



RECENT DEVELOPMENTS IN ANTIBIOTIC RESISTANCE: AN INCREASING THREAT TO PUBLIC HEALTH – A REVIEW

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Abstract

Antibiotic resistance (ABR) is a major global health threat that puts decades of medical progress at risk. Bacteria develop resistance through various means, including modifying their targets, deactivating drugs, and utilizing efflux pump systems. The main driving forces behind ABR are excessive antibiotic use in healthcare and agriculture, environmental contamination, and gaps in the drug development process. The use of advanced detection technologies, such as next-generation sequencing (NGS), clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostics, and metagenomics, has greatly improved the identification of resistant pathogens. The consequences of ABR on public health are significant (increased mortality rates, the endangerment of modern medical procedures) and resulting in higher healthcare expenses. It has been expected that ABR could potentially drive up to 24 million individuals into extreme poverty by 2030. Mitigation strategies focus on antibiotic stewardship, regulatory measures, research incentives, and raising public awareness. Furthermore, future research directions involve exploring the potential of CRISPR-Cas9 (CRISPR-associated protein 9), nanotechnology, and big data analytics as new antibiotic solutions. This review explores antibiotic resistance, including mechanisms, recent trends, drivers, and technological advancements in detection. It also evaluates the implications for public health and presents strategies for mitigating resistance. The review emphasizes the significance of future directions and research needs, stressing the necessity for sustained and collaborative efforts to tackle this issue.

Key words: antibiotic resistance, resistant pathogens, antibiotic stewardship, multi-drug resistance, artificial intelligence

Antibiotic resistance (ABR) is a significant global health challenge in the 21st century, intricately connected to the complex dynamics among humans, animals, and the environment (Ahmed et al., 2024; Ferdinand et al., 2024). ABR specifically refers to the ability of bacteria, primarily, to withstand a broad spectrum of antibiotics (Salam et al., 2023; Naghavi et al., 2024; Ruckert et al., 2024). This means bacteria can either evade the action of antibiotics that would normally inhibit their growth or kill them (Lobanovska and Pilla, 2017).

Antibiotics have played a crucial role in modern medicine ever since the discovery of penicillin by Alexander Fleming in 1928 (Fleming, 1944). Their efficacy in treating bacterial infections has saved countless lives.

Unfortunately, the widespread and often indiscriminate use of antibiotics has resulted in an alarming surge in antibiotic-resistant bacteria. Consequently, decades of medical progress are now at risk. The World Health Organization (WHO) has recognized ABR as a “global health emergency” and emphasized the urgent need for comprehensive action across sectors and nations (Ferdinand et al., 2024).

The scale of the problem is staggering. In 2019 alone, ABR infections were responsible for causing over five million deaths worldwide (Murray et al., 2022). If present patterns persist, this number could rise significantly. Forecasts indicate that by 2050, ABR could result in a global economic cost of up to \$100 trillion (Chen et

al., 2023; Ahmed et al., 2024). As bacteria develop resistance to antibiotics, the treatment of common infections becomes ever more difficult. Consequently, patients experience longer illness durations, healthcare costs soar, and mortality rates escalate.

Particular concern is directed towards ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) due to their virulence and capacity to resist multiple drugs. The development of new antibiotics to combat these pathogens is indispensable in the fight against ABR (Jadimurthy et al., 2022). The initial instances of ABR were documented in the early 1940s, shortly after penicillin gained widespread use in clinical settings.

In 1944, several scientists independently reported the presence of an enzyme produced by certain bacteria that could render penicillin inactive (Lobanovska and Pilla, 2017). The emergence of ABR in developing nations is closely linked to several factors, including the prevalence of nosocomial infections, high rates of infectious diseases, and limited access to medications. Nosocomial infections in healthcare settings often involve bacteria that have been exposed to multiple antibiotics, increasing the likelihood of resistance. In developing countries, inadequate infection control measures and overcrowded healthcare facilities exacerbate the transmission of resistant strains.

Furthermore, the high burden of infectious diseases leads to the widespread use and misuse of antibiotics, including inappropriate prescriptions and incomplete treatment courses, which places selective pressure on bacterial populations and promotes the survival of resistant organisms (Holmbom et al., 2020; Hussein et al., 2024). Limited access to effective medications, particularly newer antibiotics, also plays a significant role. Patients in resource-poor settings may rely on older antibiotics that are less effective due to existing resistance patterns (Otaigbe and Elikwu, 2023). In addition, the use of counterfeit or substandard antibiotics, which may contain inadequate active ingredients, further contributes to the development of resistance (Cavany et al., 2023). These combined factors create a conducive environment for the emergence and rapid spread of ABR in developing nations.

Since then, the emergence of ABR has accelerated due to factors such as inappropriate medication use, misuse by both humans and animals, inadequate public health systems, nosocomial infections, high rates of infectious diseases, limited access to medications in developing nations, and profit-oriented motivations (Holmbom et al., 2020; Hussein et al., 2024).

There is consistent evidence from epidemiological research linking antibiotic usage to the emergence and spread of drug-resistant microorganisms (Castro-Sánchez et al., 2016; Holmbom et al., 2020). Despite the development of novel antibiotic agents, resistance continues to surge due to genetic alterations. Multicenter

studies have substantiated a significant upsurge in both community-acquired and nosocomial ABR (Moubareck and Halat, 2020; Murray et al., 2022).

To counteract the increasing threat posed by ABR, advancements in diagnostic and surveillance technologies are of paramount importance (Nashwan et al., 2024 a). Methods such as microscopy, polymerase chain reaction (PCR), DNA microarray, and whole-genome sequencing are utilized to swiftly identify ABR and monitor its spread (Singh et al., 2022). These tools allow for quicker and more accurate detection of resistant pathogens, thereby facilitating more targeted and effective treatment strategies. The ramifications of ABR extend beyond its direct influence on the treatment of infectious diseases. The dwindling efficacy of antibiotics jeopardizes numerous aspects of contemporary medicine, including organ transplants, joint replacements, and cancer treatments, all of which rely heavily on antibiotics to prevent or combat infections (Akram et al., 2023).

Mechanisms of ABR

The susceptibility of bacteria to antimicrobial agents varies significantly within populations and is measured by parameters such as the minimum inhibitory concentration (Wen et al., 2016; Hussein et al., 2023). Bacteria can have intrinsic resistance, encoded in their genomes, or acquire resistance genes through horizontal transfer (Schrader et al., 2020). Intrinsic resistance operates independently of prior exposure and includes mechanisms such as restricted outer membrane permeability and efflux pumps (Cox and Wright, 2013). On the other hand, induced resistance develops in response to environmental stimuli, particularly antibiotic exposure, enhancing bacterial resilience (Teng et al., 2023).

