Elucidating the role of Acetylation in Antimicrobial Resistance Mechanisms of Pathogenic *Escherichia coli*

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Abstract

Antimicrobial resistance (AMR) pathways in pathogenic bacteria are among the many cellular functions that are regulated by the well-known post-translational modification acetylation. Because acetylation can pick up and disseminate the resistance and regulation of gene expression, it plays a complex role in altering antibiotic resistance pathways in *Escherichia coli (E. coli),* which is a major public health concern. The acetylation patterns of key proteins are linked to antibiotic resistance to gain insight into the molecular mechanisms by which *E. coli* adapts to and resists antibiotic treatments. Researchers emphasize how these enzymes affect microbial cellular functions and antibiotic response abilities and their mechanisms for modifying metabolically significant proteins. Research approaches to combat bacterial antibiotic resistance gain new possibilities after scientists gain comprehension of these acetylation-mediated regulatory networks. This chapter focuses on acetylation since researchers view it as a vital regulatory point while reviewing recent studies alongside novel findings and successful treatments that aim to minimize *E. coli* strains resistant to antibiotics.

Keywords: *E. coli,* Pathogenic bacteria, Antimicrobial resistance, Post-translational modification, Antibiotic susceptibility, Acetylation

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Introduction

The global health crisis of antibiotic resistance strongly affects third-world countries because bacteria demonstrate resistance to medications used for eradication or controlling proliferation. The problem arises from improper waste and water management practices which Afzal (2017) identifies as a constant source of human contact with dangerous bacteria and pathogens. The continued antibiotic use as selective pressure has triggered the development of resistant bacterial populations which demonstrate multi-drug resistance according to Chen and Jiang (2014). Healthcare systems worldwide maintain stress due to this situation forcing drug-resistant bacterial infections to become harder to treat thus increasing healthcare expenses while requiring more resources (French, 2005). Treatment of antibiotic resistance poses a threat to public health since it is essential for maintaining public health, cutting healthcare expenses, and guaranteeing the efficacy of future antibacterial treatments. The main objective is to recognize why antibiotic resistance occurs within low-resource environments while determining its impact on worldwide healthcare systems. The effective control measures should emphasize better waste and water management together with antibiotic regulation while supporting studies into bacterial resistance development mechanisms (WHO, 2023).

The Global AMR Crisis: A Molecular Perspective

The rise in various drug-resistant organisms is being closely monitored due to the serious clinical implications of infections caused by antibiotic-resistant bacteria (Lerminiaux et al., 2024). The two most popular techniques for molecularly typing antimicrobial-resistant bacteria are whole genome sequencing (WGS), which provides detailed information, and multilocus sequence typing (MLST), which offers high resolution (Ruppé et al., 2023).

Post-translational Modifications and Their Implications in Pathogen Resistance

Chemical alterations on the side chains of amino acids or protein terminuses are known as post-translational modifications (PTMs). A vast variety of proteo-forms can be produced by more than 200 identified PTMs significantly affect many biological processes and the complexity of proteomes (Deribe et al., 2010; Duan and Walther, 2015; Olsen and Mann, 2013). A proteome-wide PTM analysis on the Swiss-Prot protein database revealed that phosphorylation, acetylation, glycosylation, amidation, and hydroxylation were the top five PTMs seen experimentally. However, as described by Khoury et al. (2011), the five most common PTMs are palmitoylation, acetylation, phosphorylation, glycosylation, and methylation. These modifications enhance proteome diversity, allowing proteins to carry out a wide range of essential physiological functions. The biological roles of PTMs, along with their involvement in cancer development, include well-known examples, their impact on protein function, and their contribution to cancer pathogenesis (Zhang & Wei, 2024).

