REVIEW ARTICLE

Epigenetic Regulation of Antibiotic Resistance in Bacteria

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ABSTRACT

Objective: The objective is to understand how epigenetic mechanisms, such as DNA methylation and nucleoid-associated protein modifications, which regulate gene expression without altering the underlying DNA sequence, are associated with persistence and antibiotic resistance phenotypes in bacteria, which enable them to evade host immune responses and resist antimicrobial agents. The study aims to understand how epigenetic processes that control bacterial gene activity without changing DNA, help bacteria survive antibiotics and avoid immune system attacks.

Methodology: Peer-reviewed research articles, systematic reviews, and meta-analyses published between 1996 and 2024 were prioritized for inclusion, along with earlier foundational studies where necessary. Global health reports from organizations such as WHO and CDC were also consulted for epidemiological context. The keywords, *"bacterial epigenetics," "DNA methylation," "histone-like protein modification," "nucleoid-associated proteins," "RNA regulation," "epigenetic inheritance," "antibiotic resistance,"* and *"antimicrobial tolerance"* were used to search literature.

Results: This review finds that while the well-researched genetic factors are major influencers of bacterial resistance, genetic factors alone do not fully determine virulence due to the growing number of resistant strains. Epigenetic mechanisms also contribute by regulating gene expression without introducing permanent mutations. Rapid bacterial adaptations to antibiotic environments, and the transmission of resistance-associated phenotypes to daughter cells, have been shown to persist in some bacterial species across multiple generations under sustained selective pressure. These findings suggest that incorporating epigenetic targets into existing antimicrobial treatment strategies may improve therapeutic outcomes against resistant bacterial infections. They highlight that this dual-targeting approach may reduce the likelihood of pathogen adaptation, as it simultaneously disrupts multiple resistance-associated mechanisms available for the cell to defend itself. The practical implications of these systems could potentially lead to a decrease in the global recurrence of resistance cases.

Conclusion: Targeting bacterial epigenetic mechanisms, either through inhibitors of methyltransferases and other regulatory enzyme, or through epigenome editing tools in combination with existing antibiotics, represents a promising way to enhance antibiotic efficacy and reduce the emergence of resistance.

Keywords: Antibiotic resistance, antimicrobial strategies, bacterial pathogens, epigenetics, gene regulation

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INTRODUCTION

Antibiotic resistance has emerged as a global modern medicinal challenge primarily due to bacterial evolution.[1] Antibiotic resistance refers to the ability of bacteria to survive or proliferate in the presence of antibiotic concentrations that would otherwise inhibit their growth or cause cell death. Bacterial cells adapt to the presence of these drugs, reducing their effectiveness and contributing to the increasing prevalence of difficult to treat bacterial infections in the population, which threatens the health of millions of people and livestock. The 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report, drawing on data from 76 countries, highlighted the widespread nature of this resistance[2]. It reported 42% third-generation cephalosporin-resistant *Escherichia coli (E. coli)* and 35% methicillin-resistant *Staphylococcus aureus*, alongside an increase in resistant *Klebsiella pneumoniae (K. pneumoniae)* strains, and decreased antibiotic susceptibility in *E. coli* isolates associated with urinary tract infections[3]. The ability of bacterial cells to adapt to antibiotics has widely been linked to genetic factors, primarily horizontal gene transfer (HGT) and genetic mutations[4]. In HGT, cells undergo theoretically similar processes by the names of conjugation, transformation, and transduction which refer, respectively, to cells acquiring resistance genes

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from other bacterial cells via a 'sex pilus', cells uptaking foreign DNA from their environment, and cells transferring DNA in a process mediated by viruses such as bacteriophages[5]. Besides that, resistance may be built by genetic mutations as bacteria have rapid reproduction rates which cause mutations to accumulate in their DNA[6].

Figure 1 illustrates the major genetic mechanisms underlying antimicrobial resistance in bacterial cells, including horizontal gene transfer, spontaneous mutations, IS element insertions, two-component systems and post-transcriptional attenuation, all of which collectively drive the acquisition and dissemination of resistance.

