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Evaluation of Genetic Mutations and Efflux-Mediated Resistance to Ethambutol in Multidrug-Resistant Mycobacterium Tuberculosis

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Abstract: *Multidrug-resistant tuberculosis (MDR-TB), caused by Mycobacterium tuberculosis (Mtb), remains a major global public health challenge. Ethambutol (EMB), a first-line antitubercular drug, inhibits arabinosyl transferase enzymes involved in mycobacterial cell wall biosynthesis. However, resistance to EMB has become increasingly prevalent among MDR-TB isolates. Resistance develops through two principal mechanisms: genetic mutations in genes associated with the arabinan biosynthesis pathway and overexpression of efflux pump systems that reduce intracellular drug accumulation.*

This review evaluates the molecular basis of EMB resistance with emphasis on mutations in embB, embA, embC, ubiA, and Rv3806c, together with the contribution of efflux pumps such as Rv1258c (Tap), Rv1410c (P55), Rv1819c, and MmpL proteins. Recent molecular diagnostic techniques, including whole-genome sequencing (WGS), line probe assays (LPA), and real-time PCR, have enhanced rapid detection of resistance-associated mutations. Understanding both mutation-based and efflux-mediated mechanisms is essential for improving MDR-TB diagnosis, individualized therapy, and development of novel therapeutic strategies.

Keywords: *Mycobacterium tuberculosis, Ethambutol, MDR-TB, embB mutation, Efflux pump, Drug resistance, Whole-genome sequencing.*

I. INTRODUCTION

Tuberculosis (TB) continues to be one of the world's deadliest infectious diseases despite the availability of effective chemotherapy. According to the World Health Organization (WHO), millions of new TB cases occur annually, with multidrug-resistant tuberculosis (MDR-TB) posing a significant obstacle to disease control. MDR-TB is defined as resistance to at least isoniazid and rifampicin, the two most potent first-line anti-TB drugs. Ethambutol (EMB) is commonly included in first-line treatment regimens because of its ability to inhibit the synthesis of arabinogalactan, a crucial component of the mycobacterial cell wall.

Ethambutol specifically targets arabinosyl transferases encoded by the embCAB operon, disrupting the polymerization of arabinan in the cell wall. Resistance to EMB has traditionally been associated with mutations in the embB gene, particularly at codon 306. However, several studies have demonstrated that embB mutations alone cannot explain all resistant phenotypes, indicating that additional genetic alterations and physiological mechanisms contribute to resistance.

Efflux pumps constitute another important resistance mechanism. These membrane transport proteins actively expel antimicrobial agents from bacterial cells, lowering intracellular drug concentrations below therapeutic levels. Increased expression of efflux pumps has been observed in many MDR-TB isolates and is believed to contribute to low-level or transient EMB resistance before stable chromosomal mutations develop.

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II. MECHANISM OF ACTION OF ETHAMBUTOL

Ethambutol inhibits the synthesis of arabinogalactan and lipoarabinomannan, two major components of the mycobacterial cell wall.

Its primary target includes:

1. Arabinosyl transferase enzymes
2. embCAB operon
3. Arabinan biosynthesis pathway
4. Cell wall integrity

Disruption of cell wall synthesis ultimately inhibits bacterial growth.

III. GENETIC BASIS OF ETHAMBUTOL RESISTANCE

Multiple chromosomal mutations contribute to EMB resistance.

Major genes involved

Gene	Protein Function	Common Mutation	Contribution to Resistance
embB	Arabinosyl transferase	M306V, M306I	High
embA	Cell wall synthesis	Promoter mutations	Moderate
embC	Arabinosyl transferase	Variable	Moderate
ubiA	Decaprenyl phosphate synthesis	Various SNPs	High
Rv3806c	Cell wall biosynthesis	Point mutations	Low–Moderate
iniA	Stress response protein	Upregulation	Associated with drug tolerance

Among these genes, embB306 remains the most frequently reported mutation worldwide.

IV. EFFLUX-MEDIATED ETHAMBUTOL RESISTANCE

Efflux pumps transport antibiotics outside bacterial cells.

