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ARTICLE

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UNRAVELING GENETIC VARIABILITY IN ANTIMICROBIAL RESISTANCE AMONG INTRACELLULAR AND EXTRACELLULAR BACTERIA

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Antimicrobial resistance (AMR) represents a critical global health threat, driven by the remarkable evolutionary adaptability of microorganisms such as bacteria, fungi, and viruses. These organisms continuously evolve to overcome the effects of antimicrobials, resulting in infections that are increasingly challenging to treat. The emergence of resistance is a dynamic process influenced by a range of factors, including diverse resistance mechanisms present in both intracellular and extracellular bacteria. This review highlights the importance of studying both types, as they employ distinct survival strategies that impact treatment outcomes. Intracellular bacteria can evade immune responses and are often protected from antibiotics, complicating infection eradication. In contrast, extracellular bacteria typically develop resistance through horizontal gene transfer and biofilm formation, presenting additional management challenges. Understanding the genetic basis and dynamics of these mechanisms is crucial for developing rapid diagnostic tools and effective treatment strategies. This review delves into the complexities of AMR, emphasizing the various strategies bacteria use to neutralize antibiotics and the implications for managing both intracellular and extracellular infections. It also addresses the role of environmental reservoirs and host-pathogen interactions in the development and spread of resistance. Key conclusions emphasize the urgent need for innovative strategies to enhance antibiotic efficacy, considering the unique challenges posed by both intracellular and extracellular bacteria. By tackling these issues, the review aims to improve approaches for managing bacterial infections in clinical and public health contexts.

Introduction

Bacteria represent an extraordinarily diverse group of microorganisms, thriving in a wide range of environments—from the external surfaces of the human body to the interiors of host cells. They are generally classified into two main categories based on their lifecycles: extracellular bacteria, which exist independently in various habitats, and intracellular bacteria, which invade and replicate within host cells. This adaptability is a testament to their evolutionary success but also adds layers of complexity to the challenge of combating antimicrobial resistance (AMR).

The discovery of antibiotics stands as one of the most transformative innovations of the twentieth century, revolutionizing the treatment of bacterial infections and significantly advancing medical care[1]. However, the overuse and misuse of antibiotics have led to a troubling rise in infections caused by extensively drug-resistant

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(XDR) and multidrug-resistant (MDR) bacteria worldwide[2]. Despite the crucial role of antibiotics in enabling effective surgical procedures, treating infection-related conditions, and supporting immunosuppressive therapies, AMR has become a significant burden on global healthcare systems[3]. According to the World Health Organization (WHO), AMR was responsible for 1.27 million deaths globally in 2019[4]. The rise in resistant infections and the escalating costs of healthcare underscore the urgent need for new strategies to address this crisis.

The WHO 2024 Bacterial Priority Pathogens List (BPPL) highlights critical concerns, including Gram-negative bacteria resistant to last-resort antibiotics, rifampicin-resistant *Mycobacterium tuberculosis*, and other high-priority resistant pathogens such as *Salmonella*, *Shigella*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*[1]. This list includes both intracellular and extracellular bacteria, underscoring the ongoing struggle against AMR and the critical need for innovative approaches in drug development and infection management. The rising prevalence of both intracellular and extracellular bacteria contributes significantly to the global health crisis of AMR, as intracellular pathogens evade immune responses and prolong infections, while extracellular bacteria rapidly share resistance genes, complicating treatment efforts.

This review explores the multifaceted challenge of AMR across different bacterial environments. It investigates the diverse resistance mechanisms employed by both intracellular and extracellular bacteria, emphasizing the need to understand these strategies to develop effective treatments. Additionally, it addresses the role of environmental reservoirs and host-pathogen interactions in the spread of resistance. By highlighting these issues, the review identifies key gaps in research, particularly in understanding the interplay between intracellular survival strategies and extracellular defense mechanisms. It also emphasizes the need for innovative solutions, such as advanced drug delivery systems and novel antimicrobial peptides, to effectively combat resistant infections and address the complexities of AMR. Finally, the review suggests future research directions, including the exploration of host targeted therapy, phage therapy and microbiome-based interventions, which could play crucial roles in addressing the complexities of AMR.

Antimicrobial resistance

Microorganisms such as bacteria, viruses, and fungi continually evolve with the primary objective of rapid replication, reproduction, and proliferation. To ensure their survival, these organisms adapt to their environments and undergo evolutionary changes. AMR is a natural consequence of this evolutionary process. It arises when bacteria develop resistance to antibiotics that were previously effective against them, often due to genetic alterations that enable their survival despite the presence of these drugs.

Bacterial resistance can be broadly categorized into three types: natural, acquired, and adaptive. Natural resistance is an inherent ability of certain bacterial species to resist specific antibiotics. Acquired resistance occurs when bacteria gain resistance mechanisms through genetic mutations or horizontal gene transfer from other resistant bacteria. Adaptive resistance involves changes in bacterial gene expression in response to environmental pressures, such as the presence of antibiotics. Understanding these mechanisms is crucial for developing effective strategies to combat AMR and ensure the continued efficacy of antimicrobial treatments[2].