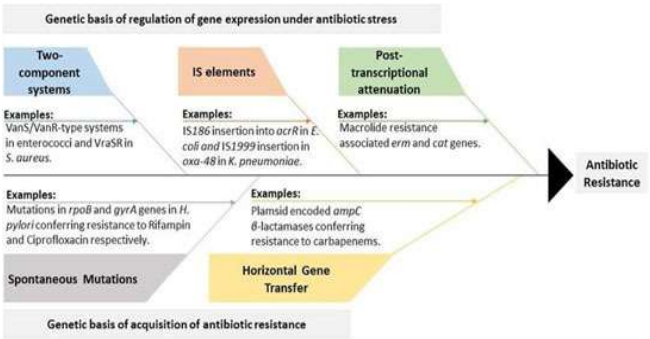

Figure 1: Shows genetic basis of antimicrobial resistance in bacterial cells. Besides mutations and HGT, transcriptional attenuation, a regulatory mechanism in which the elongation or termination of an RNA transcript is modulated in response to cellular signals before transcription is completed. (Adapted from Ghosh et al,[7]).

Purpose of the Review

The purpose of this review is to examine the expanding role of epigenetic mechanisms in shaping bacterial responses to antibiotic exposure. While genetic mutations and horizontal gene transfer are well-recognized contributors to antimicrobial resistance, emerging evidence shows that bacteria also employ non-mutational regulatory pathways to modulate gene expression, enhance survival, and transmit adaptive traits across generations. This review aims to synthesize current knowledge on how epigenetic mechanisms such as DNA methylation, nucleoid-associated protein (NAP) modifications, nucleoid remodeling, and RNA-mediated regulation contribute to virulence, persistence, and the development of resistance phenotypes. By integrating insights from molecular microbiology, genomics, and clinical research, the paper seeks to highlight underexplored pathways that may serve as novel diagnostic markers or therapeutic targets. Ultimately, the review intends to encourage the incorporation of epigenetic perspectives into conventional antimicrobial approaches to better address the global rise in resistant pathogens.

METHODOLOGY

This review employs a narrative review methodology with a defined search and inclusion protocol, drawing on secondary sources across major scientific databases, to consolidate evidence on epigenetic regulation in bacterial antibiotic resistance. A comprehensive literature search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, Google Scholar, and PakMediNet with the search last updated in March 2024.

Boolean search strategies were applied using combination of keywords such as "bacterial epigenetics," OR "DNA methylation," OR "histone-like protein modification," OR "nucleoid-associated proteins," OR "RNA regulation," OR "epigenetic inheritance," AND "antibiotic resistance," OR "antimicrobial tolerance". An example search query used in PubMed was "bacterial epigenetics," OR "DNA methylation," OR nucleoid-associated proteins," AND "antibiotic resistance," OR "antimicrobial tolerance". The search strategy was adapted for each database to account for indexing differences. Filters were applied to include English-language, peer-reviewed articles including research articles, systematic reviews, and meta-analyses.

Studies published between 1996 and 2024 were prioritized for inclusion, along with earlier foundational studies where necessary. The starting point of 1996 was selected to capture early foundational work in bacterial gene regulations and the emergence of epigenetic concepts in microbiology, which gained increasing scientific attention during the late 1990's. Global health reports from organizations such as WHO and CDC were also consulted for epidemiological context.

Inclusion criteria encompassed studies that

1. investigated epigenetic mechanisms in bacteria;
2. examined their relationship to antibiotic resistance, persistence, or virulence; and
3. provided molecular, genetic, or biochemical evidence relevant to epigenetic regulation.

Studies were excluded if they

1. focused exclusively on eukaryotic epigenetics;
2. lacked mechanistic insights or empirical data; or
3. were non-English publications.

Firstly, abstracts of collected articles were reviewed for appropriateness. Studies with fewer than 10 subjects, unpublished abstracts lacking complete data, editorials, and review articles were excluded. All selected sources

were critically evaluated for scientific rigor, relevance and clarity. Where heterogeneity was encountered in study design, bacterial species examined or outcome definitions were interpreted cautiously and differences noted in the narrative synthesis. Extracted findings were thematically categorized into major areas like DNA methylation systems, RNA-based regulation, chromatin-associated proteins, epigenetic memory and inheritance, and therapeutic implications to create a cohesive synthesis of current knowledge. Meta-analysis was not possible because of the heavy heterogeneity of the selected studies.