Bacteria employ several genetic mechanisms to acquire and disseminate antibiotic resistance traits. These include transformation, transposition, conjugation, and transduction (Arnold et al., 2022). Transformation involves the uptake of free DNA from the environment, which can include antibiotic resistance genes from lysed bacterial cells. Once incorporated into the bacterial genome or plasmids, these genes can confer resistance, allowing bacteria to survive antibiotic pressures (Da Silva and Domingues, 2016). Transposition plays a key role by allowing the movement of genetic elements known as transposons within and between DNA molecules. Transposons can carry resistance genes and, by inserting themselves into different locations in the bacterial genome or plasmids, they enable the rapid spread of resistance traits (Budiman et al., 2023). This mechanism enhances the bacteria's ability to accumulate resistance traits over time. In conjugation, resistance genes are transferred through direct cell-to-cell contact via plasmids, facilitating horizontal gene transfer between bacteria (Liu et al., 2023). Transduction, which involves bacteriophages (viruses that infect bacteria), also plays a crucial role in spreading resistance genes between bacteria, adding another layer of complexity to resistance management (San Millan,

2018). Together, these mechanisms underscore the adaptive capabilities of bacteria and the multifaceted nature of antibiotic resistance dissemination. Stress-induced mutations and genetic elements like insertion sequences and integrons also contribute to resistance spread (Stalder et al., 2012; Baquero et al., 2021). Resistance mechanisms mainly involve the modification of drug targets, limiting drug uptake, inactivating drugs, and active drug efflux (Figure 1).

Microorganisms demonstrate a remarkable ability to adapt by modifying the binding sites of antibiotics, resulting in a significant decrease in drug efficacy. This adaptation is achieved through genetic mutations that alter target proteins or enzymes. For instance, the acquisition of the *mecA* gene by *S. aureus* leads to structural changes in penicillin-binding proteins, rendering β -lactam antibiotics ineffective (Fisher and Mobashery, 2020). Similarly, the presence of *van* genes in enterococci and *S. aureus* causes structural modifications that hinder glycopeptide binding, including drugs like vancomycin (Lebreton and Cattoir, 2019; Li et al., 2022). These modifications essentially prevent antibiotics from interacting with their intended targets, enabling microorganisms to survive and proliferate in the presence of drugs.

Bacteria have evolved sophisticated defense mechanisms to evade antibiotics, particularly in Gram-negative species, demonstrating their remarkable adaptability. The outer membrane of these bacteria, which contains a high concentration of lipopolysaccharides, serves as a formidable barrier against large antimicrobial molecules (Horne et al., 2020). This protective shield obstructs the absorption of external compounds, providing inherent resistance to various antibiotics (Huszczynski et al., 2019). Even in Gram-positive bacteria like *S. aureus*, which

lack an outer membrane, intrinsic resistance mechanisms exist due to difficulties in the penetration of polar molecules and changes in cell wall composition (Saleemi et al., 2023). These adaptations significantly enhance bacterial survival against antibiotics.

Bacterial efflux pump systems, which actively remove drugs from the cell to maintain low intracellular concentrations, play a crucial role in ABR. These protein structures are divided into five main families: ABC, MATE, SMR, MFS, and RND, each exhibiting diverse structural and functional characteristics (Sharma et al., 2023). While the MATE and MFS families are primarily utilized by Gram-positive bacteria, Gram-negative bacteria possess a more complex array of efflux systems, with the clinically significant RND family being particularly abundant (Athar et al., 2023). These pumps adapt to environmental signals and substrate availability, underscoring their essential role in bacterial survival and the development of pathogenicity.

Bacteria employ a variety of enzymes to render antibiotics ineffective by breaking them down or modifying their chemical structure. An exemplar is the diverse group of β -lactamases, which degrade β -lactam drugs. Similarly, enzymes like *tetX* can render tetracycline inactive (Markley and Wenciewicz, 2018; Tooke et al., 2019). Chemical group transfer mechanisms also play a significant role, involving the addition of acetyl, phosphate, or adenylyl groups to antimicrobial molecules, thereby diminishing their efficacy (Abushaheen et al., 2020). It is noteworthy that acetylation primarily serves as the mechanism for inactivating aminoglycosides, chloramphenicol, and fluoroquinolones, often followed by phosphorylation and adenylation (Gaubha and Rahman, 2023).

Bacterial communities utilize biofilms as a complex

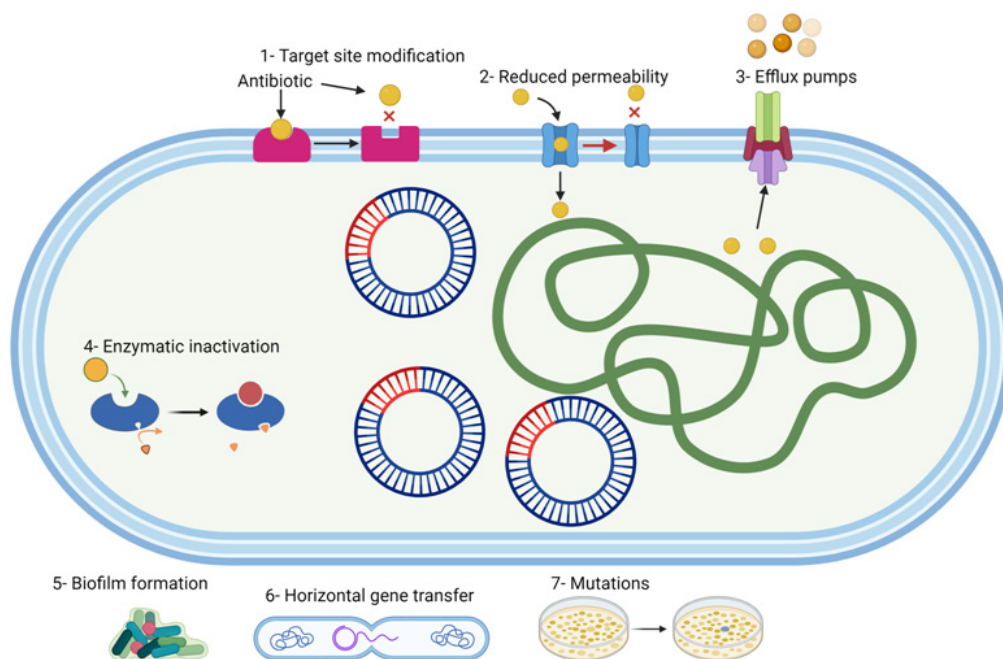


Figure 1. Mechanism of ABR in bacteria

defense mechanism to evade antimicrobial interventions. This process entails the aggregation of bacterial cells within a self-produced matrix composed of polysaccharides, proteins, and nucleic acids (Salam et al., 2023). The protective matrix not only impedes the penetration of antibiotics but also facilitates horizontal gene transfer (HGT) among bacterial populations, thereby promoting the dissemination of resistance traits (Wang et al., 2023). Consequently, biofilm-associated infections pose significant challenges in clinical settings, necessitating tailored therapeutic strategies to effectively combat these resilient microbial communities (Barawi et al., 2021; Fattah et al., 2022).