Molecular Basis of Efflux Pump Regulation in AMR

The most significant resistance mechanism employed by resistant bacteria is the efflux pump mechanism, which uses transporter proteins to move drugs from the cells to the outside world (Duarte et al., 2024). The growing necessity for antibiotics has made communities focus worldwide on blocking such efflux pump mechanisms. Medical solutions known as efflux pump inhibitors serve as tools to fight against resistant bacteria by interrupting the resistance pathways created by efflux pumps (Sharma & Piddock, 2023). The authors Munita and Arias (2016) highlighted that these inhibitors originate from natural and synthetic compounds which use specific inhibitory mechanisms to operate.

Antibiotic Target Modifications by Bacterial Enzymes

Transporter proteins from the cellular environment are transferred outside by resistant bacteria to defend themselves. The world shows growing interest in studying efflux pump inhibitions while the requirement for antibiotics continues to rise (Sharma & Piddock, 2023). Efflux pump inhibitors function as useful weapons against microorganisms demonstrating resistance because they disrupt mechanisms that lead to resistance. These inhibitors exist in natural as well as artificial forms and usually adopt specific modes of action (Munita and Arias, 2016). The most important of them are transpeptidases, which target β-lactams, and transglucosylases, whose substrates target glycopeptides.

Lipid Acetylation and outer Membrane Modifications

Lipopolysaccharides accomplish their two primary goals. First, as a defensive mechanism against harsh environmental conditions, LPS is affixed to the exterior bacterial membrane of Gram-negative bacteria. This greatly increases the stiffness and tightness of the outer membrane, which in turn affects how resistant the bacteria are to outside stimuli (Alexander and Rietschel, 2001). Second, across all Gram-negative bacterial species, LPS is one of the most conserved structures. This significant immune response helps animals recognize this PAMP by displaying comparatively conserved molecular structures (Akira and Takeda, 2004). The activation of the host immune system is largely explained by the prompt detection and identifying of LPS from invasive Gram-negative bacteria (Netea et al., 2002). Beyond membrane modifications, internal protein modifications also contribute significantly to antibiotic resistance. While Protein function can be effectively altered by PTM. There are certain changes that can be undone, while others cannot. Because a protein's activity may be up- or down-regulated in response to internal or external stimuli, avoiding the energy-intensive processes of protein breakdown, gene expression, and protein synthesis, reversible changes are particularly beneficial to the cell. Histones from the calf thymus were the first to be found to include acetyl groups on proteins. When *Staphylococcus aureus* culture was not entirely sterilized by penicillin (1% of the population remained intact) (Christensen et al., 2023).

Drug Resistance to Different Antibiotics Regulate by Acetylation

An antibiotic is a chemical substance produced by bacteria that inhibits or kills other germs (Hopwood, 2007; Davies, 2010). Approximately 80% of antibiotics now in use are produced by soil-dwelling bacteria of the genus Streptomyces, which are members of the phylum Actinobacteria (Barka et al., 2016). The discovery of streptomycin marked the start of the heyday of antibiotic research and development, which lasted from 1940 until 1990. To maintain antibiotic resistance, acetylation reduces bacterial metabolism while increasing bacterial motility.

Acetylation Reduces the Metabolic Activity of *E. coli* to Regulate Antibiotic Resistance

Antibiotics are used extensively to treat infections and have greatly decreased illness rates, enhanced life quality and lowering death. Unfortunately, antibiotic-resistant strains are rapidly emerging as a result of regular antibiotic overuse, seriously endangering human health (Baron et al., 2014). Bacteria have developed a variety of defense mechanisms against different antibiotics. The differences in acetylation intensities throughout the whole proteome between antibiotic-resistant Escherichia coli and wild-type (WT) bacteria were investigated. The highly resistant bacterial strains' minimum inhibitory concentration (MIC) was almost 60 times higher than the WT strain's, according to measurements of the MIC (Wang et al., 2023).