Epigenetics and Mechanisms

In recent years, interest has grown in investigating mechanisms that regulate gene expression without altering the underlying DNA sequence, contributing to antibiotic resistance phenotypes in bacterial pathogens. This is due to the increasing prevalence of bacterial strains which are unaffected by the action of antibiotics and due to the fact that genetic changes alone are proving to be incapable of fully explaining the rapidity of the cell's response to developments in its environment[8].

When bacteria are exposed to sub-inhibitory concentrations of an antibiotic, they may gradually develop adaptive responses. These responses allow the cells to tolerate increasing drug concentrations without the acquisition of permanent genetic mutations, but as soon as the antibiotic is removed, they revert back to their susceptible phenotype, once again becoming a target for the drug. This phenomenon is termed adaptive resistance, defined as an auto-regulated process characterized by induction of resistance upon drug exposure and reversion to a susceptible phenotype upon its removal.[9]

These observations suggests that the bacterial cell is capable of large-scale adaptation without any significant genetic changes and the swift transition displayed by the cell also highlights the level of flexibility it shows, which contrasts with traditional views of genetic mutations leading to permanent changes, hence suggesting that there may be different factors such as epigenetics also influencing the development of resistance.

As illustrated in Figure 2, adaptive resistance is a reversible, epigenetically mediated process in which bacteria exposed to sub-inhibitory antibiotic concentrations gradually develop tolerance, only to revert to their susceptible phenotype upon removal of the antibiotic, underscoring the role of non-mutational mechanisms in resistance development.

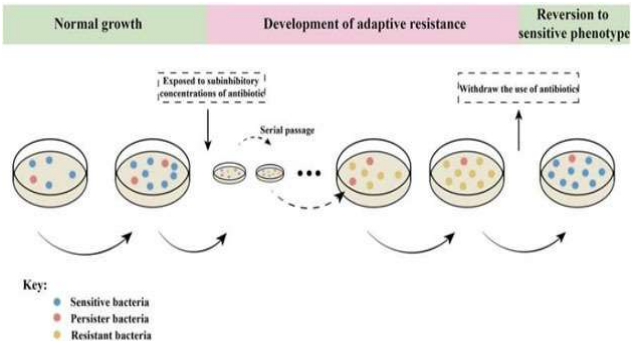

Figure 2: Adaptive Resistance is a term used to define the rapid nature of bacterial cell adaptations to its environment. *(Adapted from Wang et al,[10]).*

Epigenetics refers to the study of reversible changes in gene expression occurring without the permanent alteration of the underlying DNA sequence. It is a relatively newer method of understanding antibiotic resistance and could potentially offer therapies for existing antibiotic drugs to increase their lifespan. In bacterial cells, epigenetic changes occur through various ways such as efflux pump expression, DNA modifications, histone (histone-like proteins) modifications, and non-coding RNAs.

Efflux Pumps

Efflux, defined as the flowing out of a substance or particle, is an action carried out by efflux pump proteins in eukaryotic and bacterial cells, to remove unwanted substances from inside the cells. The expression of efflux pump proteins is controlled by operons, a group of cells transcribed together and controlled by a single promoter, and regulated by repressors and activators which are tasked with limiting and activating gene transcription[11]. Repressors and activators auto-regulate their transcription by binding to the promoter region[8]. When antibiotics are not present, the operon is expressed at low levels because repressor molecules have a binding affinity which is at least four times higher than that of activator molecules[12,13]. This limits the pump's expression.

When antibiotics enter the cell through membrane porins, they may act as inducer molecules, binding to a transcriptional repressor and preventing it from associating with the promoter region. This derepression permits RNA polymerase to initiate transcription of resistance-associated genes, including those encoding efflux pump activators or modifying enzymes. Owing to the activators' ability to express itself, its concentration multiplies along with the production rate of the efflux pumps, the activators also reduce the expression of membrane porins, which disallow antibiotics from entering the cell[8].

To date, six major families of efflux pumps have been discovered in bacteria. Namely, major facilitator superfamily (MFS), resistance nodulation division

(NDS), small multidrug resistance (SMR), ATP binding cassette transporter (ABC), multidrug and toxin extrusion (MATE), and proteobacterial antimicrobial compound efflux (PACE)[14,15]. Some of these are specific to their substrate while others can carry various, structurally dissimilar molecules.