Certain species, like *Streptococcus pneumoniae*, demonstrate natural competence, enabling them to uptake extracellular DNA from their surroundings and incorporate it into their genome, further amplifying resistance gene acquisition (Lin et al., 2024). Spontaneous mutations in bacterial DNA are crucial in the emergence of antibiotic resistance, particularly when bacteria are exposed to subtherapeutic concentrations of antibiotics. These subtherapeutic concentrations refer to doses below the minimum inhibitory concentration (MIC), which is the lowest concentration of an antibiotic that prevents visible growth of a bacterium. These lower doses can occur in various clinical scenarios, such as inadequate dosing, prolonged exposure due to treatment regimens, or environmental sources of antibiotics. Under these conditions, mutations can induce changes in essential molecular targets, transporters, and regulatory elements, thereby altering the bacteria's response to antibiotics. This mutation-driven evolution significantly contributes to the genomic landscape associated with antibiotic resistance (Baquero et al., 2021). However, these adaptations often have adverse effects on the fitness of bacteria, leading to reduced growth rates in strains that have developed resistance. Despite these disadvantages, bacterial populations consistently manage to endure and adapt to the presence of antibiotic pressure by exploring various evolutionary pathways.

Drivers of ABR

Infectious disease treatment with antibiotics is standard practice in many healthcare facilities, including hospitals, clinics, and intensive care units. They are also used in veterinary facilities, feedlots, and animal farms (Ahmed and Gareib, 2016; Hoelzer et al., 2017). Nevertheless, the widespread and irresponsible utilization of antibiotics in many settings has led to the emergence of ABR and resilient pathogens that can defy nearly all known antibiotics (Davies, 1996). The main factors driving ABR include the excessive and inappropriate use of antibiotics in clinical and veterinary medicine, the availability of low-quality drugs, inadequate regulations concerning hospitalization, and insufficient research in antibiotic discovery (Salam et al., 2023; Nashwan et al., 2024 b).

Other contributing factors include environmental elements and the persistent presence of antibiotic residues as

pollutants (Samreen et al., 2021). Overuse and misuse of antibiotics can lead to the emergence of resistant strains. It is estimated that 50% of antibiotic prescriptions in clinical settings may be inappropriate. Non-prescription use of antibiotics remains common in many countries outside of North America and Europe, accounting for 19% of total usage and up to 100% in certain situations (Sohail et al., 2016). In hospital wastewaters, horizontal gene transfer often occurs, allowing for the transmission of virulence factors and antibiotic-resistant genes between different species and within the same species (Bruno and Mackay, 2012). Preventative measures, growth promoters, and feed additives are some of the modern uses for veterinary medications that were once used to control animal infections (Landers et al., 2012). Animal urine and feces account for the excretion of about 30–90% of antibiotics administered to animals. Excessive use of antibiotics in animal agriculture leads to the accumulation of significant levels of residual antibiotics in agricultural waste. This contamination of the surrounding environment facilitates the proliferation of antibiotic-resistant bacteria (Berendsen et al., 2015). This situation facilitates the selection and transfer of antimicrobial resistance genes (ARGs) to humans and the environment through the food supply and waste products (Smith et al., 2013).

ABR can spread among humans, animals, and the environment through ecosystems that connect different animal compartments to soil, water, and sewage. The environment serves as a reservoir for the mixing of mobile genetic elements, which can come into contact and transfer to different areas or to human and animal hosts (Góchez et al., 2019). Antibiotics are released into the environment from various sources, such as antibiotics discharged in landfill leachates, animal farming practices, agricultural runoff, livestock manure, and municipal and hospital waste (Goel, 2015). Antibiotics have varying half-lives, ranging from several hours to several hundred days, making them persistent environmental contaminants (Sanderson et al., 2019). Unused or expired antibiotics are often discarded, similar to other pharmaceutical products. Because they do not easily degrade, they can enter groundwater or aquatic ecosystems during wastewater treatment. Even at low concentrations, these discarded antibiotics can exert selective pressure by altering target sites or promoting horizontal gene transfer (Samreen et al., 2021).

The percentage of clinically relevant antibiotics administered for treatment and subsequently discharged into the environment is positively associated with higher rates of ARGs in pharmaceutical wastewater. Hospitals, along with other medical facilities are significant contributors to the production of antimicrobial waste, which might come from the disposal of drugs or indirectly through the disposal of patient secretions (Diwan et al., 2010; Ahmed et al., 2023). Inappropriate antibiotic consumption in hospitals continues to be a significant factor contributing to ABR, especially in developed nations. Antibiotics are consumed broadly in clinical settings not just to treat pa-

tients with bacterial infections but also used prophylactically to decrease the risk of infections during surgeries. The development and spread of drug-resistant infectious diseases are significantly influenced by the overuse of antibiotics, coupled with close patient-to-patient contact and the requirement for improved cleanliness and sanitation (Chokshi et al., 2019). The incidence of resistance in nosocomial infections is increasing, with the main drivers being the misuse of antibiotics and cross-transmission among patients (Weinstein, 2001).

Although ABR is a serious and growing global concern, there has been a slowdown in the development of novel antibiotics for the treatment of infections (Aminov, 2010). From January 1998 to December 2002, only 3% of the new drugs approved by the Food and Drug Administration (FDA) were antibacterials (Spellberg et al., 2004). Many pharmaceutical companies have withdrawn from the antibiotic industry, leading to a decrease in the quantity and diversity of research teams (Ventola, 2015 a). The discovery of antibiotics in the early 1900s marked the beginning of the “golden age” of antibiotic research. However, there is presently not much of an incentive to proceed in this field. It is believed that the antibiotics being developed for clinical use are inadequate in effectively combating the rise of ABR (Chokshi et al., 2019). Financial incentives and regulatory challenges are the primary barriers to developing new antibiotics. Additionally, a significant number of currently available antibiotics lack patent protection, resulting in their production by generic pharmaceutical companies (Dutescu and Hillier, 2021). Furthermore, physicians typically prescribe new antibiotics as a last resort when older alternatives have failed, resulting in reduced sales for pharmaceutical companies (Ventola, 2015 a).

Finally, the substandard quality of antibiotics is another significant driver of ABR. Many developing countries in Africa lack the necessary quality assurance processes to ensure the provision of high-quality antibiotics. Logically, substandard antibiotics will raise the risk of adverse patient outcomes, cause economic problems, and drive ABR (Zabala et al., 2022). Moreover, it has been observed that using expired antibiotics results in higher rates of resistance compared to using non-expired drugs. When expired medications are used, resistance rates can increase by 2–6 times (Ogunshe and Adinmonyema, 2014). Improper storage conditions, such as exposure to high temperatures, sunlight, and humidity, contribute to the deterioration and degradation of drugs. Additionally, counterfeit antibiotics, which may lack or have minimal active ingredients, and the influx of these counterfeit antibiotics into the pharmaceutical markets may play a role in the development of resistance to antibiotics (Cavany et al., 2023).