Natural Resistance: Natural resistance can be classified as intrinsic and acquired forms of resistance. Intrinsic can be defined as an inherent features or characteristics, already present within bacterial species and not related to any prior exposure to anti-microbial drugs[3]. Acquired resistance, is a part of adaptive resistance wherein resistance develops through the acquisition or modification of genes triggered by exposure to therapeutic or sub-lethal levels of antibiotics[3]. For instance, due to the absence of cell wall in *Mycoplasma sp.*, antibiotics like β-lactam and glycopeptides are ineffective against them[4] whereas the use of glycopeptide antibiotics are effective in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) as they are Gram-positive but ineffective in treating Gram-negative bacteria as Gram-negative bacteria possess an outer membrane and its composition includes phospholipids, negatively charged lipopolysaccharides (LPS), porins and Braun's lipoprotein which is altogether attributable to inherent resistance to glycopeptide antibiotics[5].

Acquired Resistance: It is based on acquiring certain new traits, whereby bacteria use a variety of strategies to adapt to antibiotics, such as changing the drug target through mutation or transmission of resistance genes through mechanisms of genetic transfer- transformation, transduction and conjugation, which collectively come under horizontal gene transfer[6]. Each of the three pathways for gene transfer is likely to happen between closely related bacterial species, while it can additionally occur between phylogenetically distant species[7]. DNA segments known

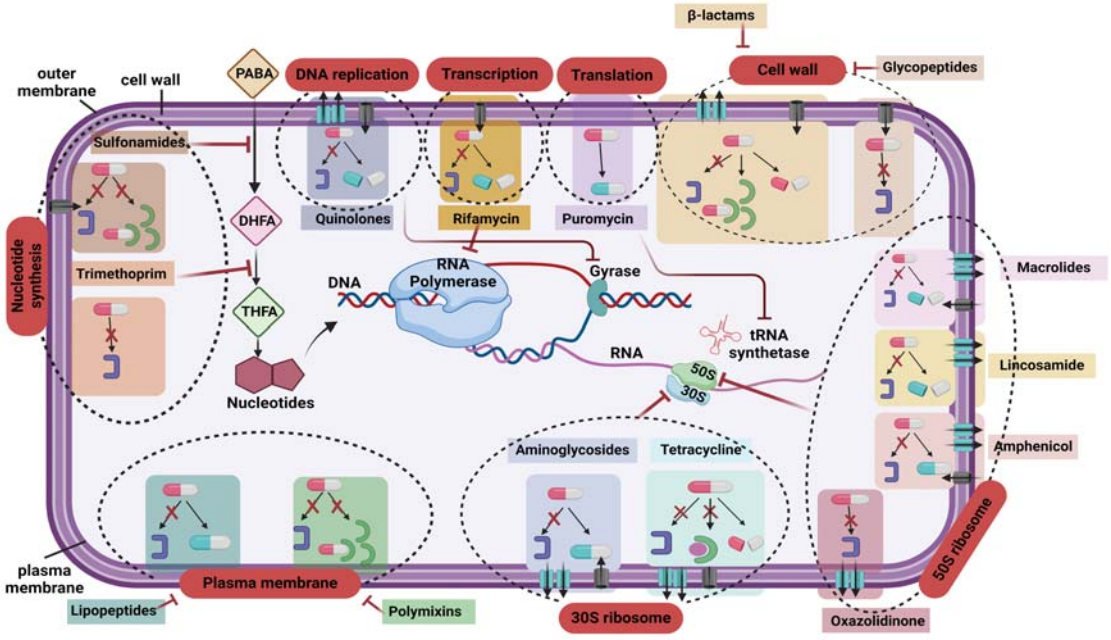

Fig. 1: This figure depicts the diverse targets and mechanisms of action of various antibiotics within a bacterial cell. It highlights the specific molecular targets and cellular processes that each antibiotic disrupts.