Acinetobacter baumannii (A. baumannii) is an opportunistic bacterial pathogen frequently associated with healthcare-associated infections in immunocompromised individuals[16]. It yields a 29% to 73% mortality rate and is an important health concern for hospitals worldwide, with the Infectious Diseases Society of America classifying it as one of the six most resistant microorganisms on the planet[17,18]. This bacterium causes an infection which has an approximate incidence rate of 100,000 cases annually, globally and is associated with multiple classes of efflux pumps: MFS, RND, MATE, PACE, ABC, and SMR, all of which contribute to its growing resistance to antibiotics[19,20].

Figure 3 depicts the six major efflux pump families - MFS, RND, MATE, PACE, ABC, and SMR - and demonstrates the movement of their substrates across the inner and outer membranes of both gram-positive and gram-negative bacteria, highlighting the structural diversity that enables multi-drug efflux across bacterial species.

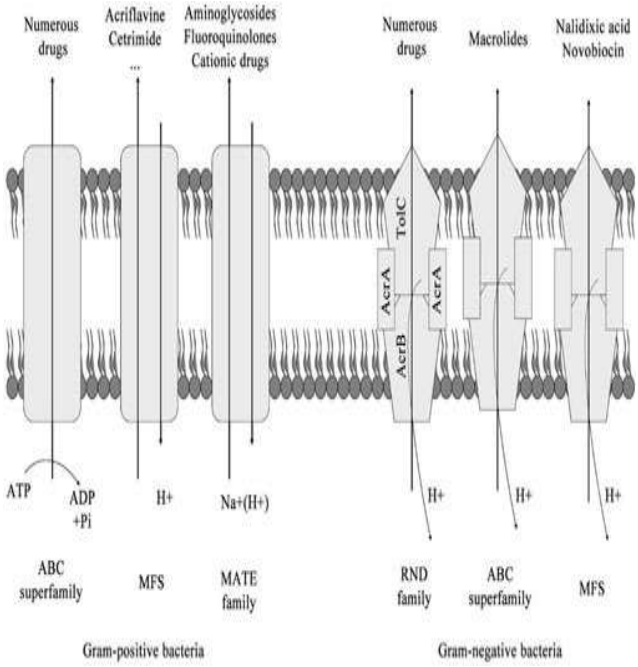

Figure 3: Shows six major families for both gram-negative and gram-positive bacteria and movement of their substrates across the inner and outer membrane (Adapted from Soto[16])

Out of these, the RND family pumps (AdeABC, AdeIJK, AdeFGH) are found to be the most clinically relevant as they are present in multiple resistant A. baumannii strains and are related to resistance to antibiotics such as aminoglycosides, chloramphenicol, tetracycline, erythromycin, and tigecycline lactams[21].

DNA Modifications

DNA Methylation: DNA methylation in prokaryotes involves the covalent addition of a methyl group to specific bases at the N6 position of adenine (yielding N6-methyladenine) or the C5 position of cytosine (yielding 5-methylcytosine), catalyzed by DNA methyltransferases (MTases)[22].

In bacterial cells, methylation can influence various factors such as virulence, cell cycle and growth, genome defense, etc. The restriction-modification (R-M) system is a well-studied model for investigating the role of DNA methylation in bacterial defense and its potential contribution to antibiotic resistance.

R-M systems are the bacterial cell's defense mechanisms that work by differentiating between self and foreign DNA through DNA methylation[23]. They consist of two components: restriction enzymes (REases) which cut foreign (unmethylated) DNA, and MTases which methylate specific sequences in the DNA, to prevent the cleavage of self-DNA (Stoddard Lab)[24]. Methylation in this system plays an important role in influencing HGT, phase variation and virulence, and persistence, all of which contribute to the cell's resistance. The methylation of R-M system, controls the uptake of foreign DNA, including plasmids which carry antibiotic resistance genes. When an incoming plasmid carries methylation patterns recognized by the host methyltransferase, it evades restriction endonuclease (REase) cleavage and is stably maintained, allowing the resistance genes it encodes to be expressed within the bacterial cell[25]. Furthermore, this process also influences the bacterial cell's responses to stress factors like antibiotics by regulating the action of persistence cells (discussed further in section 'Phenotypic Heterogeneity'), and forming biofilm, which is a cluster of bacteria encased in a slime-like coating, which increases the cell's resistance by decreasing permeability[26].

