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# A Review on the Influencing Factors of Antibiotic Tolerance

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**ABSTRACT**
Antibiotics are secondary metabolites produced by microorganisms or higher organisms that can interfere with the growth of other cells and are used to treat infections caused by pathogenic microbes. With the global surge in antibiotic usage, antibiotic resistance has become a widely recognized concern. However, antibiotic tolerance—where bacteria survive under antibiotic pressure by slowing metabolism and entering dormancy, only to resuscitate after antibiotic removal and cause recurrent infections—has received insufficient attention. Unlike resistance, where bacteria maintain reproductive ability,Tolerant bacteria are characterized by their ability to survive antibiotic exposure without undergoing growth or death. Misuse or overuse of antibiotics intensifies both antimicrobial resistance and tolerance, presenting a significant challenge to public health. This review aims to elucidate the concept, mechanisms, and significance of antibiotic tolerance and emphasizes the urgency of further research in this field.

**KEYWORDS**
Antibiotic usage; Cellular dormancy; Antibiotic tolerance; Recurrent infection

## 1 Introduction

The emergence of antibiotic-tolerant bacterial populations has become progressively more prevalent in clinical therapies in recent times，especially under conditions of antibiotic misuse. While the factors contributing to tolerance have not been thoroughly summarized or categorized in most existing literature, this study aims to classify and review known causes of bacterial tolerance and explore additional potential mechanisms. Additionally, leveraging the tolerance phenomenon may provide a novel approach to beneficial microbial engineering, laying a foundation for deeper investigation into the mechanisms of antibiotic tolerance.

Currently, the known influencing factors can be broadly classified into:

- Genetic factors,
- Antibiotic exposure,
- Nutrient limitation,
- Triclosan (antibacterial agent) treatment,
- Acidic environments,
- Low magnesium ion concentration,
- High-fat diet.

This paper systematically discusses the role of each category and lays a basis for future antibiotic tolerance research. Given the lack of clarity regarding its molecular mechanisms, classifying known factors may provide direction for further mechanistic studies.

## 2 Genetic factors influencing antibiotic tolerance

The development of antibiotic tolerance is closely associated with the bacterial genotype. The presence and expression regulation of specific genes can significantly alter bacterial susceptibility to antibiotics by affecting metabolic activity, modifying drug targets, enhancing efflux capacity, or strengthening stress responses. This section categorizes seven classes of relevant genes and explores their mechanisms of action along with representative studies.

### 2.1 Direct effects of antibiotic resistance genes (ARGs)

Antibiotic resistance genes contribute to decreased antibiotic efficacy by encoding drug-inactivating enzymes, target-modifying proteins, or protective factors.

#### 2.1.1 β-lactamase Genes (e.g., blaTEM, blaCTX-M)

The horizontal transfer of the *blaCTX-M-15* gene in *Klebsiella pneumoniae* has been shown to confer resistance to cefotaxime [1].

Additionally, plasmid-borne *blaNDM-1* mediates carbapenem resistance by encoding a metallo- β -lactamase that degrades the antibiotic molecule [2].

Mechanism: Hydrolyzes the β-lactam ring of β-lactam antibiotics, rendering them inactive.

#### 2.1.2 Methicillin resistance gene (mecA)

In methicillin-resistant *Staphylococcus aureus* (MRSA), the *mecA* gene is integrated into the SCCmec element, serving as the key mechanism for methicillin tolerance [3].

Mechanism: Encodes a low-affinity penicillin-binding protein (PBP2a), which prevents effective binding of β-lactam antibiotics, thereby conferring resistance.

### 2.2 Regulation by metabolism-related genes

Bacterial metabolic activity is closely associated with antibiotic susceptibility. Specific genes can induce tolerance by modulating energy metabolism or promoting dormancy.

#### 2.2.1 Toxin-antitoxin (TA) system genes (e.g., hipAB, tisB/IstR)

Genetic mutations within the *hipAB* locus of *Escherichia coli* have been shown to markedly elevate the formation of persister cells, consequently increasing bacterial tolerance to ciprofloxacin-mediated killing [4].

Under amino acid starvation, expression of the *tisB* toxin depolarizes the membrane, allowing cells to evade the bactericidal effects of β-lactam antibiotics [5].

Mechanism: Toxin proteins such as HipA inhibit translation or DNA replication, thereby inducing the formation of dormant persister cells.