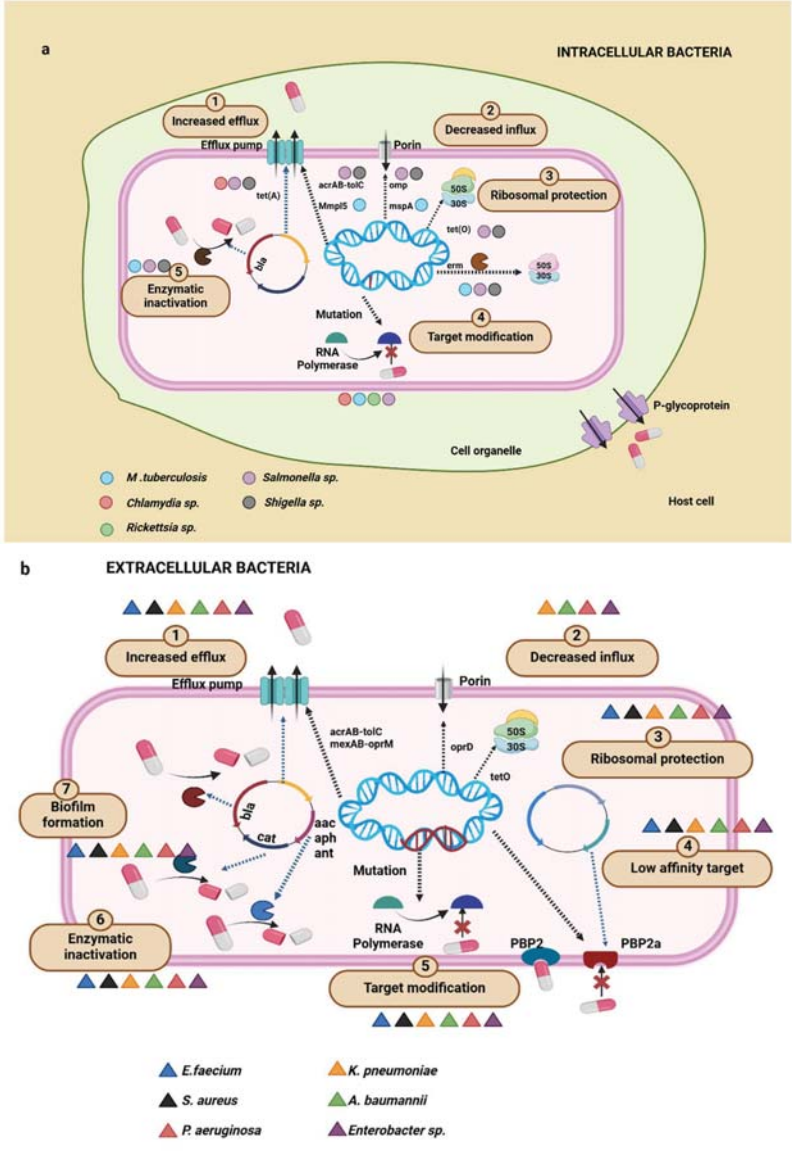

Fig. 2: : This figure compares the mechanisms of antibiotic resistance between intracellular bacteria and extracellular bacteria. **a** For intracellular bacteria, mechanisms include increased efflux, decreased influx, ribosomal protection, target modification, and enzymatic inactivation. **b** Extracellular bacteria exhibit additional resistance strategies such as low-affinity targets and biofilm formation, alongside increased efflux, decreased influx, ribosomal protection, target modification, and enzymatic inactivation. Specific bacterial species employing these mechanisms are highlighted.

as mobile genetic elements (MGEs) allow the movement of resistant genes both inside and across cells[8]. Certain MGEs, like transposons and insertion sequences, frequently enable mobility within the genome. The main carriers for extracellular mobility include- plasmids, prophages and integrative and conjugative/ mobilizable elements[9]. HGT spreads harmful characteristics including virulence genes and aids in biofilm formation, which leads to severe infections[10].

Adaptive Resistance: Adaptive resistance is a phenotype that emerges in response to environmental changes. It is typically developed transiently and usually reverts to the original state depending on the strength and duration of the selection pressure. Recent research indicates that adaptive resistance depends on epigenetic inheritance and variability in gene expression patterns, particularly those related to the production of porins and efflux pumps[11,12].

Bacterial Pathogenesis: Intracellular and Extracellular Dynamics

Bacteria are the most abundant microorganism found in the environment. These organisms exhibit remarkable diversity in their sizes and shapes. Based on their mode of existence or lifecycle, they are categorized into two groups: extracellular bacteria, which live freely in their environments, outside the host cell and intracellular bacteria, which infect and multiply within host cells[13,14]. The classification of bacteria as "extracellular" or "intracellular" is based on in vitro observations but some extracellular bacteria like *Staphylococcus aureus, Streptococcus pneumoniae* and many other can invade host cells during infection. On the other hand, bacteria usually categorized as "intracellular," such as *Mycobacterium tuberculosis*, can exhibit extracellular phases in their lifecycle, notably in cavitary lesions during infection[15].

Intracellular Bacteria: Intracellular bacteria represent a substantial challenge as they often lead to chronic or latent infections, which are challenging to diagnose and treat. Based on their ability to survive and replicate within host cells, they are classified into two categories: obligate and facultative intracellular bacteria. Obligate intracellular bacteria, such as *Chlamydia sp.*, *Rickettsia sp.*, and many others, rely entirely on host cells for their replication and cannot replicate outside a host cell[13]. On the other hand, facultative intracellular bacteria such as *Mycobacterium tuberculosis, Salmonella spp., Francisella spp., Legionella pneumophila* and others have the capability to replicate within host cells as well as in extracellular environments[13]. Intracellular bacteria have evolved intricate mechanisms to initiate and sustain infections within host cells by evading host immune system[16]. They often thrive within mononuclear phagocyte which are among the most efficient cells in antimicrobial defense[17]. The process begins with bacterial adhesion to specific receptors on the host cell surface which is facilitated by proteins called "adhesins" present on bacterial surface[18]. Upon adherence, bacteria induce their uptake into host cells through processes such as phagocytosis, micropinocytosis, endocytosis or by inducing actin rearrangements[19,20]. Once inside the host cell, some bacteria escape from the phagosome into the host cell cytoplasm using pore-forming proteins (listeriolysin O, in case of *Listeria monocytogenes)*[21] or other specialized mechanisms to avoid lysosomal degradation. This escape allows intracellular bacteria to replicate freely within the host cell, often manipulating host cell processes to create a favorable intracellular niche leading to persistent infection.