Non-Coding RNA: Non-coding RNAs (ncRNAs) are a structurally and functionally diverse class of RNA molecules that do not encode proteins, but play important roles in regulating gene expression at the transcriptional and post-transcriptional levels in both eukaryotic and prokaryotic cells. Certain ncRNAs bind to target mRNA transcripts, influencing their stability and translational efficiency by targeting transcripts that have a complementary base pairing[27]. In bacterial cells, small RNA (sRNA) carries out this function, affecting cellular responses to environmental stimuli. An example of such regulation in bacteria can be observed in the activities of the sRNA, MicF, a stress-induced gene present in E. coli and similar cells. It post-transcriptionally regulates OmpF expression by base-pairing with the ompF mRNA, thereby inhibiting its

translation and promoting its degradation. OmpF encodes an outer-membrane porin involved in antibiotic uptake[28]. This inhibition leads to reduced ompF levels which, in turn, decreases the permeability of the cell membrane, limiting the entry of certain external compounds into the cell such as antibiotics.

Table 1 summarizes the major categories of bacterial epigenetic modifications, including DNA and RNA-based mechanisms, the enzymatic systems responsible for each, their biological functions and representative examples across bacterial species, providing a consolidated reference for the mechanistic diversity discussed in this review.

Table 1: Summary of bacterial epigenetic modifications through employing various RNA and DNA modification methods (Adapted from Wang et al,[28])

Modifications	Type	Enzymatic Systems	Functions	Examples
DNA	Methylation	R-M Systems	Defense mechanism, regulate gene expression, virulence, biofilm formation	M.EcoGII. ModS, ModM, ModA, M.HpyIII, M2.HpyAII
	Phosphorothioation	Orphan Methyltransferases	Maintain EcoRII Plasmid stability, DNA repair, chromosome replication, Adenine and Cytocine Methyltransferases cause regulation of cell cycle	Dam, CcrM, Dcm, VchM, YhdJ.
		DNA Degradation	Defense mechanism, Oxidative stress, balance intracellular redox homeostasis, influence the transcriptional efficiency	dndABCDEFGH
RNA	Methylation	N^6– Methyladenosine Modification, N^1-Methyladenosine modification, 2 – Methylthiocytidine, modification, 5 – Methylcytosine modification	Regulate RNA stability, localization, transport, splicing, antibiotic resistance and translation	RlmF, RlmJ, RlmCD
	Non-Coding RNAs	Suppress or activate translation	Prevent RNA degradation	Fino/ProQ family, CsrA/RsmA family, Omp/ACF, MicACF

Phase Variability: Dam methylase (DNA adenine methyltransferase) is a well-characterized methyltransferase that adds methyl groups to adenine residues at GATC sequences throughout the bacterial genome. Methylation of promoter-proximal GATC sites can modulate transcriptional activity in a context-dependent manner either facilitating or impeding transcription factor binding and RNA polymerase recruitment, thereby enabling or restricting gene expression.

The modulation of genes in the aforementioned context is referred to as phase variability, a reversible and heritable process some bacteria undergo to adapt to the changes in their environment. *Neisseria meningitidis (N. meningitidis)* has phase variable MTases (mods) which are key epigenetic factors influencing the cell's gene expression, virulence, and immune evasion. The methylation of the promoter region impacts gene expression, altering bacterial phenotype and the interaction of the cell with its host[29]. The modA11 phasevarion regulates the expression of those genes involved in virulence and immune evasion, allowing the cell to adapt to the changes in its environment by evading immune defenses[30].

Type 1 fimbriae are hair-like protrusions present on Escherichia coli (E. coli) which act as links to host epithelial cells and are a crucial factor for the bacterium's virulence. Their expression is controlled by an inverted DNA element called fimS which contains promoters for the genes encoding the fimbrial subunits and is hence credited with being responsible for meditating the phenomenon of phase-variation in Type 1 fimbriae. As shown in Figure 4, fimS functions as a molecular switch controlling Type 1 fimbrial expression in E. coli; when the invertible promoter element is oriented in the 'ON' position, transcription of the fimbrial subunit genes is active, while reversal to the 'OFF' orientation silences their expression, exemplifying epigenetically regulated phase variation without any alteration to the underlying DNA sequence.