Major transporter families include:

1. Major Facilitator Superfamily (MFS)
2. ATP Binding Cassette (ABC)
3. Resistance-Nodulation-Division (RND)
4. Small Multidrug Resistance (SMR)

Important efflux pumps

Efflux Pump	Gene	Family	Role in EMB Resistance
Tap	Rv1258c	MFS	Drug extrusion
P55	Rv1410c	MFS	Multidrug resistance
Rv1819c	ABC transporter	ABC	ATP-dependent transport
MmpL7	mmpL	RND	Lipid transport
EfpA	efpA	MFS	Broad-spectrum efflux

Over expression of these transporters significantly decreases intracellular ethambutol concentration.

V. MAJOR GENETIC MUTATIONS IDENTIFIED IN MDR-TB

Mutation	Frequency (%)	Clinical Importance
embB306	40–70	Major resistance marker
embB406	10–20	Moderate
embB497	8–15	Moderate
ubiA mutations	15–30	High-level resistance
embA promoter	5–15	Increased expression

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Rv3806c	<10	Additional resistance

VI. MOLECULAR DIAGNOSTIC METHODS

Method	Target	Advantages	Limitations
Line Probe Assay (LPA)	embB mutations	Rapid	Limited mutation coverage
Real-Time PCR	Known SNPs	Sensitive	Detects only targeted mutations
Whole Genome Sequencing	Entire genome	Comprehensive	Expensive
Sanger Sequencing	Specific genes	Accurate	Time consuming
Targeted NGS	Resistance genes	High throughput	Bioinformatics required

VII. INTERACTION BETWEEN MUTATIONS AND EFFLUX PUMPS

The interaction between genetic mutations and efflux pump systems represents one of the most important mechanisms underlying the development and progression of ethambutol resistance in multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB). Rather than functioning as independent processes, chromosomal mutations and efflux-mediated drug transport often operate in a coordinated and sequential manner, allowing *M. tuberculosis* to survive prolonged antibiotic exposure and gradually acquire high-level resistance. Ethambutol (EMB) primarily inhibits arabinosyl transferases encoded by the embCAB operon, disrupting the synthesis of arabinogalactan and lipoarabinomannan, two essential components of the mycobacterial cell wall.

Classical studies initially attributed EMB resistance mainly to mutations in the embB gene, particularly at codon 306, but accumulating evidence has demonstrated that these mutations alone cannot explain the diversity of resistance phenotypes observed in clinical isolates. Many ethambutol-resistant strains lack detectable mutations in the emb genes, while some isolates carrying embB mutations remain susceptible, suggesting that additional resistance mechanisms, particularly efflux pump activation, play a significant role in determining the final resistance phenotype.

Efflux pumps are membrane-associated transport proteins that actively expel toxic compounds, including antimicrobial agents, from bacterial cells. These transporters reduce intracellular drug concentrations to sub-inhibitory levels, enabling bacteria to survive under antibiotic pressure. In *M. tuberculosis*, several families of efflux pumps have been identified, including the Major Facilitator Superfamily (MFS), ATP-Binding Cassette (ABC) transporters, Resistance-Nodulation-Division (RND) transporters, and Small Multidrug Resistance (SMR) proteins. Important efflux systems implicated in ethambutol resistance include Rv1258c (Tap), Rv1410c (P55), Rv1819c, EfpA, and members of the MmpL transporter family. These proteins actively export ethambutol and other antitubercular drugs, decreasing their intracellular accumulation and reducing the inhibitory effect on cell wall synthesis. The activation of efflux pumps is often considered an adaptive response that allows bacterial survival during the early stages of antibiotic exposure before stable genetic mutations become established.

The relationship between mutations and efflux pumps is dynamic and complementary rather than mutually exclusive. During the initial exposure to ethambutol, bacterial cells experience physiological stress caused by inhibition of cell wall biosynthesis. In response, regulatory pathways induce the expression of efflux pump genes, leading to enhanced drug extrusion. This transient increase in efflux activity lowers intracellular ethambutol concentrations sufficiently to permit survival of a subpopulation of bacteria.