Extracellular Bacteria: On the other hand, extracellular bacteria representing a broad spectrum of pathogens are capable of causing a range of infections, from superficial wounds to life-threatening systemic illnesses. These pathogens typically replicate in spaces outside of cells, such as mucosal surfaces, vascular and lymphatic fluids and body cavities[22,23]. Recent studies found that extracellular bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* developed ability to enter and replicate in host cell[24,25]. Certain extracellular bacteria do not invade body tissues but instead adhere to epithelial surfaces and release toxins, leading to a disease[26]. Extracellular bacteria employ several evasion strategies to subvert host immune responses. One common strategy is the formation of polysaccharide capsules, such as those seen in *Streptococcus pneumoniae* and *Haemophilus influenzae*, which inhibit phagocytosis and complement-mediated killing by neutrophils and macrophages[27,28]. Additionally, biofilm formation is utilized by bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, creating a protective matrix that shields them from immune cells and antibiotics, promoting chronic infections[29]. Virulence factors like *S. aureus* pyrogenic toxin superantigens, play crucial roles in causing tissue damage and suppressing immune responses, thereby promoting bacterial survival[30]. These evasion strategies collectively enhance the ability of extracellular bacteria to establish and maintain infections, highlighting the need for targeted approaches in therapeutic to combat infection caused by extracellular bacteria.

Antimicrobial Resistance Mechanisms in Intracellular and Extracellular Bacteria

AMR presents a formidable challenge that exacerbates infections, heightening disease severity and reducing the effectiveness of antibiotics, thereby posing substantial risks to global public health. In case of extracellular bacteria, antibiotics must penetrate extracellular barriers such as biofilms or bacterial capsules to reach their targets effectively[31]. As intracellular bacteria reside inside the host cell, antibiotics targeting intracellular bacteria face unique challenges[32]. For the antibiotics to exert their therapeutic effect, they must first traverse the host cell membrane and attain concentrations sufficient to inhibit bacterial growth within the host environment[33,34]. Various classes of antibiotics enter host cells through different pathways: β-lactams, macrolides and quinolones penetrate via diffusion[34], while aminoglycosides use receptor-mediated endocytosis[35]. Aminoglycosides, β-lactams and glycopeptides have limited ability to penetrate cells due to their hydrophilic nature, whereas others like fluoroquinolones and macrolides can diffuse more easily but they show low intracellular retention[36]. In terms of AMR, bacteria have evolved distinct mechanisms. These mechanisms often involve modifications in bacterial physiology and metabolism that enable them to withstand antimicrobial pressures within the host environment.

Altered Cell Wall Permeability: Altered cell permeability is an antimicrobial resistance mechanism employed by bacteria to evade the effects of antibiotics and persist in hostile environments. This mechanism involves several strategies that collectively reduce the intracellular accumulation of antimicrobial agents, thus mitigating their efficacy. This can take place by reducing the expression or activity of transport proteins (porins) that facilitate the uptake of antibiotics across the bacterial membrane or overexpression of efflux pumps that expel the antibiotic out of cell[12,37]. By minimizing antibiotic influx, bacteria can maintain lower intracellular drug concentrations, which reduces the efficacy of antibiotics that rely on high intracellular concentrations to exert their antimicrobial effects. Intracellular bacteria, such as *Mycobacterium tuberculosis* utilize efflux pumps to actively extrude antibiotics from their cytoplasm due to mutation in MmpR respressor leading to excessive production of efflux pump Mmpl5[38]. In case of *Chlamydia suis*, gene encoding tetracycline efflux pump tet(C) prevents tetracycline inhibition of protein synthesis by exporting the antibiotic from bacterial cells[39]. Efflux pumps in extracellular bacteria are crucial for expelling antimicrobial agents before they reach harmful concentrations. For example, most of the extracellular bacteria such as *Pseudomonas aeruginosa* uses the MexAB-OprM and arcAB-tolC efflux system to resist a broad range of antibiotics and antimicrobial peptides[40] while reduced porin protein OprD results in decreasing influx of antibiotic, developing resistance to imipenem. Biofilms, which are structured communities of bacteria encased in a self-produced matrix of extracellular substances[41,42] also contribute to AMR. It acts as a physical barrier, impairing the penetration of antibiotics[43], altered chemical microenvironments[44] and the presence of differentiated subpopulations or persisters[45,46] allow bacteria to survive even high doses of antibiotics.