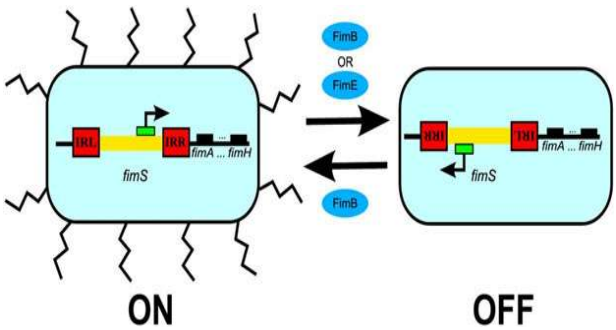

Figure 4: Shows position of fimS in bacterial cells (E. coli). FimS contains a promoter used to encode subunits like fimA and fimH (shown) and is in between two repeat invert sequences IRL, IRR. When the promoter is positioned correctly (upright) (as shown on LHS) transcription of structural fim genes is possible, enabling the expression of fimbriae (extensions on the surface of the cell). When the promoter is downwards, the system is 'off' (shown on RHS) and no fimbriae are visible. *(Adapted from Kuwahara[33]).*

When fimS is on, the promoter is active and the fimbrial genes are expressed. When it is off, the fimbrial genes remain unexpressed[31]. The applicability of the phenomenon

in this particular context is interesting to note as the expression of fimbrial genes allows E. coli to adapt to different locations in the host body without undergoing significant genetic alterations or permanent mutations.

Salmonella enterica (S. enterica), a gram-negative bacterium, has an outer membrane from which lengths of lipopolysaccharides (LPS) extend. These are large molecules made up of lipids and sugars which protect the cell from chemical attacks and stimulate the innate immune system[32]. The o-antigen present on the furthermost part of the LPS is highly changeable, with different lengths and compositions that influence the bacteria's response to multiple external factors such as the host immune system or the presence of antibiotics[33]. For the o-antigen to have changeable characteristics, it undergoes phase variability during which the expression of genes involved in o-antigen synthesis such as opvAB operon is regulated[34]. The o-antigen, although primarily associated with the evasion of the host immune system has been hypothesized to contribute to antibiotic resistance, as increased o-antigen chain length may reduce outer membrane permeability and promote biofilm formation, potentially limiting antibiotic penetration into the cell; however, this relationship requires further experimental validation.

Figure 5 shows the lipopolysaccharide (LPS) structure of gram-negative bacteria, with the o-antigen chain extending from the outer lipid layer; as described above, phase variability in o-antigen expression in S. enterica is epigenetically regulated through the opvAB operon, contributing to immune evasion and potentially to antibiotic resistance by altering outer membrane permeability.

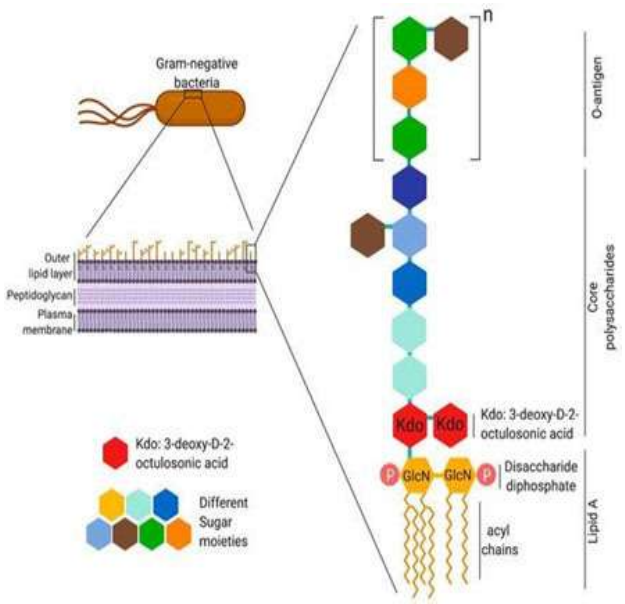

Figure 5: Shows gram-negative bacteria, magnifying the structure liposaccharide chain which is emanating from the outer lipid layer of the bacteria. The outermost part of the chain can be identified as the o-antigen. *(Adapted from Mazgaeen et al*[36])

Phenotypic Heterogeneity

Phenotypic heterogeneity refers to the variation in phenotype observed among genetically identical cells exposed to identical environmental conditions[35,36]. In the context of the bacterial population, this difference in expressed characteristics ensures the survival of the overall population as some individuals may be able to fare better under strenuous conditions such as antibiotics, allowing the species to thrive in the host.

