteomics and antibiotic resistance, metabolomics and antibiotic resistance, metagenomics and antibiotic resistance, multi-omics and antibiotic resistance, and multi-omics challenges in characterizing antibiotic resistance.

All of the articles used were peer-reviewed and published in English, between 2000 and 2026. All articles published before 2000, non-English articles, and abstracts with no full article were excluded. A total of 185 articles were found initially using the databases. After the reviews, 165 articles were included in the analysis part of the study. Seven articles were excluded as they were duplicates, eight articles due to the publication year (pre-2000), and five articles as they were in a language other than English. The focus of this article is on integrative multi-omics approaches concerning the problem of antibiotic resistance and highlights some of the challenges of the research in this field.

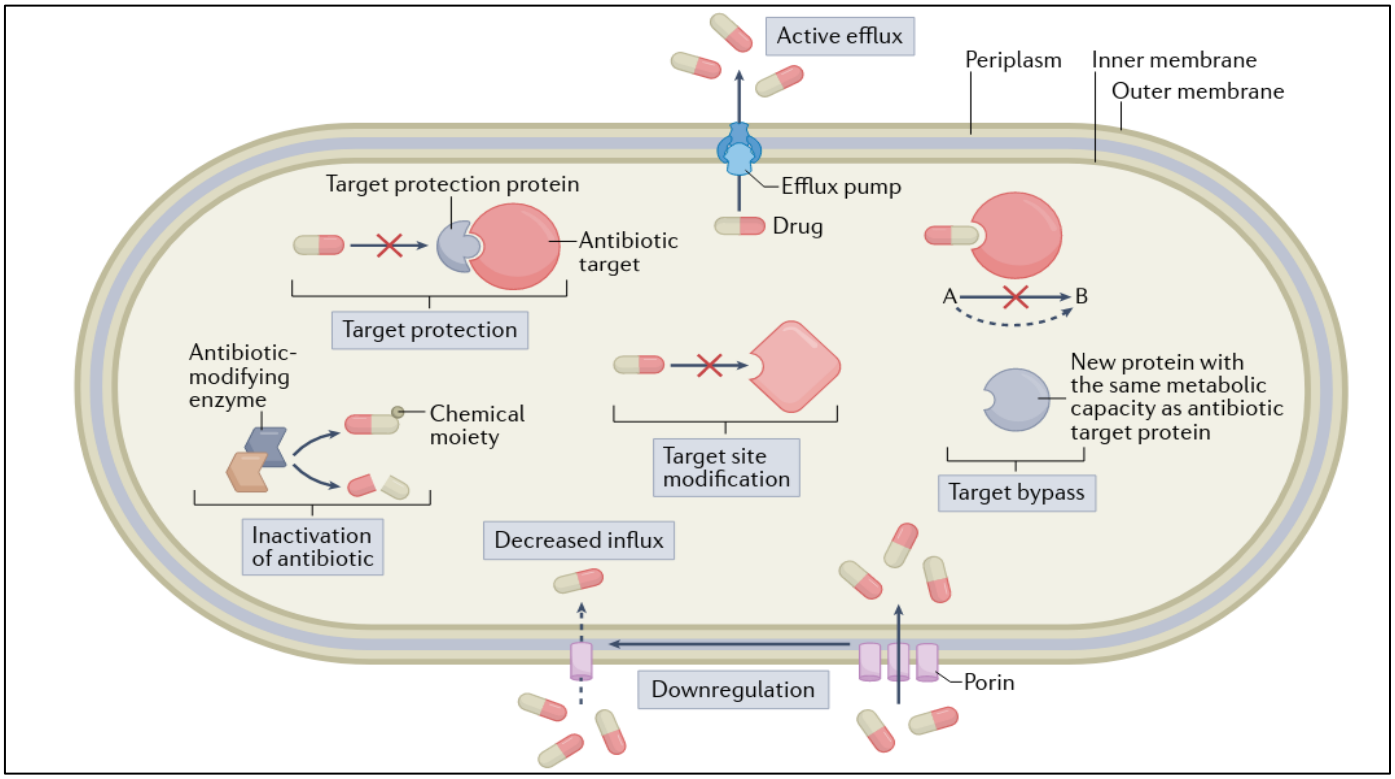


Fig. 1. Mechanisms of antibiotic resistance in a Gram-negative bacterium. Resistance occurs through six main strategies: drug inactivation by modifying enzymes, target site modification, and target protection by shielding proteins, target bypass via an alternative protein with equivalent function, active efflux through pumps, and decreased influx caused by porin downregulation [27].

3. OBJECTIVE OF THE REVIEW

The objective of this review is to assess critically the integrative multi-omics approach in providing insights into the mechanisms of antibiotic resistance in microbes, while accounting for existing challenges and suggesting paths for future research.