Antibiotic Target Modifications: Target site modifications refer to genetic alterations in bacteria that affect the binding affinity of antibiotics to their intended targets, rendering the drugs less effective in inhibiting bacterial growth[47]. Target site modifications causing antibiotic resistance vary significantly between intracellular and extracellular bacteria, influenced by their distinct habitats and adaptive strategies. The modifications are accomplished through various mechanisms, such as replacing target sites, enzymatically modifying target sites or mutating the genes that encode these target sites. In intracellular bacteria, such as *Mycobacterium tuberculosis*, resistance often arises from genetic mutations in key enzymes like DNA gyrase[48] and RNA polymerase[49]. Rifampicin resistance in *M. tuberculosis* arises from mutations in the *rpoB* gene, which encodes the β subunit of RNA polymerase[50]. These genetic alterations modify antibiotic binding sites, reducing the effectiveness of rifampicin within host cells. Putative compensatory mutations in the *rpoA* and *rpoC* genes, which encode the α-subunit and β2 -subunit of RNA polymerase respectively, has also been identified in rifampin-resistant *M. tuberculosis* isolates[51–53]. In contrast, extracellular bacteria like *Staphylococcus aureus* employ surface protein modifications, such as altering penicillin-binding proteins (PBPs), to evade the effects of antibiotics like β-lactam[54]. PBP2a encoded by the acquired *mecA* gene[55], has a low affinity for β-lactam antibiotics[56,57] and serves as a replacement for the other PBPs (PBP1–4), allowing *S. aureus* to persist even in the presence of high levels of β-lactam drugs, including methicillin, that target cell wall biosynthesis[58].

Antibiotic Inactivation: Antibiotic inactivation refers to the ability of bacteria to neutralize or degrade antibiotics and decreasing their effectiveness in combating bacterial infections. Antibiotics can be inactivated through enzymatic degradation or through modifications. Bacteria produce

enzymes that alter and inactivate antibiotics, including β-lactamases, aminoglycoside-modifying enzymes (such as acetyltransferases (AAC), aminoglycoside nucleotidyltransferases (ANT) and aminoglycoside phosphotransferases (APH))[59] and chloramphenicol acetyltransferases[60]. β-lactamases are particularly well-researched in the context of antibiotic resistance due to their ability to cleave the amide bond within the four-membered β-lactam ring[61]. These enzymes including TEM, SHV and CTX-M types are prevalent in extracellular Gram-negative bacteria[62]. Similarly, in *Salmonella*, β-lactamases, including extended-spectrum β-lactamases (ESBLs) (encoded *by blaSHV, blaTEM, blaCTX*, and *blaCMY*) and AmpC types, degrade β-lactam antibiotics, leading to resistance.

Factors Influencing Antimicrobial Resistance Dynamics

AMR dynamics are shaped by a complex interplay of factors spanning biological, environmental and societal dimensions. The dissemination of multidrug-resistant bacteria strains and genes carrying antibiotic resistance is currently considered to be one of the most important global problems. These resistant organisms render once-effective treatments ineffective, leading to prolonged illnesses, increased healthcare costs, and higher mortality rates.

Environmental Reservoirs : Recently, the significance of the environment contributing both as a source and route for spreading AMR has been given wide attention[63,64]. Scientists have emphasized the importance of a comprehensive approach in understanding AMR encompassing humans, animals and the broader environment—a holistic approach often referred to as "one-health"[65]. The environment serves as a pool where different antimicrobial resistance genes (ARGs) and mobile genetic elements can interact and further disseminate to different parts and hosts. A global study revealed significant regional differences in the diversity of ARGs linked to the country's economy. Wealthier nations had a limited range of macrolide resistance genes, while lower-income countries like India and Brazil displayed a broader spectrum of resistant genes[66]. Hospitals are sources of resistant infections and are epidemiologically hubs for extensive antimicrobial intake[67]. The widespread use of antibiotics in hospital units creates selection pressure that encourages the establishment of multi-drug-resistant bacteria. Pesticide use is also linked to antibiotic resistance; *E. coli* and *S. typhimurium*, for example, became resistant to multiple antibiotics after exposure to herbicides like Kamba, 2.4-dichlorophenoxyacetic acid and Roundup[68].