Persister cells are a well-researched example of phenotypic heterogeneity in bacterial cells. These cells represent a small subpopulation of bacteria that enter a transient state of dormancy, enabling them to survive lethal concentrations of antibiotics that would otherwise eliminate genetically identical cells without acquiring permanent genetic changes associated with resistance[37]. Persister cells can emerge from a range of mechanisms, including stochastic processes, where certain conditions favor the formation of these variants[38].

Notably, studies have highlighted that one of the leading causes of persister cell formation is the induced antibiotic pressure on the bacteria, which facilitates individual survival and increases chances for the accumulation of genetic mutations in daughter cells[39]. Persister cells may contribute to resistance evolution by providing a surviving population under antibiotic pressure; however, the relationship between persistence rates and mutation rates is complex and not simply proportional. This association should be interpreted cautiously in the absence of direct evidence.

Furthermore, recent research shows that diversity exists within the population of persister cells as well. This suggests variation in cell responses when the population is exposed to different environmental and stress factors. The complexity of these phenotypic characteristics emphasizes the importance of personalized approaches in antibiotic therapy, as chances of treatment failure could increase if the diversity is left unaccounted for.

Histone Modifications

Histones are proteins, present in eukaryotic cells which help condense DNA into chromosomes. Though histones are absent in bacterial cells, they are replaced by nucleoid-associated proteins (NAPs) which, coupled with DNA methylation systems, can regulate gene expression by altering chromatin structure, thereby impacting the transcription of resistance-related genes[40]. Changes in histone marks could significantly impact the expression of antibiotic resistance genes, demonstrating that specific histone modifications are directly related to the development of resistance mechanisms in bacteria[37].

Bacteria package their DNA into a nucleoid, the bacterial equivalent of chromatin, through the help of NAPs such as HU in E. coli. The acetylation and methylation of NAPs, influence the expression of genes such as resistance genes, in the nucleoid, by either activating them (loosening the chromatin structure) or repressing them (tightening the chromatin structure)[41].

This could regulate the expression of resistance-related genes, influencing the cell's susceptibility to antibiotics.

Clinical Implications

The increasing prevalence of antibiotic-resistant bacterial strains represents a critical challenge in clinical medicine. When a pathogen shows resistance in vivo, current treatment guidelines suggest exploring alternative antibiotic regimens, combination therapy, or agents with broader or differing mechanisms of action. It is well established in the literature that infection with a resistant bacterial strain is linked with higher patient mortality rates. Conventional antibiotic therapy should be complemented by strategies targeting the epigenetic mechanisms that modulate bacterial susceptibility with the goal of sustaining and restoring antimicrobial efficacy[42,43,31].

Inhibition Drugs

Research into drugs designed to inhibit epigenetic modifications in bacterial cells to increase their susceptibility to antibiotics is progressing. It is driven by the findings that antimicrobial resistance is not solely genetic.

Epigenetic modifications which are characteristically reversible, present an important therapeutic opportunity. These drugs primarily operate in three ways[44].

i) by targeting enzymes like MTases or histone deacetylases which could potentially reverse resistance

ii) by altering the chromatin landscape and its associated proteins iii) by enhancing the effects of therapeutic agents to reduce drug resistance and boost the host immune response

By inhibiting enzymes responsible for DNA methylation such as methyltransferases and by disrupting NAP-mediated gene silencing, the expression of genes associated with antibiotic sensitivity may be restored, rendering the bacterial cell susceptible to antimicrobial agents. Combining epigenetic-targeting strategies with host-directed immunomodulatory therapies, may help augment immune-mediated clearance of resistant pathogens, allowing the pathogenic cell to become a target and the host system to fight the infection[45,46].