Protein Acetylation and the Formation of Persister in *E. coli*

A reversible post-translational alteration that is conserved from bacteria to humans is acetylation of lysine residues (Christensen et al., 2023). Although hundreds of lysine-acetylated proteins have been identified in a variety of bacteria by recent investigations, nothing is known about the physiological significance of these changes (Liu et al., 2024). Protein conformation may be impacted by lysine acetylation as it alters the size and charge of proteins. The impact of protein acetylation on *E. coli* cell survival, protein aggregation, and persister formation using suitable mutant strains lacking in non-enzymatic acetylation and enzymatic acetylation or deacetylation processes. It was discovered that lysine acetylation enhanced cell survival and protein aggregation during the late stationary phase (Wang et al., 2023).

Acetyl-CoA: The central Metabolite in Acetylation Reaction

Acetyl-CoA is a membrane-impermeant molecule made up of an acetyl moiety (CH_3CO) connected by a thioester bond to coenzyme A (CoA), a product of vitamin B5 and cysteine. The chemical structure of Acetyl-CoA makes it easier for the acetyl moiety to move to a range of acceptor molecules, such as amino groups on proteins, since thioester linkages are energy-rich (Shi and Tu, 2015). One possible co-translational process is acetylation (Hollebeke et al., 2012). Acetyl-coenzyme A (Acetyl-CoA) is essential for intermediate metabolism, it is suggested in speculative reconstructions of the start of life that it was involved in ancestral methanotrophic processes carried out by the prokaryotic last common precursor (Nitschke and Russell, 2013).

Lysine Acetylation a Molecular Overview

The hydrophobic side chain of the tiny amphipathic molecule lysine has an extra positively charged amino group linked to it. The evolutionarily conserved PTM of protein acetylation on lysine residues is intimately associated with the physiology and function of cells. One highly controlled posttranslational change that is reversible is lysine acetylation. In addition to playing important roles in basic biology, lysine acetylation and its regulatory enzymes, histone acetyltransferases and histone deacetylases (HDACs), are also strongly linked to aging and several serious illnesses, such as dementia, cardiovascular disease, and cancer (Blander and Guarente, 2004, Carrozza et al., 2003, McKinsey and Olson, 2004). After 10 years of thorough research, less than 90 lysine-acetylated proteins have been identified (Kouzarides, 2000).

Acetylation cross Functional Proteins Classes Enzymes, Structural Proteins, Regulators

A significant post-translational modification, acetylation impacts many different protein types, including regulators, structural proteins, and enzymes. The addition of acetyl groups to lysine residues by acetyltransferases, such as CBP/p300 and Gcn5, can alter the interactions and functions of proteins (Kouzarides, 2000). Acetylation also improves the stability and transcriptional activity of many transcription factors, including p53 and NF-κB, in response to cellular inputs (Gu & Roeder, 1997). Cells must always be alert to changes in their internal and external environments, some of which need prompt action. In these cases, reversible PTMs of proteins can convey conditional changes from sensors to effectors (Wang & Zhang, 2024).

Role of Acetylation in Antibiotic Resistance Mechanisms

A growing public health concern is antibiotic resistance (Christensen et al., 2023). There have been numerous studies on the role that those PTMs play in controlling bacterial metabolism (Zhao et al., 2022). However, it is yet unknown how acetylation is controlled in bacteria that are resistant to antibiotics. Antibiotics are commonly used for bacterial infections to decrease mortality and enhance quality of life. Human health is under peril due to the widespread abuse of antibiotics, which is causing antibiotic-resistant microorganisms to emerge quickly (Laxminarayan & Chaudhry, 2023). Numerous defensive mechanisms against different antibiotics have been developed by bacteria (Munita & Arias, 2022).

Acetylation in the Regulation of β-lactamase Activity

The β-lactamases provide their bacterial hosts with a high level of antibiotic resistance by hydrolyzing the amide bond of the four-membered β-lactam ring. In Gram-negative bacteria, these enzymes are very important since they represent the primary defense mechanism against β-lactam-based drugs (Bush, 2023). As beta-lactamase develops, a number of dangerous bacterial strains with high degrees of resistance to b-lactam medicines appear. Numerous enzymes have been found, exhibiting a broad variety of basic structures and catalytic properties (Tooke et al., 2022). Though they appear to have the same chemical functionalities at the same key places, all known three-dimensional structures of active-site serine b-lactamases have a striking number of similarities (Palzkill, 2023).