In this review, the authors provide a thorough systems approach to understand antibiotic resistance arising from bacterial networks of molecular processes. The authors emphasize the role of genomics, transcriptomics, proteomics, metabolomics, and metagenomics and highlight that the approach helps to overcome issues faced by traditional genomic approaches. Concisely, the authors demonstrate the challenges of using the multi-omics approach in clinical settings while showing how the previous studies can be improved.

4. ANTIBIOTIC RESISTANCE GENES AND THEIR DISSEMINATION

The global dissemination of antibiotic-resistant bacteria, particularly those resistant to beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, constitutes a serious public health problem. This issue is caused by three closely interconnected elements: the development of resistance genes encoding harmful beta-lactamases, mechanisms of the action of beta-lactamases able to inactivate antibiotics, and the mobile genetic elements responsible for the propagation of the resistance genes. Beta-lactamases decrease the efficacy of antibiotics using one of two methods. There are serine beta-lactamase enzymes (classes A, C, D) that use serine for hydrolysis, and metallo-beta-lactamases (class B) that are zinc-dependent. The latter is particularly alarming as it is capable of breaking carbapenems, along with the ability to evade most ß-lactamase inhibitors (e.g. clavulanate and avibactam). Mobile genetic elements (MGEs), which mediate horizontal gene transfer, mainly determine how quickly resistance genes are acquired. Rather than being inherited traditionally, resistance genes can move from one bacterial species to another. MGEs drive the rapid spread of beta-lactam resistance in bacteria, making it more likely than inheritance alone. Mobile genetic elements include plasmids, transposons, insertion sequences, and integrons. Plasmids, which transmit genes by contact, including those with *blaNDM, blaKPC*, and *blaCTX-M*, are the main drivers of the global dissemination of these genes. Transposons, such as Tn3, Tn4401, and Tn125, help move genes within the genome. Insertion sequences such as ISEcp1 increase gene activity and cause resistance. Integrons are also part of the MGEs, especially class 1 integrons, which capture and express genes. Together, they contribute to the dissemination of genes responsible for the synthesis of enzymes that destroy medicines. Notably, understanding the nature of MGEs is vital in the fight against antimicrobial resistance.

Bacterial transformation: A donor cell releases naked DNA into the surrounding environment upon lysis. A recipient cell takes up this DNA, which may carry an antibiotic-resistance gene, and integrates it into its chromosome or plasmid. **Bacterial transduction**: A bacteriophage infects a donor cell (Fig. 2), packaging bacterial DNA (including resistance genes) into new