Technological and scientific advances in ABR identification

Culture-based methods

Culture-based methods remain a cornerstone for identifying antibiotic resistance in bacterial pathogens.

These include the disk diffusion (Kirby-Bauer) method, E-tests, and broth microdilution, all of which provide reliable phenotypic evidence of resistance by measuring the minimum inhibitory concentrations (MICs) of antibiotics (Humphries et al., 2019). MIC represents the lowest concentration of an antibiotic required to inhibit visible bacterial growth after overnight incubation (Eucast, 2000).

In the disk diffusion (Kirby-Bauer) test, a standardized bacterial suspension is inoculated onto Mueller-Hinton agar plates, and antibiotic-impregnated disks are placed on the surface. After incubation, inhibition zones around the disks are measured and compared to established breakpoints to determine resistance (Jonasson et al., 2020). This method is simple, cost-effective, and widely used. E-tests (Epsilometer tests), on the other hand, provide a more precise quantification of MICs. An antibiotic gradient strip is placed on an inoculated agar plate, forming an elliptical inhibition zone. The MIC is read where the ellipse intersects the strip, making this method particularly useful for testing complex resistance patterns (Abolghasemi et al., 2021). Broth microdilution involves serial dilution of antibiotics in a liquid growth medium within 96-well plates, where the MIC is identified as the lowest concentration of antibiotic that inhibits bacterial growth (WHO, 2022). This method allows simultaneous testing of multiple antibiotics and provides quantitative MIC results.

While these culture-based methods are reliable and clinically relevant, their main drawback is the time required to obtain results (18–24 hours or longer for slow-growing organisms) (Florio et al., 2019). These delays can affect treatment decisions, and certain complex resistance mechanisms may be overlooked. Despite these limitations, culture-based methods remain crucial for providing direct evidence of bacterial growth inhibition and detecting novel resistance mechanisms (Banerjee and Humphries, 2017).

Automated systems

Automated systems like VITEK 2 and BD Phoenix have significantly improved the speed, accuracy, and standardization of traditional culture-based methods (Bianco et al., 2020). These systems utilize miniaturized broth microdilution techniques with integrated optical and detection technologies. VITEK 2 uses plastic reagent cards containing antibiotics and growth medium. After inoculation, bacterial growth is monitored through optical scanning, and results are produced within 4–8 hours for fast-growing organisms (Charnot-Katsikas et al., 2018). Similarly, BD Phoenix employs microfluidic cartridges with antimicrobial agents, detecting growth turbidimetrically and colorimetrically, with results available in 6–16 hours depending on the organism (Fitzpatrick et al., 2021). Other platforms like MicroScan WalkAway and Sensititre ARIS 2X offer comparable automation and throughput, excelling at reducing turnaround times, standardizing results, and minimizing manual labor

(Evangelista and Karlowsky, 2016). These systems are also integrated with laboratory information systems, enabling efficient data management and surveillance (Karprou et al., 2021).

However, automated systems have limitations. They depend on predefined antibiotic panels and databases, which may not cover the latest antibiotics or emerging resistance mechanisms. Their high cost can also restrict use in resource-limited settings. Additionally, the proprietary algorithms used by these systems can obscure result interpretation, making it essential to maintain a balance between automation and traditional microbiology skills (Su et al., 2019).

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an alternative method to traditional genotypic or phenotypic bacterial characterization for detecting ABR (Gajdacs et al., 2017; Justesen et al., 2018). MALDI-TOF MS profiles proteins, primarily ribosomal ones (2–20 kD), from whole bacterial cell extracts to create a unique bacterial spectral fingerprint. This allows discrimination at the genus, species, and subspecies levels, and can detect antibiotic resistance mechanisms (Angeletti, 2017; Welker and van Belkum, 2019). Standardization is crucial for reproducibility (Hoyos-Mallecot et al., 2014; Rodríguez-Sánchez et al., 2019). In the assay, the sample is mixed with an energy-absorbing matrix solution and crystallizes upon drying. When hit by a laser, the sample ionizes, producing protonated ions that are accelerated and separated based on their mass-to-charge (m/z) ratio. The time taken for each ion to travel is measured, generating a “peptide mass fingerprint” (PMF). This PMF is compared to a reference database, enabling sample identification based on specific genera and species (Vrioni et al., 2018; Rodríguez-Sánchez et al., 2019).

Whole genome sequencing (WGS)

WGS provides comprehensive data on the genetic makeup of bacteria, enabling the identification of resistance genes and mutations. This method offers high accuracy and can detect resistance to multiple antibiotics simultaneously. The extracted DNA undergoes sequencing using high-throughput sequencing technologies, which produce extensive sequence data encompassing the entire bacterial genome. Subsequently, bioinformatics tools are employed to analyze this sequence data to pinpoint genetic markers indicative of antibiotic resistance. This includes identifying resistance genes by comparing the bacterial DNA sequences against known resistance gene databases, as well as detecting mutations associated with resistance mechanisms (McDermott et al., 2016). Whole genome sequencing (WGS) cannot fully replace phenotypic susceptibility testing for several reasons. First, the cost of WGS remains relatively high, making it less accessible for routine use. Additionally, there is the possibility of new antibiotic resistance genes or mutations

emerging, which may not be immediately identifiable by current genomic databases. Furthermore, sequencing using platforms like Illumina can take several days to complete, resulting in a slower turnaround compared to traditional susceptibility testing methods (Ellington et al., 2017).

PCR-RFLP

In restriction endonuclease analysis (REA), one of the initial methods for restriction fragment length polymorphism (RFLP), bacterial DNA is digested by restriction enzymes after being amplified with PCR, resulting in smaller fragments that are subsequently separated by size through gel electrophoresis (Hema et al., 2022). This method, when performed under a standardized protocol, is relatively quick, discriminatory, and reproducible. However, the complex patterns generated can make result interpretation challenging and hinder data exchange among different research groups. To enhance result interpretation, combining RFLP with ribotyping is recommended (Kakuta et al., 2020). This process can be used to verify the target sequence of genes that encode ABR through RFLP analysis. For example, the *bla*_{CTX-M} gene, renowned for its capacity to bestow resistance against extended-spectrum beta-lactam antibiotics, was detected using PCR-RFLP (Yamin et al., 2023).

CRISPR-Cas system

Several CRISPR-Cas systems have been developed for the detection of nucleic acids and biomarkers in bacteria (Li et al., 2019). These systems are capable of accurately identifying genotypes and single nucleotide polymorphisms (SNPs) in pathogenic bacteria, detecting antibiotic resistance genes (ARGs) and virulence genes, and diagnosing bacterial infections (Sashital, 2018). Advances in nanotechnology have enabled the development of nanosized CRISPR complexes, facilitating rapid on-site diagnosis. Compared to traditional nucleic acid detection technologies, these CRISPR-based methods offer enhanced specificity and sensitivity (Zhao et al., 2020).