Acetylation-mediated Modulation of Efflux Pumps (e.g., AcrAB-TolC)

There are five families of bacterial drug efflux pumps: the resistance-nodulation-division (RND) superfamily, the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, and the small multidrug resistance (SMR) family, which is a subgroup of the drug/metabolite transporter superfamily (Duarte et al., 2024). Particularly important for clinically meaningful resistance in Gram-negative bacteria are drug exporters from the RND family (Sharma & Piddock, 2023). An efflux pump is a proteinaceous transporter machinery system found in the cell membrane of the microorganism. Because of their ability to extrude many medicines into bacteria, either separately or in combination, they are frequently associated with multidrug resistance (Zwama et al., 2023).

Influence of Acetylation on Porins and Permeability Barriers

Gram-negative bacteria have a unique outer membrane (OM) structure composed of β-barrel porins, lipoproteins, phospholipids, and lipopolysaccharides (Henderson et al., 2016). O'Shea and Moser (2008) and Pages et al. (2008) claim that the OM acts as an additional barrier to keep dangerous substances like antibiotics and bile acid from passing through. According to O'Shea and Moser (2008), materials weighing more than 600 Da are often unable to penetrate the envelope of Gram-negative bacteria. The outer barrier of gram-negative bacteria blocks the passage of daptomycin and vancomycin, both of which have molecular weights more than 1400 Da.

Regulation of Acetylation by Sirtuins-like Deacetylases (CobB) and Pat Proteins

Using NAD^+, lysine de-acylase enzymes called Sirtuins create Nitin amide (NAM) and 2-O-acetyl-ADP-ribose. The presence of the first Sirtuins, called Sir2 (Silent informatory regulator 2), in *Saccharomyces cerevisiae (S. cerevisiae)* was identified in the 1970s (Christensen et al., 2023). But its critical role in deacetylase activity and replicative lifespan was not recognized until much later. After Sir2 was identified, more Sir2-like proteins in prokaryotic and eukaryotic species were found; these proteins were called Sirtuins (Yang et al., 2024).

Genome-wide CRISPR Screens to Identify Acetylation Targets in AMR Pathways

The *S. pyogenes* Cas9 system in CRISPR-Cas technology allows researchers to create precise genomic modifications and perform CRISPRi suppression and CRISPRa activation processes for enhanced functional genetic screening methodologies. These methods have been applied in several research to investigate a range of species, including eukaryotes (Bassett et al., 2015; Gilbert et al., 2014) and prokaryotes (Lee et al., 2019). To connect CRISPR techniques with the acetylation regulation of antibiotic resistance, additional investigation is necessary. Genes that regulate acetylation processes, like Pat and CobB, are made accessible by CRISPR editing. The genes under study can be switched on or off using CRISPRi and CRISPRa systems to observe their effects on AMR (Wang et al., 2024). By employing CRISPR, which makes acetylation gene targets accessible, researchers may investigate how acetylation controls bacterial resistance mechanisms. This strategy facilitates the investigation of the molecular connections between acetylation and AMR since it exhibits potential as a therapeutic target in infection resistance strategies. For studies pertaining to AMR and its management, researchers may accurately alter bacterial genomes using CRISPR technology (Li et al., 2023). By modifying the acetylation pathways of genes Pat, CobB, and other acetyltransferase genes, scientists can modify their expression using the CRISPR Toolkit. By controlling these genes, researchers may learn how acetylation influences bacterial resistance patterns and investigate AMR resistance functions made feasible by CRISPR (Doudna et al., 2023).