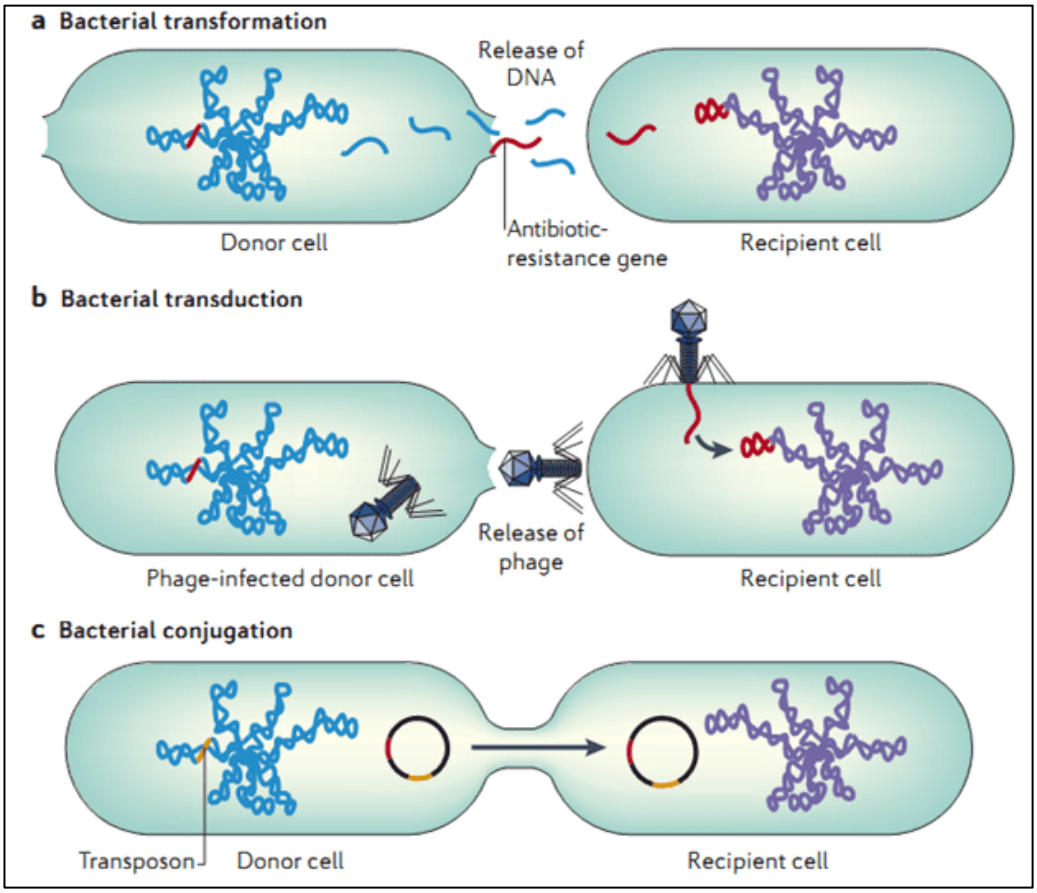


Fig. 2. Horizontal gene transfer mechanisms between bacteria. The three classical horizontal gene transfer mechanisms: transformation, transduction, and conjugation, contribute to the spread of antibiotic resistance among bacteria [39].

phage particles. Upon release, these phages infect a recipient cell, where the resistance gene may be integrated into the bacterial chromosome via lysogeny. **Bacterial conjugation**: Direct contact occurs between a donor and a recipient cell. The donor transfers DNA through a pilus. Plasmids carrying antibiotic-resistance genes are exchanged, and transposons, mobile genetic elements, can further mobilize resistance genes between the plasmid and the recipient's chromosome [39].

Integrative multi-omics to combat Antibiotic Resistance: From Static Gene Catalogs to Dynamic Molecular Networks

Antibiotic resistance is a multifactorial issue that requires a systems approach [39, 40]. Traditional approaches (such as culture-based susceptibility testing or single-genome sequencing) do not usually reflect the full dimension and complexity of the antibiotic resistance phenomenon, which is based not only on genetic mutations but also on epigenetics, metabolic adaptations, and interactions at the community level in microbiomes [41,42]. The ability of integrative multi-omics is to combine data from multiple omics platforms, such as genomics, transcriptomics, proteomics, metabolomics, and metagenomics, providing a holistic view of the antibiotic resistance issue [43–45].

Rather than focusing on "what genes" (genomics) or "what proteins" (proteomics) are present, there is an emphasis on integration, which provides a picture of causative networks showing how genetic potential is translated into antibiotic resistance in vivo [43,46]. Fig. 3 illustrates how single-cell multi-omic technologies capture several molecular layers simultaneously. The computational frameworks that support such integration (genomic-scale metabolic models, resistome-mobilome analysis and multi-omics factor analysis) are summarized in Table1.

Antibiotic resistance remains a worldwide health crisis that is both ongoing and worsening, driven by complex biological and social processes [48,49]. From an ecological perspective, antibiotic use drives the emergence of antibiotic-resistant bacteria and complicates the treatment of infections [50,51]. Resistance can be transmitted between individuals through poor hygiene or ineffective infection control measures, leading to outbreaks and wider spread. Combating antibiotic resistance requires replacing outdated gene-mapping practices with sophisticated multi-omics techniques [52], which integrate information from genomics, transcriptomics, proteomics, metabolomics, and other omics to provide a holistic picture of living organisms [53]. Table 2 provides an overview of the five omics layers discussed below (genomics, proteomics, transcriptomics, metabolomics and metagenomics), with their definitions, applications and advantages.

5. GENOMICS

Genomic analysis determines antibiotic resistance by identifying specific genes that confer resistance, as well as mutations affecting the bacteria's DNA. Unlike phenotypic testing, which relies on bacterial growth in the presence of antibiotics, genomics reveals an organism's genetic potential for resistance [54,55]. The main elements of this technique involve sequencing