Techniques like SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) utilizes the CRISPR-Cas13 system, where Cas13 is an RNA-guided RNA-targeting enzyme. Unlike Cas9, which targets DNA, Cas13 targets RNA and has a collateral cleavage activity that is crucial for the SHERLOCK assay. By designing gRNAs to target RNA transcripts of antibiotic resistance genes (ARGs), SHERLOCK can identify the presence of these genes in clinical or environmental samples (Kellner et al., 2020).

The DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) technique utilizes CRISPR-Cas systems to detect nucleic acids, enabling rapid identification of resistant strains. DETECTR can identify ARGs by targeting specific DNA sequences associated with resistance. This is crucial for diagnosing infections caused by antibiotic-resistant bacteria and monitoring resistance

patterns. Examples include the detection of resistance genes like *bla*_{CTX-M}, *bla*_{NDM}, and *bla*_{KPC}, which confer resistance to beta-lactam antibiotics (Chen et al., 2018).

A notable technique, FLASH (Finding Low Abundance Sequences by Hybridization) (Quan et al., 2019), employs CRISPR-Cas9 for the multiplexed detection of ABR sequences. This method is particularly effective for assessing the resistance of *Staphylococcus aureus* strains and is crucial in the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis*. Researchers have further advanced this approach by integrating a single-step optical DNA mapping assay with CRISPR-Cas9, enabling the direct identification of specific resistance genes (*bla*_{CTX-M}, *bla*_{NDM}, and *bla*_{KPC}) on plasmid molecules. This assay provides sequence information within hours, making it an ideal tool for the rapid tracking of infections (Müller et al., 2016).

Next-generation sequencing (NGS)

NGS allows for rapid and thorough assessment of bacterial genomes to identify ABR genes. It offers a more advanced approach compared to traditional drug response techniques. NGS can track the spread of resistance through plasmids in *Escherichia coli* and monitor outbreaks of drug-resistant bacteria. With high-resolution data available in just 2–5 days, NGS has the potential to become the standard for infection diagnosis and predicting effective antibiotics, potentially replacing culture methods. Furthermore, NGS can identify new resistance mechanisms, giving it a significant advantage over traditional methods (Yamin et al., 2023). For example, NGS was used to identify transmissible plasmids in multidrug-resistant (MDR) *E. coli* isolates that exhibited an extended-spectrum beta-lactamases (ESBLs) phenotype, demonstrating its usefulness in resistance surveillance (Saracino et al., 2021).

Metagenomics

Metagenomic approaches, particularly utilizing short-read next-generation sequencing (NGS) data, enable the measurement of numerous transmissible resistance genes in a single sample without relying on predefined genes. This methodology offers deeper insights into the diversity of bacteria, diseases, and virulence genes present in a sample (Rout et al., 2024). The collected data can be reanalyzed in the future to incorporate newly discovered genes of interest. Studies have demonstrated the superiority of metagenomics over conventional methods for ABR surveillance in pig herds and for comparing ABR in animals (Munk et al., 2018). Metagenomics also serves as an efficient tool for investigating epidemiological data (Van Gompel et al., 2019). Due to its numerous advantages, metagenomics is widely considered a promising method for ABR surveillance. It is conceivable that a centralized surveillance system for ABR may emerge, facilitating the detection of all resistance genes (Uddin

et al., 2021). Techniques for the diagnosis of ABR, their applications, impacts, and principles have been shown in Table 1.

Emerging technologies in ABR identification

Recent advancements in biosensor technologies, artificial intelligence (AI), machine learning (ML), and rapid point-of-care (POC) diagnostics are transforming antibiotic resistance (ABR) detection, providing faster, more accurate alternatives to traditional methods.

Biosensor technologies

Biosensors, enhanced by nanotechnology, offer sensitive, rapid, and portable ABR detection. Incorporating nanomaterials like gold nanoparticles, quantum dots, and carbon nanotubes has significantly improved sensitivity and signal amplification (Welch et al., 2021). A gold nanoparticle-based colorimetric biosensor demonstrated the detection of β -lactamase-producing bacteria within 15 minutes with a detection limit of 10^3 CFU/mL (Wang and Alocilja, 2015; Miller et al., 2022). Optical biosensors like surface plasmon resonance (SPR) and surface-enhanced Raman spectroscopy (SERS) also offer label-free, rapid detection. An SPR-based biosensor, for example, identified vancomycin-resistant *Enterococcus* (VRE) with 98% accuracy in under 30 minutes (Das et al., 2022; Saxena et al., 2022). SERS is a powerful analytical technique that significantly enhances the Raman scattering signal of molecules adsorbed on certain nanostructured metallic surfaces, typically gold or silver. The mechanism behind SERS involves both electromagnetic and chemical enhancement processes (Hassanain et al., 2022; Qurbani et al., 2022). Bacterial detection using SERS can be conducted either without labels (intrinsic) or with labels (extrinsic). In label-free SERS, the bacterial sample is either mixed directly with metallic nanostructures in a solution or applied as a thin film onto a solid SERS substrate, allowing the bacteria to attach. This approach generates SERS signals directly from the bacterial sample itself (Hassanain et al., 2017). This technique fully exploits the fingerprint information provided by SERS, offering detailed structural insights into the bacterial sample (Yang et al., 2016).

The bacterial sample was directly mixed with silver nanoparticles (AgNPs) under optimized incubation conditions, including specific shaking speed, time, and temperature, to achieve the strongest SERS enhancement. In contrast, label-based SERS involves functionalizing nanostructures with specific target-recognition molecules (such as antibodies, aptamers, or enzymes) and a Raman label to create a SERS nanotag. This nanotag selectively captures the target antigen from complex matrices and produces a strong, characteristic Raman signal from the label, indirectly indicating the presence and/or concentration of bacteria in the sample (Liu et al., 2017).

Table 1. Recent scientific and technological developments in the detection of antibiotic resistance