Host-pathogen interaction: An important aspect of host-pathogen interactions is the formation of biofilms. In addition to protecting bacteria against changes in pH, osmolarity, nutritional scarcity and mechanical stress, biofilms also prevent antibiotics and host immune cells from penetrating bacterial biofilm communities[69,70]. Consequently, the biofilm matrix provides bacteria with an extra layer of resistance, enabling them to withstand harsh environments and combat antibiotics[71]. Resistance of antibiotic and functional characteristics of biofilm are closely linked to its structure, the configuration of its matrix, the three-dimensional organization of the microorganism within it. Bridier et al., using confocal laser scanning microscopy (CLSM), observed distinct biofilm structures among different microbes[72]. Intracellular bacteria like *L. monocytogenes* formed rough, variable-thickness biofilms, while *Salmonella enterica* produced small, dispersed clusters[73]. In contrast, extracellular bacteria such as *P. aeruginosa* and *Enterococcus faecalis* formed mushroom-shaped and flat, compact biofilms, respectively, highlighting microbe-specific differences in biofilm architecture[74]. Recent studies on biofilm metabolism have highlighted important metabolites and pathways, suggesting that metabolic engineering could play a vital role in managing biofilms. Lu et al. examined the metabolic differences between biofilm and planktonic populations of UTI89, a uropathogenic *E. coli*, using mass spectrometry and cytological imaging techniques[75]. Emerging therapies, such as phage therapy and CRISPR-Cas9, show promise; phages can break down the extracellular matrix, while CRISPR-Cas9 can specifically target genes associated with biofilm formation. Photodynamic therapy (PDT) employs reactive oxygen species to disrupt biofilms with minimal harm to host tissues. Together, these innovative strategies present promising alternatives to conventional treatments for infections related to biofilms. Another aspect of host-pathogen interaction is immune escape. One of the studies described a metabolic adaptability mechanism in *S. aureus* that enables them to evade both antibacterial and host immunological attacks at the same time[76]. Similarly, *M. tuberculosis* can persist in growing and thriving as an intracellular bacterium inside the host's macrophages despite receiving antimicrobial medication, evading the immune system and causing repeated infections[77]. In *M. tuberculosis*, the transmission of antibiotic resistance has not reported through horizontal gene transfer instead primarily occurs through vertical gene transfer[78]. Understanding complex systems and opening up new avenues for treatment depends on an examination of the host-pathogen interactions, including how they have changed over time and in response to various therapeutic approaches.

Impact of Heterogeneity on Treatment Strategies

Antimicrobial resistance (AMR) remains a major challenge in clinical and public health settings, exacerbated by the heterogeneous nature of bacterial populations. This heterogeneity refers to the variability in antimicrobial susceptibility within bacterial communities, arise from a variety of factors including genetic diversity, epigenetic regulation and adaptive stress responses. Understanding this heterogeneity is crucial for developing effective treatment strategies that can mitigate the emergence and spread of resistant strains. Antibiotics that are effective against extracellular bacteria may not adequately target intracellular pathogens, leading to incomplete eradication and potential relapse. The ineffectiveness of conventional antibiotics against intracellular infections can be attributed to challenges such as their inability to penetrate cells adequately, accumulate sufficiently within subcellular compartments, or withstand inactivation by acidic conditions within those compartments[79]. Due to these factors, certain antibiotics can effectively kill extracellular bacteria but fail to effectively eliminate intracellular bacteria[79]. In a study assessing the minimum inhibitory concentrations (MICs) of vancomycin (VCM), doxycycline, linezolid and rifampicin against methicillin-resistant *S. aureus* (MRSA), it was found that the MICs required to inhibit intracellular MRSA were 25 ~ 10^4 times higher compared to those needed for extracellular MRSA[80]. Currently, the primary approach for treating intracellular infections involves extended courses of antibiotics at high doses[81]. Intracellular bacteria may serve as sources for recurrent infections and community spread as they can shift to a non-replicating or slowly replicating state[82]. *M. tuberculosis* can enter a dormant state in the host, leading to latent infections that are resistant to standard treatments[83]. These physiological adaptations diminish the effectiveness of antibiotics. Thus, to effectively eradicate intracellular infections involving non-replicating bacteria, antibiotics must be capable of targeting both active and dormant bacterial states. For the treatment of infection caused by intracellular bacteria certain classes of antibiotics, including macrolides, fluoroquinolones, tetracyclines are effective against both obligate and facultative intracellular organisms[84]. Macrolides and quinolones are efficiently absorbed by phagocytic cells. However, despite their accumulation inside these cells, efflux pumps found on the surfaces of mammalian cells and on endosomal membranes, such as P-glycoproteins reduce the intracellular concentration of drugs within the host cell[85,86]. In contrast, other antibiotics such as beta-lactams and aminoglycosides are either ineffective or have limited activity against intracellular pathogens[84] as they face difficulty in penetrating eukaryotic cells. Extracellular bacteria are generally more accessible to antibiotics and immune defenses compared to intracellular bacteria, which are sheltered within host cells like macrophages[87]. The immune response to extracellular bacteria involves recognition by pattern recognition receptors, phagocytosis, inflammation and antibody-mediated destruction. AMR complicates this process by allowing bacteria to evade immune detection and resist treatments, diminishing the effectiveness of both the immune system and antibiotics. This interplay between immune defense and resistance highlights the challenge of treating infections and underscores the need for new strategies to manage resistant strains.

New Strategies for Combatting Intracellular and Extracellular Bacterial Infections

New approaches to combat both intracellular and extracellular bacterial infections are being explored, focusing on improving drug delivery into cells, inhibiting bacterial efflux mechanisms, and developing combination therapies. These strategies aim to enhance treatment efficacy against infections caused by bacteria in diverse environments within the body.