Because the intracellular drug concentration is reduced, these surviving organisms continue to replicate despite the presence of antibiotics, increasing the probability of acquiring spontaneous chromosomal mutations in genes associated with drug resistance. Consequently, efflux-mediated tolerance provides a window of opportunity during which stable mutations can accumulate through natural selection.

Mutations occurring in the embCAB operon remain the most extensively studied genetic determinants of ethambutol resistance. Among these, mutations affecting embB306, embB406, and embB497 alter the structure of arabinosyl transferase enzymes, decreasing their affinity for ethambutol while maintaining sufficient enzymatic activity for cell

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wall biosynthesis. Additional mutations in embA, embC, ubiA, and Rv3806c further modify the arabinan synthesis pathway and contribute to varying levels of resistance.

When these chromosomal mutations arise in bacterial populations already exhibiting elevated efflux activity, the combined effects substantially increase resistance levels. Efflux pumps reduce intracellular drug exposure, while structural mutations reduce drug-target binding, resulting in synergistic protection against ethambutol.

Several experimental studies have demonstrated that inhibition of efflux pumps can partially restore ethambutol susceptibility in resistant isolates, even in strains possessing emb gene mutations. Efflux pump inhibitors such as verapamil, reserpine, carbonyl cyanide m-chlorophenylhydrazone (CCCP), chlorpromazine, and thioridazine have been shown to increase intracellular accumulation of ethambutol by blocking active transport mechanisms.

When these inhibitors are combined with ethambutol in laboratory experiments, the minimum inhibitory concentration (MIC) often decreases significantly, indicating that active drug efflux contributes substantially to resistance. However, complete restoration of susceptibility is generally not observed in isolates carrying high-level emb mutations, confirming that both mechanisms jointly determine the resistant phenotype. This complementary relationship illustrates that mutations and efflux systems are not competing explanations but rather interconnected components of a multifactorial resistance process.

Gene expression analyses have further strengthened the understanding of mutation-efflux interactions. Exposure of *M. tuberculosis* to sub-lethal concentrations of ethambutol induces significant upregulation of efflux pump genes such as Rv1258c, Rv1410c, and efpA, along with stress-response genes including iniA, iniB, and iniC. The iniBAC operon is activated by agents that interfere with cell wall synthesis and is considered an important adaptive response to antimicrobial stress. Increased expression of these genes enhances bacterial survival under drug pressure and facilitates the eventual selection of resistant mutants. As antibiotic exposure continues, chromosomal mutations become fixed within the bacterial population, producing stable resistance that persists even if efflux activity later decreases. Thus, transient physiological adaptation mediated by efflux pumps often precedes permanent genetic adaptation through mutation.

Whole-genome sequencing studies have revealed that ethambutol-resistant clinical isolates frequently possess multiple resistance-associated mutations together with altered expression of efflux-related genes. Instead of relying on a single mutation, many MDR-TB strains accumulate numerous genetic alterations affecting drug targets, regulatory proteins, metabolic pathways, and membrane transport systems. Mutations in regulatory genes controlling efflux pump expression may also enhance transporter activity, producing constitutive overexpression even in the absence of antibiotic exposure. Such regulatory mutations further strengthen multidrug resistance by continuously lowering intracellular drug concentrations. This complex genetic architecture explains why resistance levels vary considerably among isolates carrying identical embB mutations and why phenotypic susceptibility testing sometimes shows discrepancies with molecular diagnostic results.

The interaction between mutations and efflux pumps has important clinical implications. Diagnostic methods based solely on detection of embB mutations may fail to identify isolates in which resistance is primarily mediated by increased efflux activity. Conversely, isolates carrying emb mutations without significant efflux activation may exhibit lower levels of phenotypic resistance. Therefore, integrating molecular detection of resistance-associated mutations with assessment of efflux pump expression provides a more comprehensive understanding of drug susceptibility. Whole-genome sequencing, transcriptomic analysis, quantitative reverse transcription PCR, and RNA sequencing are increasingly being used to investigate both chromosomal mutations and differential gene expression, thereby improving prediction of ethambutol resistance in clinical settings.