Technologies	Applications	Impacts	Principle	References
Culture-based methods	1. Disk diffusion (Kirby-Bauer) method. 2. E-tests. 3. Broth microdilution.	1. Provide reliable phenotypic evidence of resistance. 2. Measure minimum inhibitory concentrations (MICs) of antibiotics. 3. Simple, cost-effective, and widely used.	Measure bacterial growth inhibition in the presence of antibiotics, either on agar plates or in liquid media.	(Jonasson et al., 2020; Abolghasemi et al., 2021)
Automated systems	1. VITEK 2, BD Phoenix, MicroScan WalkAway, and Sensititre ARIS 2X systems. 2. Miniaturized broth microdilution techniques with integrated optical and detection technologies.	1. Improved speed, accuracy, and standardization of traditional culture-based methods. 2. Reduced turnaround times and manual labor. 3. Efficient data management and surveillance.	Use plastic reagent cards or microfluidic cartridges containing antibiotics and growth medium, monitoring bacterial growth through optical scanning or turbidimetric/colorimetric detection.	(Chamot-Katsikas et al., 2018; Fitzpatrick et al., 2021)
NGS	1. Comprehensive analysis of bacterial genomes to identify resistance genes and mutations. 2. Tracking the spread of resistance genes through populations and environments. 3. Identifying novel resistance mechanisms.	1. Faster and more precise diagnosis of resistant infections. 2. Improved surveillance and epidemiological tracking of resistance. 3. Enhanced understanding of resistance evolution, leading to better-targeted therapies.	Align reads to a reference genome, identify genetic variations, and process data with bioinformatics tools.	(Saracino et al., 2021)
CRISPR-based diagnostics	1. Detection of specific DNA sequences associated with ABR using CRISPR-Cas systems. 2. Development of rapid point-of-care tests.	1. Highly specific and rapid diagnostics, potentially usable in low-resource settings. 2. Ability to detect resistance genes directly from clinical samples without the need for culture.	Using a guide RNA to direct the Cas enzyme to a specific DNA sequence, where it cuts the DNA, allowing for targeted gene editing.	(Jiang et al., 2013; Wu et al., 2021)
WGS	1. Comprehensive analysis of bacterial genomes. 2. Identification of resistance genes and mutations. 3. Detection of resistance to multiple antibiotics simultaneously.	1. High accuracy in detecting resistance genes. 2. Comprehensive genetic data for epidemiological studies. 3. Potential to replace phenotypic susceptibility testing in the future.	Extracted DNA undergoes high-throughput sequencing, followed by bioinformatic analysis to identify genetic markers of antibiotic resistance.	(Doyle et al., 2020; Uelze et al., 2020)
PCR-RFLP	1. Verification of target sequences of genes encoding ABR. 2. Detection of specific resistance genes (e.g., <i>bla_{CTX-M}</i>).	1. Relatively quick, discriminatory, and reproducible method. 2. Enhanced result interpretation when combined with ribotyping.	Bacterial DNA is amplified with PCR, then digested by restriction enzymes. The resulting fragments are separated by size through gel electrophoresis.	(Kakuta et al., 2020; Hema et al., 2022; Yamin et al., 2023)
MALDI-TOF MS	1. Rapid identification of bacterial species and detection of resistance mechanisms. 2. Profiling the proteome of bacteria to create unique spectral fingerprints.	1. Quick and accurate identification of pathogens and their resistance profiles. 2. Reduced time to diagnosis, leading to more timely and appropriate treatment decisions.	Involves ionizing proteins from a sample with a laser, then measuring the time it takes for the ions to travel to a detector, creating a mass spectrum for identification.	(Karas and Hillenkamp, 1988; Singhal et al., 2015)
Metagenomics	1. Metagenomics is an efficient tool for investigating epidemiological data. 2. Metagenomics is commonly regarded as a highly promising approach for ABR surveillance due to its numerous advantages. 3. Using metagenomics, a centralized surveillance system for ABR might develop to help identify all resistance genes.	1. Detection of novel and emerging resistance mechanisms. 2. High sensitivity and specificity 3. Comprehensive detection of resistance genes	Involves sequencing the collective genetic material from a mixed microbial community, enabling the study of microbial diversity, functions, and interactions.	(Van Gompel et al., 2019; Uddin et al., 2021)

Artificial intelligence and machine learning

Artificial intelligence (AI) tools, such as predictive modeling, natural language processing, and mobile applications, present new opportunities for early disease detection, risk assessment, personalized care planning, and real-time patient support (Fareeq et al., 2024). These innovations are increasingly vital in the fight against ABR. These technologies analyze large-scale genomic and phenotypic data, enhancing detection accuracy and speed (de la Lastra et al., 2024; Olatunji et al., 2024). Machine learning models, such as convolutional neural networks, predict resistance profiles with high accuracy from genomic data (Kuang et al., 2022). AI integration into traditional molecular diagnostics has reduced time to results. AI-enhanced PCR systems can detect and classify resistance genes, cutting processing times by 40% (Ali et al., 2023 a). AI's role extends to automating antibiotic susceptibility testing (AST) with high accuracy and predicting future resistance trends, enabling proactive control measures (Kaushal and Gupta, 2022). Moreover, natural language processing (NLP) algorithms can mine biomedical literature to reveal new resistance mechanisms (Wong et al., 2018). Integrating AI with biosensor technologies further improves accuracy and efficiency, reducing error rates compared to traditional methods (Khan et al., 2024). While AI has made strides in ABR detection, data quality, model interpretability, and standardization remain active areas of research (Branda and Scarpa, 2024).

Rapid point-of-care (POC) diagnostics

Rapid POC diagnostics are essential in the fight against ABR, particularly in resource-limited settings. Isothermal amplification techniques, such as loop-mediated isothermal amplification (LAMP), provide quick ABR detection without the need for thermal cycling equipment, making them ideal for POC applications (Shang et al., 2020). A multiplexed LAMP assay, for instance, detected carbapenemase genes in under 30 minutes (Lahiri et al., 2019). LAMP has also been incorporated into microfluidic devices for comprehensive sample preparation and detection on a single chip (Shang et al., 2020). Colorimetric LAMP assays, which utilize dyes that change color upon amplification, enable easy interpretation and can be paired with smartphone apps for further convenience (Park, 2022). Lateral flow assays (LFAs), another POC technology, offer fast, paper-based detection of resistance markers. A recent study demonstrated a multiplex LFA for detecting β -lactamase genes within 15 minutes with 95% accuracy (Gupta and Ghrera, 2021; Kanokudom et al., 2021). LFAs have been further enhanced using nanoparticles, improving sensitivity and reducing background noise. For example, a gold nanoparticle-based LFA detected VRE in clinical samples with a detection limit of 10^2 CFU/mL (Wang et al., 2017). While these diagnostics

hold promise, challenges such as assay stability and interpretation in varied environments remain. The combination of POC diagnostics with digital health platforms could revolutionize ABR surveillance, as demonstrated by a cloud-connected LFA system enabling real-time tracking of resistance patterns across healthcare facilities. Table 2 summarizes the emerging technologies in ABR identification, including biosensor technologies, artificial intelligence and machine learning applications, and rapid point-of-care diagnostics, highlighting their key applications, impacts, and underlying principles.

Public health implications

ABR is a growing global public health concern with widespread implications (Figure 2). The economic burden and healthcare costs associated with ABR are significant and increasing (Roope et al., 2019; Pulingam et al., 2022). According to a WHO report, ABR could push up to 24 million people into extreme poverty by the year 2030 (WHO, 2020). This is mainly due to the rise in healthcare expenses and productivity losses (Dadgostar, 2019). In the United States alone, antibiotic-resistant infections are expected to result in annual direct healthcare costs of \$20 billion (Martens and Demain, 2017). Additionally, the impact of ABR on morbidity and mortality is substantial. A study published in the Lancet estimates that bacterial antibiotic resistance was responsible for approximately 1.27 million deaths globally in 2019 (Aguilar et al., 2023). Antibiotic-resistant infections are associated with longer hospital stays, increased risk of complications, and higher mortality rates compared to infections caused by susceptible bacteria (Peters et al., 2019). The global antimicrobial resistance data available is depicted in Figure 2.