#### 2.2.2 Stringent response genes (relA, spoT)

In *Pseudomonas aeruginosa*, deletion of *relA* results in defective stringent response and increased sensitivity to aminoglycoside antibiotics [6].

Mechanism: These genes synthesize alarmone molecules (p)ppGpp, which suppress ribosome synthesis and downregulate metabolic activity.

### 2.3 Stress response and DNA repair genes

Stress response genes facilitate the evolution of antibiotic tolerance by promoting damage repair or regulating mutation rates.

#### 2.3.1 SOS response genes (lexA)

*lexA* non-cleavable mutants (e.g., $Ind^{-}$) constitutively express SOS-related genes, increasing the proportion of tolerant strains [7].

Mechanism: DNA damage activates RecA, which relieves LexA repression and initiates error-prone repair (SOS repair pathway).

#### 2.3.2 Hypermutation genes (mutS, mutL)

*P. aeruginosa* strains lacking *mutS* rapidly evolve antibiotic resistance under sub-inhibitory concentrations of ciprofloxacin [8].

Mechanism: Deficiency in mismatch repair (MMR) leads to elevated spontaneous mutation rates.

### 2.4 Efflux pump genes

Overexpression of efflux pump genes enables bacteria to actively expel a wide range of antibiotics, thereby reducing intracellular drug concentration. Overexpression of *acrB* increases *E. coli* tolerance to β-lactams and fluoroquinolones [9].

### 2.5 Genes related to membrane permeability

Mutations in outer membrane porin or lipopolysaccharide (LPS) biosynthesis genes can restrict antibiotic entry into bacterial cells.

#### 2.5.1 Porin genes (e.g., ompF, ompC)

Ciprofloxacin accumulation is reduced and its MIC elevated in *Escherichia coli* strains lacking *ompF*, due to impaired outer membrane permeability [10].

Mechanism: Downregulation or structural alteration of porins hinders the transmembrane passage of hydrophilic antibiotics, such as fluoroquinolones.

#### 2.5.2 Lipopolysaccharide (LPS) modification genes (e.g., pmrAB, phoPQ)

Activation of the *phoPQ* two-component regulatory system in *Salmonella* has been shown to confer resistance to polymyxin B [11].

Mechanism: Modifications such as the addition of 4-amino-arabinose or phosphoethanolamine to lipid A reduce the

binding affinity of cationic antibiotics like polymyxins.

### 2.6 Epigenetic regulatory genes

Epigenetic modifications modulate gene expression in a reversible manner and can influence phenotypic antibiotic tolerance.

#### 2.6.1 DNA methyltransferase genes (e.g., dam)

Deletion of the *dam* gene in *E. coli* increases susceptibility to aminoglycoside antibiotics [12].

Mechanism: Alters the expression of virulence and metabolic genes, thereby indirectly affecting antibiotic sensitivity.

#### 2.6.2 CRISPR-cas system genes

Targeted disruption of the *blaKPC* gene using CRISPR-Cas9 in *Klebsiella pneumoniae* restores susceptibility to carbapenem antibiotics [13].

Mechanism: Selective degradation of resistance gene-carrying plasmids inhibits horizontal gene transfer (HGT).

### 2.7 Quorum sensing (QS) regulatory genes

QS systems modulate group behaviors such as biofilm formation, indirectly contributing to enhanced antibiotic tolerance.

#### 2.7.1 QS signal synthesis genes (e.g., lasI, rhlI)

*Pseudomonas aeruginosa* mutants lacking *lasI* exhibit defective biofilm formation and increased susceptibility to tobramycin [14].

Mechanism: QS-regulated autoinducer molecules (e. g., C4-HSL, 3-oxo-C12-HSL) promote biofilm development, enhancing bacterial protection.

#### 2.7.2 Biofilm matrix biosynthesis genes (e.g., pel, psl)

Double mutants lacking *pel* and *psl* genes display disrupted biofilm architecture, which enhances the penetration of vancomycin [15].

Mechanism: The secretion of extracellular polysaccharides (EPS) creates a physical barrier that limits antibiotic diffusion.

## 3 Antibiotic Exposure

Exposure to antibiotics is a major driving force in the development of bacterial tolerance. It primarily promotes the enrichment of resistance genes and phenotypic adaptation through selective pressure. The mechanisms by which antibiotic exposure induces tolerance involve genetic adaptation, phenotypic switching, and community-level behaviors. Based on recent studies, the key processes are outlined below:

### 3.1 Formation of persister cells

Antibiotic pulse treatment can induce a subset of bacterial populations to enter a metabolically dormant state—often mediated by toxin-antitoxin (TA) systems such as HipA—allowing them to evade bactericidal activity and resuscitate once antibiotic pressure is removed [16].