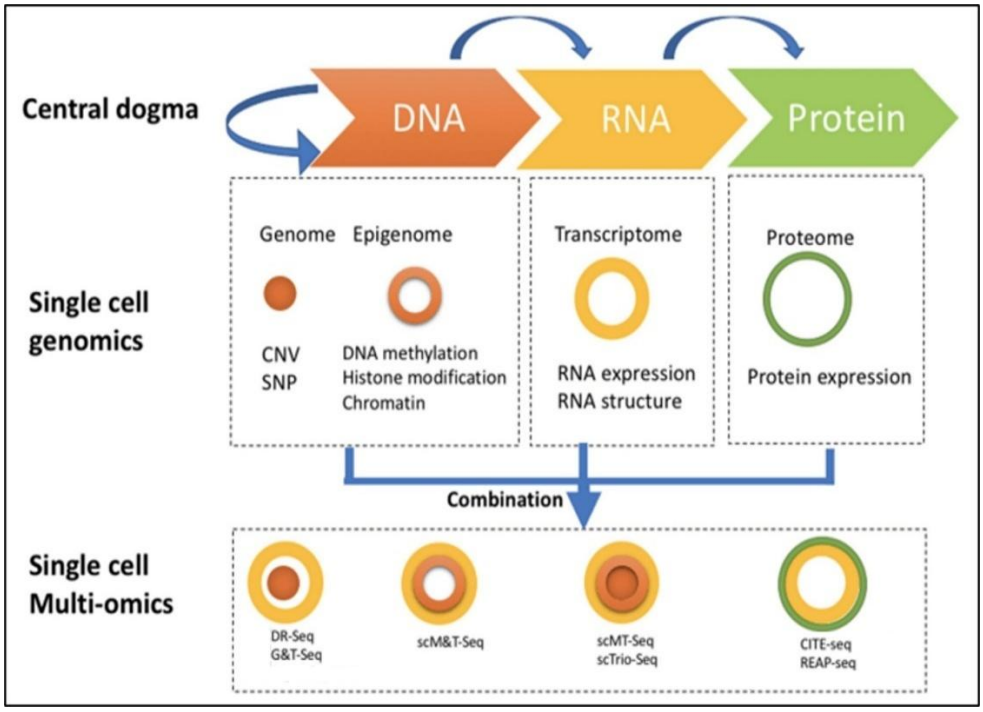


Fig 3. The illustration clarifies that contemporary technologies related to individual cells go beyond solely measuring RNA or DNA. Rather, these technologies capture several layers of information, such as DNA mutations and RNA environments, or RNA and proteins, simultaneously, hence escaping the shortcomings of static, single-layer diagnostics pointed out in previous studies on antibiotic resistance [47].

the genome of the bacteria in question and examining the genomic information for comparisons with available databases of antibiotic-resistance genes. When a match is found, the bacteria in question can be said to possess the ability to resist the antibiotic in question [56,57]. This approach applies to both acquired resistance genes that many bacteria possess on mobile elements or on their chromosomes, and to the presence or absence of important genes for susceptibility. The major disadvantage of this methodology is that it cannot predict the existence of resistance mechanisms that have yet to be found [58–60]. Although the study of bacterial genomics has revolutionized the detection of antibiotic resistance genes and the distribution of AMR via WGS, understanding the mechanisms underlying resistance requires integrative multi-omics approaches [61].

Scientists can utilize genomics and proteomics to study the process of AMR emergence as they employ a systematic approach [62]. By determining the presence of certain genes, genomics serves the purpose of revealing the blueprint for resistance, whereas proteomics demonstrates active mechanisms of resistance by showing which proteins are indeed produced and utilized [63,64]. Thus, these techniques create more complete information that goes beyond the knowledge of the presence of antitreatment genes to the verification of their expression [65,66]. For instance, genomics can reveal the presence of carbapenem-resistant genes, while proteomics confirms whether the genes are used, thus demonstrating the causes of treatment outcomes and distinguishing between the cases of genetic resistance and actual resistance [67].

6. PROTEOMICS

Proteomics improves the comprehension of antibiotic resistance by revealing the real protein levels, the modifications after translation, and the interactions that lead to resistance [68]. While the use of genomics can show the possible presence of resistance, proteomics explains the mechanisms operating in the bacteria. It uses mass spectrometry to study antibiotics-resistant proteins and to identify and quantify the enzymes responsible for resistance [68,69]. Thus, it differentiates types of β-lactamase variants (such as TEM, SHV, and CTX-M) according to protein markers from mass spectrometry, and identifies and quantifies the expressions of carbapenemases (KPC, NDM, OXA, and VIM) [70,71].

Proteomics assesses enzyme activity and substrate specificity through structural analysis. Clinically, it enables rapid identification of β-lactamases to guide precise inhibitor therapy [72,73]. It characterizes efflux pumps by identifying their components, measuring expression, and detecting regulatory proteins (e.g., AcrR, MarR, SoxR) [74]. All three pump parts such as inner transporter, periplasmic adaptor, and outer channel are identified [74]. Overexpression in resistant strains is quantified. For example, in *Pseudomonas aeruginosa*, proteomics profiles MexAB-OprM, MexCD-OprJ, and MexEF-OprN systems to inform inhibitor choice [75,76].

Proteomics also directly detects antibiotic-modifying enzymes like aminoglycoside acetyl/phospho/nucleotidyl transferases, chloramphenicol acetyltransferases, and macrolide esterases [77,78]. It quantifies outer membrane permeability changes, including porin loss (OmpC, OmpF, OmpK), structural modifications, and compensatory porin expression [79,80]. Porin profiling predicts β-lactam penetration and guides dosing [81]. Additionally, proteomics uncovers cell wall biosynthesis alterations, such as modified penicillin-binding proteins, peptidoglycan-modifying enzymes, and Lipopolysaccharide (LPS) changes that affect antibiotic binding [82–84]. Persister cell proteomics identifies dormancy proteins (e.g., metabolic shutdown factors). In *Acinetobacter baumannii*, it quantifies species-specific OXA carbapenemases (OXA-23, OXA-40) [12,85].