Furthermore, ABR poses a threat to modern medicine, including surgical procedures, cancer treatment, and organ transplantation (Akram et al., 2023; Ali et al., 2024). These medical interventions often rely on the availability of effective antibiotics for preventing and treating infections. Without effective antibiotics, routine medical procedures could become high-risk endeavors, potentially resulting in a significant setback in medical advancements (Ahmed et al., 2024). Vulnerable groups, such as immunocompromised individuals, the elderly, and children, are particularly susceptible to the consequences of ABR (Maillard et al., 2020; Francis et al., 2023). The danger of infection is greater for patients whose immune systems are already compromised, as is the case with individuals receiving chemotherapy or organ transplants. Moreover, their treatment options may be limited due to ABR. The elderly population is also disproportionately affected, as they often have multiple comorbidities and are more prone to healthcare-associated infections (Pulcini et al., 2019).

Table 2. Emerging technologies in ABR identification

Technologies	Applications	Impacts	Principle	References
Biosensor technologies	1. Sensitive, rapid, and portable ABR detection. 2. Gold nanoparticle-based colorimetric biosensors for β-lactamase-producing bacteria detection. 3. Optical biosensors (SPR, SERS) for label-free, rapid detection.	1. Improved sensitivity and signal amplification. 2. Rapid detection (e.g., 15 minutes for β-lactamase-producing bacteria). 3. High accuracy (e.g., 98% for VRE detection in under 30 minutes).	Incorporate nanomaterials (gold nanoparticles, quantum dots, carbon nanotubes) to enhance sensitivity. Use optical techniques like SPR and SERS for label-free detection.	(Hassanain et al., 2022; Miller et al., 2022)
Artificial intelligence and machine learning	1. Analysis of large-scale genomic and phenotypic data. 2. Prediction of resistance profiles from genomic data. 3. Enhancement of traditional molecular diagnostics. 4. Automation of antibiotic susceptibility testing (AST). 5. Mining biomedical literature for new resistance mechanisms.	1. Enhanced detection accuracy and speed. 2. Reduced processing times (e.g., 40% reduction in PCR processing time). 3. High accuracy in predicting resistance profiles. 4. Improved efficiency and reduced error rates compared to traditional methods. 5. Proactive control measures through prediction of future resistance trends.	Use machine learning models (e.g., convolutional neural networks) to analyze genomic data. Integrate AI with traditional diagnostic methods and biosensor technologies. Apply natural language processing (NLP) to mine biomedical literature.	(Kaushal and Gupta, 2022; Ali et al., 2023 a)
Rapid point-of-care diagnostics	1. Quick ABR detection in resource-limited settings. 2. Isothermal amplification techniques (e.g., LAMP) for rapid detection. 3. Microfluidic devices for sample preparation and detection. 4. Colorimetric assays paired with smartphone apps. 5. Lateral flow assays (LFAs) for fast, paper-based detection of resistance markers.	1. Rapid detection (e.g., under 30 minutes for carbapenemase genes). 2. High accuracy (e.g., 95% for β-lactamase gene detection). 3. Improved sensitivity and reduced background noise with nanoparticle-enhanced LFAs. 4. Real-time tracking of resistance patterns across healthcare facilities.	Use isothermal amplification techniques that do not require thermal cycling equipment. Incorporate colorimetric detection methods for easy interpretation. Enhance LFAs with nanoparticles for improved sensitivity. Integrate with digital health platforms for real-time surveillance.	(Shang et al., 2020; Park, 2022)

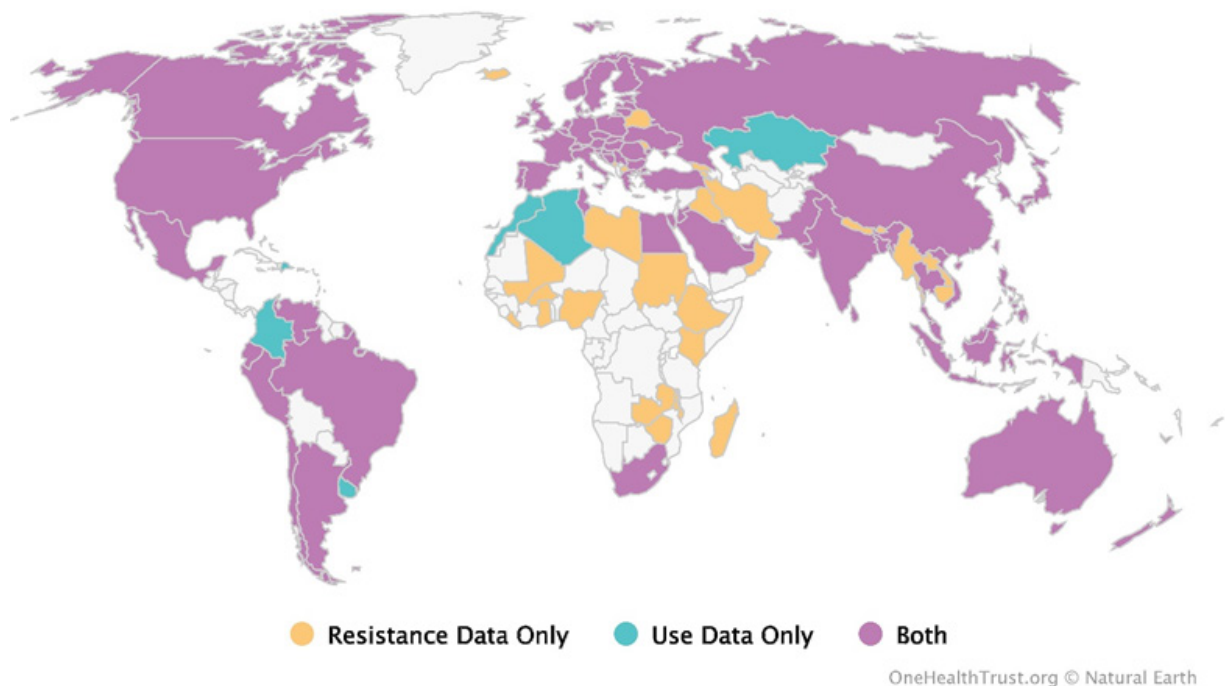


Figure 2. Global antimicrobial resistance map (<https://resistancemap.onehealthtrust.org>)

Additionally, children, especially those in low- and middle-income countries, are vulnerable to the effects of ABR (Fink et al., 2020; Ramblière et al., 2021; Sulis et al., 2022). Infections caused by resistant bacteria can lead to prolonged illnesses, increased healthcare costs, and higher mortality rates among this vulnerable group (Pacios et al., 2020). Moreover, the psychological and social implications of ABR should not be overlooked. Patients with antibiotic-resistant infections may experience increased anxiety, depression, and decreased quality of life due to the challenges associated with treatment and the potential for adverse outcomes (Fregonese et al., 2023). Consequently, the psychological burden extends beyond patients to their families and caregivers, who may experience significant emotional and financial stress (Thomasen et al., 2023).