A promising strategy to administer these drugs to patients is, combining the inhibitors with existing antibiotics to revive older antibiotics already present in the system which are rendered ineffective due to their resistance[47]. This strategy could prolong the effectiveness of ongoing treatments and prove particularly useful when dealing with bacterial strains that are known to be resistant to multiple antibiotics such as K. pneumoniae and A. baumannii[48]. Studies dealing with the impact of natural compounds like epigallocatechin-3-gallate (EGCG) on Staphylococcus aureus show, that these compounds can disrupt cellular processes in the bacteria, which parallels the idea of using inhibitor drugs to alter and restore gene expression for increasing sensitivity to drugs[47].

However, despite this, the challenge remains in fully understanding the complex interplay between these epigenetic mechanisms and their influences on both the microbe and host responses, so off-target effects on host cells could be avoided and the effective delivery of the drug to bacterial cells could be ensured.

Epigenome Editing

Epigenomic editing involves the targeted modification of specific bacterial epigenetic markers such as, DNA methylation patterns at defined genomic loci or NAP-binding sites, to alter the expression of resistance-associated genes. Tools, such as, dCas9-fused effectors, have been investigated for their potential to selectively activate or repress such genes, though robust experimental evidence in bacterial systems remains limited. This method provides a more targeted form of therapy than inhibition drugs as it specifically focuses on a few genes rather than the entire cell.

CRISPR dCas

The CRISPR dCas system is a modified version of the CRISPR Cas system. In it, the Cas9 enzyme is catalytically inactivated (dCas9) by mutations in its nuclease domains, abolishing its ability to cleave DNA, while retaining its guide RNA-directed DNA-binding capability[44]. This system, when coupled with epigenetic regulators, can be programmed to target specific resistance-related genes in the bacterial cells and influence their expression[46].

While this approach offers greater target specificity, a significant challenge lies in developing effective delivery mechanisms. CRISPR-dCas systems are large macromolecular complexes, that face difficulties penetrating the bacterial cell envelope. Furthermore, bacteria harbor endogenous defense mechanisms including restriction-modification systems and CRISPR-based immunity that can recognize and degrade foreign nucleic acids, limiting the intracellular persistence of delivered constructs. The extent of these barriers varies considerably across bacterial species and warrants species-specific evaluation[47]. One delivery mechanism, genetically engineered bacteriophages, shows some promise and is currently being researched[48].

Bacteriophages can serve as delivery vectors for CRISPR-dCas constructs, exploiting their natural ability to infect bacterial cells and introduce foreign nucleic acids. Their ability to solely infect bacterial cells makes them an ideal carrier in cases of precision therapy because they do not harm human cells or beneficial microbiota.

However, even if the construct successfully traverses bacterial defense systems including restriction-modification barriers and endogenous CRISPR immunity, which may recognize and eliminate it as a foreign entity, it would still face challenges in reaching the target and evading additional bacterial defense responses[49].

Although innovations in biotechnology have enabled researchers to successfully modify previously existing medical techniques and use them to regulate the epigenetic mechanisms in bacteria, in-depth research still needs to be conducted in this field to perfect these techniques and make them suitable for clinical practice.

CONCLUSION

The emerging and rapidly growing crisis of antibiotic resistance calls for a deeper understanding of bacterial adaptation mechanisms, with epigenetic regulation emerging as a crucial factor. This paper has explored how DNA modifications, efflux pumps, phenotypic heterogeneity, and histone modifications, contribute to bacterial survival under antibiotic stress. Targeting these epigenetic mechanisms through inhibition drugs or epigenome editing to suppress enzyme function, alter DNA and gene expression, alongside administering preexisting antibiotics, presents a promising strategy to enhance antibiotic efficiency and reduce resistance. However, the development of precise therapeutic interventions remains a challenge, as bacterial adaptability and the risk of off-target effects continue to limit clinical translation. Detailed research and medical trials are needed to provide researchers with a well-rounded understanding of the interplay between genetic and epigenetic resistance mechanisms. Such insights may aid the development of medical techniques capable of controlling epigenetic changes and inform the rational design of next-generation antimicrobial strategies targeting both genetic and epigenetic determinants of resistance.

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Authors' Contribution: SFZ conceptualized the review, conducted literature search and study selection, performed data synthesis and interpretation, and wrote and revised the manuscript.

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