7. TRANSCRIPTOMICS

Transcriptomic analysis comprises the sequencing of RNA transcripts to recognize gene expression and it is also used to identify key regulatory networks involved in antibiotic resistance [86,87]. Transcriptomics offers comprehensive insights into the gene expression patterns governing resistance mechanisms in bacterial pathogens [88]. This approach has revolutionized our understanding of antibiotic resistance, enabling researchers to identify, characterize, and decipher its molecular basis at the RNA level [89,90]. Key bacterial genes and regulatory networks involved in bacterial antibiotics are identified by distinguishing the expressed resistance and susceptible genes, and thus transcriptomics is used to compare these genes [91].

As it allows to compare gene expression between resistant and sensitive strains and to monitor the transcriptional reaction to antibiotics, transcriptomics indicates which resistance genes are expressed at higher or lower level [92,93]. This has provided valuable information, including the discovery of the expressed efflux pumps and increased production of the β-lactamases [94,95]. In addition, transcriptomics has been used to study the regulatory networks of antibiotic resistance [96]. For instance, there are studies that have applied this methodology to analyze the networks responsible for ciprofloxacin resistance in *Escherichia coli* [97].

The rise in activity of pump genes, an essential resistance mechanism, grabs the attention during transcriptomics [98, 99]. For example, in *Pseudomonas aeruginosa*, mexAB-oprM has been shown to be overexpressed by a factor of 5 in resistant strains [100]. In addition, transcriptomics indicates expression of genes involved in the synthesis of β-lactamases as a reaction to β-lactam antibiotics [98].

8. METABOLOMICS

The field of metabolomics is highly interesting and rapidly developing, as it employs sophisticated analytical chemistry and microbiological principles together [101,102]. It uses technology to provide comprehensive information on small-molecule metabolites inside bacterial cells and can reveal how bacteria alter their metabolic processes to survive the effects of antibiotics [103,104]. By doing so, the study of bacterial resistance to antibiotics has been transformed, as it goes beyond the scope of conventional genetic and proteomic studies [27,40]. Antibiotic-resistant bacteria undergo a fundamental change in their metabolism to resist treatment [105,106]. This involves the modification of central energy metabolism through changes in glycolysis, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle [9,107]. They also change their amino acids biosyn-thesis and nucleotide metabolism, allowing them to continue functioning during stressful conditions [108,109].

In addition, metabolomics has made significant advances by establishing a new method to understand resistance in biological systems [110]. The technique evaluates changes associated with efflux pump activity, determines the metabolic demands required to operate them, and observes metabolic changes that indicate their high levels of activity [111]. Moreover, metabolism has also been used to monitor changes in cell membrane synthesis by accumulating data on the amount of peptidoglycan precursor involved in cell wall formation, alongside measuring fat metabolism, which results in alterations in the fatty acid pattern observed in the membrane [112,113]. Another interesting aspect of the use of metabolism in drug resistance is the discovery of metabolites formed from drug degradation [114]. Through this detailed metabolic profiling, metabolomics provides a powerful, functional readout of bacterial antibiotic resistance. Table 3 consolidates the genomic, transcriptomic, proteomic, and metabolic signatures associated with each major resistance mechanism.

9. METAGENOMICS

Metagenomics is the study of a metagenome, the complete set of microbial genomes present in a given sample. Unlike traditional genomics, which sequences DNA from a single, isolated bacterial colony, metagenomics sequences all the DNA from a mixed sample [115]. This approach offers unique advantages in the fight against antibiotic resistance. As a culture-independent method, metagenomics can capture the large majority of bacteria (often estimated at about 99%) that are not readily cultured in the laboratory [116,117]. It enables the identification of the complete resistome, the full collection of ARGs within a microbial community, beyond just those found in a single pathogen of interest [118,119]. Moreover, metagenomics can track the mobilome, which includes mobile genetic elements such as plasmids, transposons, integrons, and phages that transfer ARGs between different bacterial species (e.g., from harmless Escherichia coli to pathogenic Klebsiella). Finally, using contextual genomics enabled by long-read sequencing technologies (e.g., Nanopore or PacBio), metagenomics can physically link a specific ARG to both its bacterial host species (via taxonomic binning) and the mobile genetic element that carries it.

Table 1. Development of sophisticated integration frameworks for conducting multi-omic research in the field of antibiotic resistance. Here we summarize three computer modelling and analytics frameworks, namely GEMs, Resistome-Metabolome Analysis, and MOFA, along with their respective input data, output, features, and uses. These frameworks provide a systems-level vision for the analysis of antimicrobial resistance via multi-layer integration of omics to allow for the prediction of metabolic weaknesses, evaluation of the potential for horizontal gene transfer events, and investigation of host-pathogen interactions.