Tackling ABR necessitates a comprehensive strategy that involves several parties, including healthcare practitioners, legislators, academics, and the general public. Encouraging the cautious and responsible use of antibiotics, allocating resources to systems that manage and monitor the use of antibiotics, and supporting the advancement of new antibiotics and alternative treatment methods are essential measures in addressing this escalating danger (Aziz et al., 2023 b; Kader et al., 2024; Qurbani et al., 2024 c). Furthermore, improving infection prevention and control measures in healthcare settings, raising public awareness about ABR, and strengthening surveillance systems to monitor resistance patterns are essential components of a comprehensive strategy (Alhumaid et al., 2021; Qurbani et al., 2024 b). ABR is a complex and multidimensional challenge that demands

immediate attention and coordinated action (Aslam et al., 2021). By recognizing the extensive implications of this issue, such as the economic burden, impact on morbidity and mortality, threats to modern medicine, vulnerability of specific populations, and psychological and social consequences, we can prioritize and implement effective interventions to safeguard public health. This will help ensure the continued efficacy of antibiotics for generations to come.

Strategies for addressing ABR

In order to effectively address the growing threat of antibiotic resistance (ABR) and its impact on public health worldwide, a comprehensive approach is required. This approach should encompass policy and regulatory frameworks, antibiotic stewardship programs, research and development incentives, public awareness campaigns, and international collaborations. Governments play a crucial role in implementing stringent regulations to control the use and distribution of antibiotics. The WHO has developed a global action plan to tackle ABR, urging member states to develop national action plans that include surveillance and monitoring systems (WHO, 2016). The widespread use of antibiotics in farming is another factor that has to be addressed by regulatory measures; this practice greatly contributes to the rise of bacteria that are resistant to these drugs (Manyi-Loh et al., 2018).

Antibiotic stewardship programs (ASPs) are essential for promoting responsible antibiotic use in both healthcare and community settings. In hospitals, these programs aim to improve prescribing practices, and research

has shown that comprehensive ASPs can reduce inappropriate antibiotic use by up to 39% (Schuts et al., 2016). Community-based ASPs focus on educating healthcare providers and the public about appropriate antibiotic usage, successfully reducing prescriptions for viral infections that do not require antibiotics (Parveen et al., 2022).

Incentives for research and development are crucial for promoting the discovery of new antibiotics and alternative treatments. Financial motivations, such as grants, subsidies, and collaborations between public and private sectors, might stimulate pharmaceutical corporations to allocate resources towards antibiotic development. The Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) is a successful example of such incentives, providing support for innovative research and development (Alm and Gallant, 2020). Enhanced collaboration between health ministries and academia, particularly in developing nations, is also essential.

Public awareness and education campaigns are fundamental in combating ABR. These initiatives can help dispel misconceptions about antibiotics and promote behavioral change. Research has demonstrated that educational interventions, such as the European Antibiotic Awareness Day, can significantly improve public knowledge and reduce antibiotic consumption (Cizman et al., 2018; Huttner et al., 2019; Kosiyaporn et al., 2020; Qurbani et al., 2024 a). These campaigns should be culturally sensitive and utilize diverse media platforms, including social media, news channels, conferences, and summits, to effectively engage diverse populations.

Global cooperation is crucial in tackling the worldwide scope of ABR. Initiatives such as the Global Antibiotic Research and Development Partnership (GARDP) and the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) bring countries together in the fight against resistance by promoting the sharing of data, resources, and best practices (Ventola, 2015 b). International agreements, such as the WHO's global action plan, provide a framework for harmonizing policies and strategies across borders.

Future directions and research needs

The rise of multidrug-resistant microorganisms poses substantial difficulties in the treatment of infectious illnesses. Urgent development of novel strategies and therapeutic agents is necessary (Ahmed et al., 2024; Yin et al., 2024; Gopikrishnan et al., 2024). CRISPR-Cas9 technology offers precise approaches to combat resistance by targeting and deactivating resistance genes in pathogenic bacteria (Palacios Araya et al., 2021; Vaghari-Tabari et al., 2022; Javed et al., 2023; Tao et al., 2023). Advancements in nanotechnology hold promise for developing antibiotics with targeted delivery systems (Chakraborty et al., 2022; Hetta et al., 2023; Muteeb, 2023). Bacteriophage therapy presents an alternative to traditional antibiotics, especially for multidrug-resistant strains (Hatfull et al., 2022; Ali et al., 2023 b).

Integrating big data analytics with predictive modeling has the potential to revolutionize our understanding and control of ABR. Machine learning algorithms can analyze extensive datasets from genomic, clinical, and environmental sources to predict resistance mechanisms and inform new antibiotic development (Kim et al., 2022). NGS, machine learning, and quantum computing have accelerated the identification of drug-resistant pathogens and deepened our understanding of genetic complexities (Gopikrishnan et al., 2024). Real-time surveillance of resistance patterns through big data analytics enables swift and accurate public health interventions (Yin et al., 2024).

To address ABR effectively, research must prioritize areas with the greatest potential impact, such as the development of innovative antibiotics and alternative therapies (Aziz et al., 2023 a; Qurbani et al., 2024 c). Understanding and mitigating socio-economic factors contributing to antibiotic misuse and the spread of resistance is crucial (Khadka et al., 2023). Achieving sustainable antibiotic use requires expanding global stewardship programs in human and veterinary medicine (Mendelson et al., 2024). Economic and regulatory incentives can promote pharmaceutical research and the development of new antibiotics (Aziz et al., 2023 b; Kader et al., 2024).

Despite advancements, substantial knowledge gaps remain in ABR research. Further investigation is needed into mechanisms of horizontal gene transfer among bacteria in diverse environments (Wang et al., 2023), the impact of sub-lethal antibiotic concentrations on resistance development (Li et al., 2021), and the role of microbiomes in resistance dynamics (Schwartz et al., 2020). Addressing these gaps requires comprehensive multidisciplinary research efforts integrating microbiology, genomics, environmental science, and clinical studies. Collaborative approaches will be essential in developing effective strategies to combat the growing threat of ABR and ensure the continued efficacy of antibiotic treatments.

Conclusion

ABR is a pressing global health challenge that requires immediate action on multiple fronts. This review has highlighted the complex mechanisms behind resistance, advancements in detection technology, and the significant impact on public health. To address this crisis, we need policy reforms, antibiotic stewardship, research incentives, public awareness campaigns, and international collaboration. Exciting technologies like CRISPR-Cas9, nanotechnology, and big data analytics show promise in fighting resistance. However, there are still knowledge gaps that require ongoing interdisciplinary research. Through the integration of these approaches and promoting international collaboration, we can safeguard the efficacy of antibiotics and the well-being of the general population for future cohorts.

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Conflicts of interest

There is no conflict to be declared.

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