### 3.2 Biofilm-mediated protection

In *Pseudomonas aeruginosa*, exposure to β-lactam antibiotics enhances the secretion of alginate through the LasI/RhlI quorum sensing system, promoting biofilm formation. This biofilm acts as a protective barrier that reduces antibiotic penetration [17].

## 4 Nutrient limitation

Nutrient scarcity (e.g., lack of carbon or nitrogen sources) can reprogram bacterial metabolism and induce tolerance.

### 4.1 Stringent response

The synthesis of (p)ppGpp under nutrient-limited conditions leads to repression of ribosome production and a decline in metabolic activity, resulting in reduced aminoglycoside antibiotic uptake [18].

### 4.2 Energy metabolism arrest

During glucose starvation, *E. coli* shuts down its tricarboxylic acid (TCA) cycle, leading to reduced membrane potential

(Δψ). This weakens the proton-motive force necessary for the uptake of certain antibiotics, such as polymyxins [19].

### 4.3 Increased persister cell formation

Under amino acid starvation, activation of toxin-antitoxin pairs like *tisB/lstR* in *E. coli* promotes bacterial dormancy and subsequently increases β-lactam antibiotic tolerance.

## 5 Triclosan exposure

Triclosan, a broad-spectrum antimicrobial agent, may indirectly promote antibiotic tolerance through cross-tolerance mechanisms after prolonged use.

### 5.1 Efflux pump activation

Triclosan exposure induces the expression of the AcrAB-TolC efflux pump in *E. coli*, leading to enhanced extrusion of antibiotics such as fluoroquinolones and tetracyclines [20].

### 5.2 Disruption of lipid metabolism

By inhibiting the fatty acid synthase FabI, triclosan forces bacteria to import exogenous fatty acids for compensation. This shift is accompanied by the upregulation of multidrug resistance regulator MarA, thereby increasing tolerance [21].

### 5.3 Biofilm induction

Sublethal concentrations of triclosan promote the synthesis of extracellular polysaccharide (PIA) in *Staphylococcus aureus* via activation of the SaeRS two-component system. This results in enhanced biofilm formation, which acts as a physical barrier to antibiotics [22].

## 6 Acidic environment (Low pH)

Acidic environments—such as those in the host gastrointestinal tract or at infection sites—can alter bacterial physiology and affect antibiotic susceptibility.

### 6.1 Disruption of proton motive force (PMF)

The uptake of proton gradient – dependent antibiotics, such as aminoglycosides, is compromised in acidic environments. *H. pylori*, for example, mitigates gastric acidity through urease activity, thereby reducing clarithromycin accumulation across its membrane [23].

### 6.2 Induction of stress response proteins

Acid stress activates the RpoS sigma factor in *E. coli*, upregulating antioxidant enzymes (e. g., KatE catalase) and molecular chaperones (e.g., DnaK), thereby reducing oxidative damage induced by β-lactam antibiotics [24].

### 6.3 Membrane remodeling

Under low pH conditions, *Lactobacillus* increases the synthesis of cyclopropane fatty acids, enhancing membrane rigidity and limiting the permeability of polymyxin B [25].

## 7 Low magnesium ion ($Mg^{2+}$) environment

Magnesium ions are essential cofactors for membrane stability and enzymatic functions in bacteria. $Mg^{2+}$ depletion can trigger multiple tolerance mechanisms.

### 7.1 Activation of the phoPQ two-component system

Low $Mg^{2+}$ levels activate the PhoPQ regulatory system in *Salmonella*, leading to outer membrane modifications such as the addition of 4-amino-arabinose to lipopolysaccharide (LPS), thereby reducing the binding of cationic antimicrobial peptides like polymyxin B [26].

### 7.2 Suppression of porin expression

In *E. coli*, $Mg^{2+}$ limitation downregulates OmpF porin expression, restricting the transmembrane diffusion of hydrophilic antibiotics such as norfloxacin [27].

### 7.3 Functional compensation via metal ion substitution

Methicillin-resistant *Staphylococcus aureus* (MRSA) upregulates the ZnuABC zinc transport system to compensate for the absence of $Mg^{2+}$-dependent enzymes, thus sustaining growth and β-lactam tolerance [28].