Framework	Inputs	Output Mechanism	Application
Genome-Scale Metabolic Models (GEMs)	Genomics + Metabolomics	Reconstructs the metabolic network in silico. Predicts essential genes and metabolic vulnerabilities specific to resistant strains	Identifying "Achilles' heels" in MDR strains (e.g., specific carbon sources required for resistance).
Resistome-Mobilome Analysis	Metagenomics + Long-read sequencing	Maps resistance genes (resistome) onto mobile genetic elements (mobilome—plasmids, phages). Determines the transfer potential and structural context of ARGs.	Surveillance of outbreak clones; predicting horizontal gene transfer risks.
Multi-Omics Factor Analysis (MOFA)	All layers (host + pathogen)	Integrates heterogeneous data to identify latent factors (e.g., a specific host immune signature correlating with a specific bacterial efflux pump expression).	Understanding host-pathogen interactions during antibiotic treatment failure.

Table 2. A summary of the various omics technologies employed in studies of antibiotic resistance. The following table provides an overview of five important omics fields (genomics, transcriptomics, proteomics, metabolomics, and metagenomics), explaining what each entail, how each of them is used in research into antibiotic resistance, and what advantages each approach has. All five techniques provide a different approach to understanding antibiotic resistance mechanisms, with one providing genetic mapping and another metabolic phenotyping.

Omics Technology	Definition	Application in Antibiotic Resistance	Key Advantages
Genomics	Study of complete DNA sequences.	Identification of resistance genes, mutations, and mobile genetic elements.	High-resolution genetic mapping, phylogenetic analysis.
Transcriptomics	Analysis of RNA expression patterns.	Gene expression changes during antibiotic exposure.	Real-time response monitoring, pathway analysis.
Proteomics	Study of protein expression and function.	Protein-level resistance mechanisms, enzyme activity.	Direct functional assessment, post-translational modifications.
Metabolomics	Analysis of small molecule metabolites.	Metabolic pathway alterations, biomarker discovery.	Phenotypic endpoints, drug metabolism.

Table 3. Brings together multi-omics exploration of the mechanisms of antibiotic resistance, including genomic, transcriptomic, proteomic, and metabolic markers related to the major mechanisms of resistance, which are categorized as follows: enzymatic inactivation, target modification, efflux pumps, changes in permeability, bypass mechanisms, and targe protection. Thus, each omics level provides additional insight into the molecular basis of resistance.

Resistance Mechanism	Genomic Markers	Transcriptomic Signatures	Proteomic Changes	Metabolomics Alterations
Enzymatic Inactivation	β-lactamase genes(bla), aminoglycoside-modifying enzymes	Upregulation of resistance genes	Increased enzyme production	Antibiotic degradation product
Target Modification	Point mutations in target genes (rpoB, gyrA)	Altered expression of target proteins	Modified protein structure/function	Changed metabolic flux
Efflux Pumps	Efflux pump genes (mexAB, acrAB)	Overexpression of pump components	Increased pump protein levels	Reduced intracellular drug concentration
Permeability Changes	Porin gene mutations (ompF, ompC)	Downregulation of porin genes	Reduced porin expression	Altered membrane composition
Bypass Pathways	Alternative pathway genes	Activation of compensatory pathways	Alternative enzyme production	Metabolic rerouting
Target Protection	Protection protein genes (e.g. tet(M).gnr)	Expression of target-protection genes	Presence of protein that shields the antibiotic target	Largely indirect target-linked metabolism maintained

10. METAGENOMICS AND MULTI-OMICS AGAINST ANTIBIOTIC RESISTANCE

Since metagenomics (gene presence) doesn't equal function (transcriptomics/proteomics/metabolomics), integrating these layers is critical to reveal real threats [120,121]. Metagenomics plus meta-transcriptomics identifies actively expressed resistance (e.g., only only a small subset of the detected ARGs may be actively expressed, guiding immediate antibiotic choice) [122]. Adding long-read sequencing and metabolomics assesses horizontal transfer risk by detecting ARGs on plasmids plus conjugation signals, which could help identify high-risk intensive care unit (ICU) patients for isolation. Metagenomics with proteomics validates actual enzyme expression (e.g., confirming Verona Integron-Encoded Metallo-β-lactamase 2 (VIM-2) is active, avoiding meropenem) [123]. Finally, integrating metagenomics with genome-scale metabolic models exposes ecological vulnerabilities, like a metabolic bottleneck, where blocking a shared compound can help reduce community resistance [124].

11. INSTRUMENTAL TECHNIQUES FOR DETECTING ANTIBIOTIC RESISTANCE GENES

These methods identify the genetic potential for resistance by directly detecting the DNA sequences of ARGs or their RNA expression. Sequencing-Based Platforms (NGS & Long-Read) are the most direct tools for ARG detection. Metagenomics profiles the full resistome, targeted amplicon sequencing (e.g., MultiSeq-AMR) enables rapid clinical diagnosis, and long-read sequencing (e.g., Oxford Nanopore) links ARGs to their host species or mobile genetic elements [125,126].