Improved Membrane Permeability and Antibiotic Concentration: Increasing the permeability of membranes and the intracellular accumulation of antibiotics is crucial for developing potent agents against intracellular pathogens. One promising approach involves the use of cell-penetrating peptides which can either be naturally occurring or artificially created peptide sequences. These peptides are known for their ability to selectively target and enter cells, cross lipid membranes, and accumulate in intracellular compartments[88]. A study demonstrated that a "metaphilic" cell-penetrating polypeptide-vancomycin conjugate efficiently eliminate intracellular bacteria both *in vitro* and *in vivo* through a dual mechanism, combining enhanced cellular penetration with potent bactericidal action[89]. Antimicrobial peptides (AMPs) represent another promising strategy for combating both intracellular and extracellular bacterial infections. AMPs are characterized as short peptides, usually under 50 amino acids in length, most of which carry a positive charge at neutral pH and contain approximately 50% hydrophobic amino acids[90]. The antibacterial effects of AMPs typically involve strong membrane disruption and pore formation, leading to leakage of cellular contents. Furthermore, AMPs can penetrate and interact with intracellular molecules within bacteria, thereby

Table 1: Overview of antibiotic classes: their mode of action, resistance mechanisms, their antibacterial activity against different bacteria.

Antibiotic class	Examples	Mode of action	Mechanism of Resistance	Antibacterial activity against
Aminoglycosides	Amikacin Gentamicin Plazomicin	attach tightly to the A-site of the 16S rRNA on the 30S ribosome, error-prone protein synthesis, which can further disrupt cell processes and damage the cell membrane[96].	Enzymatic drug modification[97],16S rRNA methylation[98], efflux-mediated resistance[99]	carbapenem-resistant Enterobacteriaceae (CRE), *P.aeruginosa* , *M. tuberculosis*, *M. fortuitum*[96].
Penicillins	Amoxicillin Dicloxacillin Meticillin Nafcillin	Disrupt peptidoglycan crosslinking by targeting peptidoglycan-binding proteins, finally causing cell lysis[100].	Synthesis of β-lactamases[101], Alteration of penicillin-binding proteins[102], enhanced efflux and decreased permeability[101]	Staphylococci, *Corynebacterium diphtheria, Haemophilus influenzae, Escherichia coli, Salmonella*, and *Shigella*[101].
Cephalosporins	Cefotaxime Ceftazidime Ceftriaxone	Disrupt peptidoglycan crosslinking by targeting peptidoglycan-binding proteins, finally causing cell lysis[117].	Mutations in penicillin-binding proteins, production of extended-spectrum β-lactamases[117].	*Staphylococcus aureus, Streptococcus pyogenes, H. influenza, P. aeruginosa Neisseria meningitidis, and S. pneumonia*[121].
Macrolides	Azithromycin Clarithromycin	They hinder protein translation by targeting the 23S rRNA within the 50S ribosomal subunit, resulting in truncated polypeptide chains[103].	Modifications at the target site, such as methylation or mutation hinder the antibiotic rom binding to its fibosomal target[104], efflux mediated[104], and drug inactivation[105].	*Helicobacter pylori, Staphylococcus, Streptococcus, Mycoplasmas, Bordetella pertussis* and *Neisseria meningitis*[122]
Fluoroquinolones	Ciprofloxacin Tosufloxacin	Inhibit DNA replication by targeting DNA gyrase and topoisomerase IV[109]	By mutations in gyrase and topoisomerase IV, efflux pump mediated, and expression of Qnr proteins[109].	*P. aeruginosa, S. pneumonia, Legionella, Chlamydia, Mycoplasma, H. pylori*[110]
Lincosamides	Clindamycin	They disrupt protein translation by specifically targeting the 23S rRNA of the 50S ribosomal subunit, leading to the truncated production of peptide chains	Via methyltransferases that alter the structure of 23S rRNA[107], efflux and drug inactivation (108).	group A and B streptococci, *Streptococcus pneumonia, Chlamydia trachomatis*[106]
Polymyxins	Colistin_IV	Binding to lipidA, which leads to impairment of LPS structure and cell death[111].	Lipid A modification and inactivating genes involved in Lipid A biosynthesis[111].	*Pseudomonas, Acinetobacter* and *Enterobacterales*[111]
Tetracyclines	Doxycycline	Bind to the 30S ribosomal subunit's 16S rRNA to inhibit translation by impeding tRNA binding at the A site[112].	Efflux pumps, ribosome-protecting proteins, drug inactivation, mutations within ribosome structure, and decreased permeability of drug[112].	*Yersinia pestis, Bacillus anthracis, Rickettsia*, MRSA, vancomycin-resistant entero-cocci, tetracycline-*resistant S. pneumoniae (*28).

Table 1 contd....