## 8 High-fat diet

Variations in host dietary intake have the potential to alter gut microbial communities and their metabolic functions, which in turn may influence the therapeutic efficacy of antibiotics and the evolution of antimicrobial resistance.

### 8.1 Disruption of bile acid metabolism

A high-fat diet elevates the secretion of secondary bile acids (e.g., deoxycholic acid), which induce the expression of efflux pumps such as BmeABC in *Bacteroides*, thereby promoting tetracycline resistance [29].

### 8.2 Gut inflammation and oxidative stress

High-fat diets provoke intestinal inflammation (e.g., increased TNF-α), which stimulates the SOS response in *E. coli* and facilitates horizontal gene transfer of resistance elements [30].

### 8.3 Microbiota interactions and metabolic exchange

Enrichment of Firmicutes (e.g., *Clostridium* spp.) by high-fat intake promotes cross-feeding of short-chain fatty acids (SCFAs), which enhance the metabolic tolerance of *Enterococcus* to vancomycin [31].

## 9 Conclusion

This review comprehensively summarizes the major factors and underlying mechanisms contributing to antibiotic tolerance. The influences of genetic determinants, antibiotic exposure, nutrient limitation, triclosan treatment, acidic conditions, low magnesium environments, and high-fat diets are systematically discussed. Current research indicates that these factors promote antibiotic tolerance through diverse mechanisms, including target modification, metabolic regulation, membrane adaptation, and host-microbiota interactions. Specifically:

### 9.1 Genetic determinants

Various gene categories—including antibiotic resistance genes, metabolism-related genes, stress response and DNA repair genes, efflux pump genes, membrane permeability-related genes, epigenetic regulators, and quorum sensing genes—affect bacterial susceptibility through different pathways. For instance, resistance genes encode drug-inactivating enzymes or protective proteins, while metabolic genes enhance tolerance by altering energy metabolism or inducing dormancy.

### 9.2 Antibiotic exposure

Antibiotic exposure is a primary driver of tolerance development. It facilitates the enrichment of resistance genes and phenotypic adaptation through selective pressure. The formation of persister cells and biofilm-mediated protection are key tolerance mechanisms induced by antibiotic exposure.

### 9.3 Nutrient limitation

Nutritional deficiencies induce metabolic reprogramming that favors tolerance, including activation of the stringent response, metabolic shutdown, and increased persister formation.

### 9.4 Triclosan treatment

Long-term exposure to triclosan can indirectly promote antibiotic tolerance by activating efflux pumps, disrupting lipid metabolism, and enhancing biofilm formation.

### 9.5 Acidic environment

Exposure to acidic conditions can significantly alter bacterial physiology and antibiotic sensitivity by impairing the proton motive force, activating stress response pathways, and reshaping membrane lipid composition and integrity.

### 9.6 Low magnesium environment

Magnesium is essential for membrane stability and enzymatic activity. The absence of this component facilitates bacterial tolerance through activation of the PhoPQ signaling pathway, reduced porin expression, and compensatory induction of enzymes reliant on metal cofactors.

### 9.7 High-fat diet

Host dietary patterns reshape the gut microbiome and metabolism, indirectly influencing antibiotic efficacy. High-fat diets promote tolerance through bile acid dysregulation, induction of intestinal inflammation, and enhanced microbial interactions and metabolic exchange.

## 10 Perspectives

Although significant progress has been made in understanding antibiotic tolerance, many questions remain unanswered. Future research should focus on the following aspects:

### 10.1 Mechanistic elucidation of tolerance

The molecular mechanisms underlying antibiotic tolerance remain incompletely understood. Further studies are needed to clarify the interplay among multiple factors and their roles in tolerance development. For example, the synergistic effect of acidic pH and magnesium deficiency on PhoPQ activation, or the role of gut microbial interactions in mediating high-fat diet–induced tolerance, warrant detailed investigation.

### 10.2 Development of novel anti-tolerance strategies

New approaches targeting the drivers of tolerance should be explored. These may include inhibition of efflux pump gene expression, enhancement of membrane permeability, or dietary modulation to restore antibiotic efficacy.

### 10.3 Clinical and public health applications

Bridging fundamental research with clinical application is crucial for addressing antibiotic tolerance. For instance, tailoring antibiotic regimens for patients consuming high-fat diets may reduce the development of tolerance. Additionally, public health initiatives should be reinforced to enhance awareness of antibiotic tolerance and discourage the misuse of antimicrobial agents.