Microfluidic & Single-Cell Technologies enable high-resolution, single-cell analysis. DoTA-seq links ARGs to specific species via droplet microfluidics. Digital-GPA pairs digital microfluidics with metabolic labeling to directly connect ARG genotype to resistance phenotype in individual bacteria [127]. Unlike genomics, which reveals resistance potential, these methods detect actual resistance expression, including drug-deactivating enzymes and metabolic changes. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) & Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) is a cornerstone for analyzing ARG downstream products [128]. MALDI-TOF detects resistance mechanisms (e.g., antibiotic breakdown) via mass spectral fingerprints [129]. LC-MS/MS identifies resistance biomarkers [130].

12. INTEGRATED MULTI-OMICS PLATFORMS

By merging the advantages of the previously mentioned techniques, advanced approaches leverage powerful bioinformatics pipelines. Tools like ABRomics-analysis and BALROG-MON are not instruments but essential software

workflows that process raw sequencing data [126]. They integrate genomic, metagenomics, and functional annotations to deliver a complete picture of the resistome, from identifying specific ARGs and assessing their mobility potential to pinpointing their microbial hosts [126, 131,132].

13. MULTI-OMICS CURRENT CHALLENGES

Multi-omics integration is a revolutionary promise in diagnosing and understanding antibiotic resistance [133,134]. However, transitioning from a research tool to a routine clinical diagnostic faces significant technical, biological, clinical, and ethical challenges [135].

The true power of multi-omics lies in integrative analysis although the integration poses a central challenge as each omics layer generates massive, heterogeneous datasets that require robust, standardized bioinformatics pipelines to synthesize [53, 136, 137]. Beyond technical integration, a significant biological and interpretive hurdle remains significant challenges to correlate complex omics signatures with definitive phenotypic outcomes [138]. Multi-omics can predict resistance mechanisms; however, often falls short of confirming actual phenotypic resistance. For instance, a bacterium may harbor a resistance gene (a genomic signal) that remains unexpressed, with no corresponding transcriptomic or proteomic activity [139]. As the clinical gold standard is still the phenotypic growth assay, this interpretive gap is critical. Furthermore, the sophisticated bioinformatics expertise and computing infrastructure needed for such analyses currently confine the technology to well-funded research hospitals, limiting its adoption in routine clinical laboratories [140,141].

Table 4. Instrumental technologies and their corresponding omics layers for ARG detection. This table summarizes key analytical platforms, ranging from genomic to metabolomic approaches, and specifies their primary applications in identifying ARG sequences, linking resistance to host organisms, and detecting resistance-associated phenotypes or biomarkers.

Technique	Omics Layer	Primary Application for ARG Detection
Metagenomic & Amplicon Sequencing	Genomics	Directly identify ARG DNA sequences in a sample [125].
Long-Read Sequencing (e.g., ONT)	Genomics	Link ARGs to specific host bacteria or plasmids [126].
Single-Cell Sequencing (DoTA-seq)	Genomics	Link ARGs to species and track their prevalence at single-cell resolution [127].
MALDI-TOF Mass Spectrometry	Proteomics	Detect enzymatic breakdown of antibiotics or bacterial protein fingerprints [128,129].
LC-MS/MS	Metabolomics	Identify unique metabolite biomarkers indicative of a resistant phenotype [132].
Digital Microfluidics (Digital-GPA)	Genomics/Phenomics	Genotype and phenotype single resistant bacterial cells simultaneously

Multi-omics faces substantial clinical and practical translation barriers, foremost among them a lack of standardized protocols for sample processing, data generation, and analysis, which undermines inter-laboratory reproducibility, which is a prerequisite for any global diagnostic [142,143]. Translating a multi-omics signature comprising hundreds of data points into a simple, clinically interpretable report is a critical unsolved problem in bioinformatics [144,145]. From a regulatory perspective, pathways exist for conventional diagnostics, but the approval process for a complex, algorithm-driven multi-omics test represents uncharted territory, posing a significant foreseeable hurdle [146,147]. There is a lack of large-scale, prospective clinical trials proving that multi-omics diagnostics lead to better patient outcomes (reduced mortality, shorter hospital stays, less antibiotic-related toxicity) compared to standard of care, and that they are cost-effective [148,149]. Perhaps the most pervasive challenge is the background interference from the host's own response; proteomics and metabolomics profiles are dominated by host signals, making the isolation of the specific bacterial resistance phenotype a formidable but essential bioinformatics task for accurate prognosis [146].

The generation of multi-omics data creates an immense informatics and privacy challenge, as the enormous file sizes associated with patient omics profiles present significant hurdles for storage, management, and secure handling [150,151]. Accelerating progress in the field depends on the establishment of shared, high-quality, curated databases that robustly link omics signatures to phenotypic resistance and clinical outcomes [152,153]. However, constructing these essential resources is a global undertaking hindered by complex issues of data ownership, stringent privacy regulations, and a persistent lack of standardization.