Acetylation as a Therapeutic Target in AMR

According to some theories, aminoglycosides, streptogramins, fluoroquinolones, and chloramphenicol all employ acetylation, which is the most often utilized technique. It is believed that aminoglycosides are the target of acetylation and phosphorylation. Using therapeutic proteins that actively contribute to the formation and upkeep of biofilms, biofilm populations may be efficiently targeted (Roy et al., 2018). Anti-biofilm drug resistance may not be very likely since focusing on biofilm formation has little influence on bacterial DNA replication or cell division (Saipriya et al., 2020). Synthesis of capsular polysaccharide (CPS), the two-component system, the cyclic-di-GMP (c-di-GMP) signaling pathway, the biofilm-associated protein, and the quorum-sensing pathway.

Drug Synergy: Combining Acetylation Modulators with Traditional Antibiotics

The combination of herbal medicines and phytochemicals with antibiotics and other therapeutically relevant therapy is a novel and effective way to treat MDR (Zhao et al., 2024). Phytochemical substances and antibiotics have been delivered jointly using this technique to prevent the development of resistance. Acinetobacter baumannii is an MDR bacterium that is more vulnerable to the antibacterial effects of aflavin-epicatechin combinations than to either substance by itself (Peng et al., 2023). Similar outcomes were also obtained when curcumin and EGCG were combined. Numerous strategies have been employed to optimize AMP efficacy and their synergistic action with antibiotics (Sharma et al., 2023).

Targeting Sirtuins-like Deacetylases in Pathogens: A Novel Therapeutic Strategy

Sirtuins are categorized as NAD-dependent class III HDACs because they need NAD^+ as a cofactor to deacetylate a range of substrates, such as transcriptional factors and histone and non-histone proteins. Sirtuins govern aging, DNA repair, oxidative stress, cell metabolism, and other physiological functions (Yang et al., 2024). Sirtuins' role in tumor biology has garnered a lot of interest lately due to mounting evidence that they control several abnormal cellular functions inside tumor environments. Recent studies that have focused a lot of attention on the Sirtuins pathway suggest that Sirtuins activator/inhibitors may be used to treat a variety of illnesses, including those caused by protozoa (Baur et al. 2012; Zheng, 2013).

CRISPR-mediated Manipulation of Acetylation Pathways for AMR Control

CRISPR-associated genes (Cas) and clustered, regularly interspaced short palindromic repeats (CRISPR) belong to a broad family of prokaryotic adaptive immune systems that have been used as biological tools and therapeutic treatments (Doudna et al., 2023). The discovery of anti-CRISPR (Acr) proteins, which are protein inhibitors of CRISPR-Cas systems, may result in the creation of more accurate and regulated CRISPR-Cas instruments. Innovative approaches are urgently needed to halt the rise of bacterial diseases and AMR (Forsberg et al., 2024). The CRISPR-Cas system, an adaptive immune system found in many prokaryotes, presents inviting opportunities to accurately and reliably target and change nucleic acids. Reports suggest that by resensitizing bacterial cells to antibiotics, tailored CRISPR-Cas systems may effectively eliminate bacteria or even reverse antibiotic resistance in bacteria (Li et al., 2023).

Conclusion

Many antibiotic-resistant strains have emerged because of antibiotic abuse, posing a severe hazard to human health. Bacterial metabolism is strictly regulated by post-translational changes of proteins, particularly acetylation. Nevertheless, little is known about the overall mechanism governing acetylation in bacterial resistance. The most widely used mechanism is acetylation, which is thought to be used by aminoglycosides, fluoroquinolones, streptogramins, and chloramphenicol. It is thought that acetylation and phosphorylation target the aminoglycosides. The most versatile method is acetylation, which has been utilized to combat aminoglycosides, fluoroquinolones, streptogramins, and chloramphenicol. The stability of peptides can be enhanced by in-natural amino acids (mostly D-form amino acids), cyclization, or alteration of the terminal sections of amino acids by amidation or acetylation

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