Antibiotic class	Examples	Mode of action	Mechanism of Resistance	Antibacterial activity against
Phosphonics	Fosfomycin_IV	Inhibits peptidoglycan biosynthesis by inactivation of MurA enzyme[113].	Mutation in the target protein, reduced drug permeability, overproducing the target, and drug modification enzymes[113].	*Proteus mirabilis*, *Klebsiella pneumoniae*, *E. coli* MDR *P. aeruginosa* and *A. baumannii*[124].
Carbapenems	Imipenem/ cilastatin Meropenem	Inhibit peptidoglycan crosslinking by targeting peptidoglycan-binding proteins, finally causing cell lysis[114]	β-lactamases, mutations in Penicillin-binding proteins, efflux mediated and alteration of porin protein[114]	*P. aeruginosa*, *Acinetobacter baumannii*, *Mycobacterium tuberculosis*, MRSA, VRSA[114].
Oxazolidinones	Linezolid Tedizolid	Hinder translation by binding to the 23S rRNA of the 50S subunit, thereby preventing the formation of a functional 70S ribosome[115].	Mutation within 23S rRNA, ribosomal protection by ABC-F proteins[115].	*S. pneumoniae*, (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci[125]
Glycopeptides	Vancomycin	They prevent the synthesis of peptidoglycan by binding to D-alanyl-D-alanine in the peptide chain, thereby inhibiting crosslinking[116].	Intrinsic resistance within Gram-negative bacteria, and in Gram-positive bacteria resistance by modifying peptidoglycan precursor molecules[116].	MRSA, *Clostridium difficile*[126]
Sulphonamides	Sulfamethoxazole	They inhibit dihydropteroate synthase, halting dihydrofolic acid synthesis and cell proliferation[118].	Resistance by gene sul1/2, which encodes sulphonamide-resistant forms of dihydropteroate synthase enzyme, mutation in *fol*P gene[119].	*Klebsiella*, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter* species[118].
Chloramphenicol	Amphenicols	Block translation by attaching to the A site of the 50S ribosomal subunit, thereby inhibiting the synthesis of proteins[120].	Efflux-mediated, changes in the 23S rRNA of the 50S ribosomal subunit due to mutations, and enzymatic deactivation of the drug by acetyltransferases[120].	*Haemophilus Influenza*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Rickettsia* and *Salmonella enterica*[127].

blocking DNA, RNA and protein synthesis[91]. An example of an AMP that can penetrate mammalian cells is cathelicidin LL-37, which is naturally produced by human skin. Research has shown that LL-37 is effective against both intracellular and extracellular *S. aureus*[92].

Drug Carrier: Drug carriers such as liposomes, nanoparticles have been studied for delivery of antibiotics. Liposomes are rounded structures varying in size from 20 nanometers to several micrometers, comprising one or multiple layers of lipid membranes enclosing aqueous compartments[93]. These are capable of transporting both hydrophilic and hydrophobic drugs. Hydrophilic drugs like aminoglycosides are enclosed within their aqueous compartment, while hydrophobic drugs such as penicillin tend to bind to or integrate into the lipid bilayer[36]. Encapsulating antibiotics in liposomes or nanoparticles may provide an effective method for enabling the drug to penetrate the systemic circulation for example via the alveolar-capillary barrier, leading to its distribution in various organ tissues, or for direct delivery to the lungs[94]. A study demonstrated the use of poly(lactide-co-glycolide)

acid (PLGA) to develop gentamicin-loaded nanoparticles for treating *in vitro* and *in vivo* infections caused by the intracellular pathogen *Brucella spp.*[95]. The potential of these technologies extends beyond simple drug delivery; they can also improve the therapeutic efficacy and reduce the side effects of antibiotics by enabling targeted delivery to infection sites and minimizing systemic exposure. Recent clinical trials are exploring the efficacy of these technologies. The ongoing studies are assessing liposomal formulations of antibiotics like amikacin for treating multidrug-resistant tuberculosis and the use of polymeric nanoparticles for localized antibiotic delivery in chronic wound infections. However, several challenges and limitations must be addressed. Toxicity is a concern, as some materials may trigger immune reactions or cause cell damage. The development process is costly, especially for advanced designs, and regulatory approval is often delayed due to the need for thorough safety evaluations. Furthermore, the stability of these formulations during storage and transport can impact their clinical viability, necessitating ongoing research to optimize formulations.

Conclusion

In conclusion, microorganisms utilize a wide range of mechanisms to overcome the inhibitory and lethal effects of antibiotics, complicating the dynamics of resistance through their interactions with environmental factors and host-pathogen relationships. To address this urgent challenge, it is crucial to implement innovative strategies that encompass enhanced drug delivery systems, the development of antibiotic potentiators, and the investigation of novel antimicrobial alternatives. For intracellular bacteria, efforts should focus on discovering natural or synthetic compounds that inhibit resistance mechanisms or improve membrane permeability. Achieving effective penetration of these compounds into bacterial cells while protecting host cell membranes will necessitate a deep understanding of drug structure-activity relationships and substantial advancements in novel drug design. Conversely, for extracellular bacteria, research must target the disruption of biofilm formation and increasing their susceptibility to existing antibiotics, as these factors present significant treatment challenges. Expanding research on antibiotic alternatives such as phage therapy and gene editing tool like CRISPR could revolutionize chronic and resistant bacterial infections. Additionally, policy initiatives should encourage collaboration among researchers, healthcare providers, and policymakers to develop guidelines that promote responsible antibiotic use and secure funding for AMR research initiatives aimed at both bacterial types.

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