The combined action of mutations and efflux pumps also has significant therapeutic implications. The incorporation of efflux pump inhibitors into anti-tuberculosis treatment regimens has been proposed as a strategy to restore antibiotic activity, shorten treatment duration, and reduce the emergence of additional resistance mutations. Although several compounds have demonstrated promising results in laboratory studies, most lack specificity or have unacceptable toxicity, limiting their clinical application. Ongoing research aims to identify novel, selective efflux inhibitors that can

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be safely combined with existing anti-tuberculosis drugs. Such combination therapies may enhance intracellular drug concentrations, suppress the survival of tolerant bacterial populations, and reduce the likelihood of mutation-driven resistance.

In summary, the interaction between genetic mutations and efflux-mediated resistance represents a complex, multifactorial process that underlies ethambutol resistance in multidrug-resistant *Mycobacterium tuberculosis*. Efflux pumps provide an immediate adaptive response by lowering intracellular antibiotic concentrations, allowing bacterial survival during initial drug exposure. This survival creates an opportunity for the accumulation and selection of chromosomal mutations in genes such as embB, embA, embC, and ubiA, which subsequently establish stable, high-level resistance. The synergistic effects of reduced drug accumulation and altered drug targets produce resistant phenotypes that are more difficult to diagnose and treat than resistance mediated by either mechanism alone. Continued investigation into the molecular interplay between efflux systems and genetic mutations will improve diagnostic accuracy, support the development of targeted therapeutics, and contribute to more effective management of multidrug-resistant tuberculosis.

Current evidence suggests that resistance develops through multiple stages:

1. Initial drug exposure induces efflux pump expression.
2. Intracellular EMB concentration decreases.
3. Surviving bacteria accumulate chromosomal mutations.
4. Stable high-level resistance emerges.

This explains why some isolates lacking embB mutations still exhibit EMB resistance.

VIII. THERAPEUTIC IMPLICATIONS

Potential strategies include:

1. Efflux pump inhibitors
2. Personalized therapy based on WGS
3. Combination drug therapy
4. Development of new arabinosyl transferase inhibitors
5. Host-directed therapies

Efflux pump inhibitors such as verapamil, reserpine, and thioridazine have shown promising activity in laboratory studies by increasing intracellular antibiotic concentrations.

IX. CONCLUSION

Ethambutol resistance in multidrug-resistant *Mycobacterium tuberculosis* is a multifactorial phenomenon involving both chromosomal mutations and efflux-mediated drug extrusion. While mutations in the embB gene remain the primary molecular markers of resistance, alterations in embA, embC, ubiA, and other genes significantly contribute to resistant phenotypes. Efflux pumps further enhance resistance by reducing intracellular drug concentrations and facilitating bacterial survival under antibiotic pressure. Modern molecular diagnostics, particularly whole-genome sequencing, have greatly improved the detection of resistance mechanisms. A comprehensive understanding of both genetic and physiological pathways is essential for optimizing MDR-TB treatment, improving diagnostic accuracy, and guiding the development of novel therapeutic interventions.

REFERENCES

1. Ahmad, S., Mokaddas, E., & Fares, E. (2022). Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Microbial Drug Resistance, 28*(3), 310–322.
2. Almeida, D., Ioerger, T., Tyagi, S., (2016). Mutations in *embB* and ethambutol resistance in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy, 60*(2), 946–953.

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3. Coll, F., McNerney, R., Preston, M. D., (2018). Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. *Genome Medicine, 10*(1), 1–13.
4. Nguyen, L. (2016). Antibiotic resistance mechanisms in *Mycobacterium tuberculosis*. *Frontiers in Microbiology, 7*, 1–15.
5. Safi, H., Lingaraju, S., Amin, A., (2013). Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations. *Nature Genetics, 45*(10), 1190–1197.
6. Telenti, A., Marchesi, F., Balz, M., (1997). Rapid identification of ethambutol resistance mutations in *Mycobacterium tuberculosis*. *The Lancet, 350*(9070), 135–136.
7. World Health Organization. (2024). *Global Tuberculosis Report 2024*. Geneva: WHO.
8. Zhang, Y., & Yew, W. W. (2015). Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *International Journal of Tuberculosis and Lung Disease, 19*(10), 1276–1289.