14. MULTI-OMICS FUTURE DIRECTIONS

Integrative multi-omics represents a cutting-edge, systems-level approach to understanding AMR [44, 154]. By analyzing the combined interactions of a pathogen's genome, transcriptome, proteome, metabolome, and resistome within its environmental context, this method moves beyond single-molecule studies to reveal how resistance arises from complex biological networks [155,156].

A key application is deciphering difficult-to-detect phenomena such as hetero-resistance and bacterial persistence [44]. Integrative omics can uncover the distinct metabolic and regulatory signatures of resistant subpopulations that conventional diagnostics often miss [157]. These insights provide biomarkers for improved detection and reveal novel therapeutic targets [157,158]. Ultimately, this approach paves the way for treatments designed to disrupt the metabolic adaptations that allow persistent bacterial populations to survive [158].

Integrative multi-omics is key to unraveling the complex mechanisms behind MDR [159]. MDR typically arises from a network of concurrent adaptations, such as efflux pump upregulation (transcriptome/proteome), cell wall alterations (proteome/metabolome), and target enzyme mutations (genome) [160]. By integrating these omics layers, researchers can map the complete resistance network of a specific strain under treatment stress [161]. This systems-level view enables the rational prediction of effective drug combinations that simultaneously target multiple vulnerabilities within the network [162].

The future of multi-omics for AMR lies in moving from descriptive to predictive and actionable integration [163]. The grand

challenge is to build a unified, dynamic model of a bacterial pathogen that can predict how it will evolve resistance under various selective pressures and within specific host environments [164]. Overcoming the current bottlenecks through technological advances in spatial/single-cell analysis, sophisticated AI integration, and a strong focus on in vivo relevance will transform our understanding of resistance from a genocentric view to a holistic systems-level understanding. This shift is essential for developing the next generation of antimicrobial strategies and diagnostics to outmaneuver bacterial evolution [165].

15. CONCLUSION

Antibiotic resistance, once regarded merely as a genetic feature, has now become a dynamic, systemic phenotype that evolves from intertwining molecular networks. Traditional diagnostic techniques like MIC tests, PCR, and even whole-genome sequencing regard resistance as static and are unable to detect phenomena such as hetero-resistance, persistence, as well as the mismatch between genotype and phenotype. In this review, we critically analyze how the use of integrated multi-omics (genomics, transcriptomics, proteomics, metabolomics, and metagenomics) allows to overcome these issues with the technology responsible for changing the paradigm from static gene catalogues to molecular networks.

The review illustrates that the process of survival by bacteria under the influence of antibiotics occurs as a result of not only the presence of genes resistant to the drug but their active expression of the function of proteins, metabolic changes, and transmission through MGEs. Thus, while genomics makes it possible to establish the potential existence of resistance, transcriptomics demonstrates which of the genes are working, proteomics assesses whether the protein is functional, and metabolomics helps gain insights into metabolic changes. Moreover, metagenomics extends this framework to community-level resistome and mobilome analysis enabling tracking of ARG transfer across species.

One important scientific contribution of this study is the introduction of innovative integrative methods such as the use of Genome-Scale Metabolic Models (GEMs), Resistome-Mobilome analysis and Multi-Omics Factor Analysis (MOFA). These methods would enable scientists to construct resistance in the form of a multilayered network and identify metabolic weaknesses of the MDR strains.

However, the review critically identifies major translational challenges: lack of standardized protocols, difficulty in distinguishing expressed vs. silent resistance genes, host signal interference (especially in proteomics and metabolomics), and the absence of large-scale clinical trials proving improved patient outcomes. Regulatory pathways for algorithm-driven multi-omics diagnostics remain undefined.

List of Abbreviations

ARGs: antibiotic resistance genes
AMR: Antimicrobial resistance
DNA: deoxyribonucleic acid
ESBLs: extended-spectrum β-lactamases
GEMs: Genome-Scale Metabolic Models
HGT: horizontal gene transfer
ICU: intensive care unit
LPS: Lipopolysaccharide
MBLs: metallo-β-lactamases
MDR: multidrug resistance
MGEs: mobile genetic elements
MIC: minimum inhibitory concentration
MOFA: Multi-Omics Factor Analysis
PCR: polymerase chain reaction
TCA: tricarboxylic acid
WGS: whole-genome sequencing

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Conflict of interest

The authors declare no conflicts of interest.

Ethical Approval

Not applicable. The review article does not involve any studies dealing with humans or animals performed by the author.

Author contributions

Woyesa SB. Methodology; Project administration; Data curation; and Writing – original draft; Writing – review and editing.

Abbai N. Formal analysis; Validation; Writing – original draft; and Writing – review and editing.

Kassa T. Visualization; and Writing – review and editing. Supervision; Project administration; Writing – original draft; and Writing – review and editing.

Abebe G. Conceptualization; Formal analysis; Investigation; Writing – original draft; and Writing – review and editing.

All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work

AI declaration

The authors used Claude AI & Gemini Pro to improve the manuscript's academic tone and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Data availability

Data will be made available on request.

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