### 10.4 Multidisciplinary collaboration

Antibiotic tolerance represents a complex and multifactorial biological phenomenon that spans the fields of microbiology, genetics, molecular biology, pharmacology, and nutrition. Advancing our understanding of this issue will require integrated, cross-disciplinary collaboration aimed at elucidating underlying mechanisms and developing targeted therapeutic interventions.

In summary, antibiotic tolerance has emerged as a critical global health concern. By systematically uncovering its driving factors and molecular basis, the design and implementation of effective anti-tolerance strategies in both clinical practice and public health policy may provide promising solutions to mitigate its growing impact.

## References

[1] D'Andrea M. M., et al. (2013). blaCTX-M-15 dissemination in Enterobacteriaceae from Italian hospitals. Antimicrobial Agents and Chemotherapy, *57*(1), 389–392.

[2] Zhou Y., et al. (2021). mSystems.

[3] Ito T., Katayama Y., & Hiramatsu K. (2001). Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Journal of Antimicrobial Chemotherapy, *48*(1), 1–12.

[4] Balaban N. Q., et al. (2004). Bacterial persistence as a phenotypic switch. Science, *305*(5690), 1622–1625.

[5] Dörr T., Vulić M., & Lewis K. (2010). Ciprofloxacin causes persister formation by inducing the tisB toxin in Escherichia coli. PLOS Biology, *8*(2), e1000317.

[6] Nguyen D., Singh P. K., Chiang W.-C., Hultgren S. J., & Sauer K. (2011). The stringent response controls tolerance of Pseudomonas aeruginosa biofilms to antibiotic treatment via the ppGpp regulators RelA and SpoT. Molecular Microbiology, *80*(6), 1470–1486.

[7] Gutierrez A., Laureti L., Crussard S., Abida H., Rodríguez-Rojas A., Blázquez J., ... & Baharoglu, Z. (2013). MrxABC promotes resistance to oxidative stress and antibiotic tolerance via SOS response in Staphylococcus aureus. mBio, *4*(3), e00853-13.

[8] Oliver A., Cantón R., Campo P., Baquero F., & Blázquez J. (2000). High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. Science, *288*(5469), 1251–1254.

[9] Nikaido H. (2018). Multidrug resistance in bacteria. Cold Spring Harbor Perspectives in Medicine, *8*(9), a025106.

[10] Pagès J. M., James C. E., & Winterhalter M. (2008). The porin and the permeating antibiotic: a selective diffusion barrier in gram-negative bacteria. Journal of Antimicrobial Chemotherapy, *62*(Suppl 1), i7–i14.

[11] Gunn J. S., et al. (1998). $Mg^{2+}$ sensing by the $Mg^{2+}$ sensor PhoQ of Salmonella enterica. Science, *280*(5367), 1392–1395.

[12] Liu A., Tran L. M., Becket E., Lee K., Chinn L., Park E., ⋯Miller J. H. (2010). Antibiotic sensitivity profiles determined with an Escherichia coli gene knockout collection: Generating an antibiotic bar code. Antimicrobial Agents and Chemotherapy, *54*(4), 1393–1403.

[13] Citorik R. J., Mimee M., & Lu T. K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nature Biotechnology, *32*(11), 1141–1145.
[14] Davies D. G., Parsek M. R., Pearson J. P., Iglewski B. H., Costerton J. W., & Greenberg E. P. (1998). The involvement of cell-to-cell signals in the development of Pseudomonas aeruginosa biofilms. Science, *280*(5361), 295–298.
[15] Colvin K. M., Gordon V. D., Murakami K., Borlee B. R., Wozniak D. J., Wong G. C. L., & Parsek M. R. (2011). The pel polysaccharide can serve a structural and protective role in the biofilm matrix of Pseudomonas aeruginosa. PLOS Pathogens, *7*(1), e1001264.
[16] Fisher R. A., Gollan B., & Helaine S. (2017). Persistent bacterial infections and persister cells. Nature Reviews Microbiology, *15*(8), 453–464.
[17] Costerton J. W., Stewart P. S., & Greenberg E. P. (1999). Bacterial biofilms: a common cause of persistent infections. Science, *284*(5418), 1318–1322.
[18] Amato S. M., et al. (2013). The role of metabolism in bacterial persistence. Frontiers in Microbiology, *4*, 70.
[19] Lobritz M. A., Belenky P., Porter C. B. M., Gutierrez A., Yang J. H., Schwarz E. G., & Collins J. J. (2015). Antibiotic efficacy is linked to bacterial cellular respiration. Proceedings of the National Academy of Sciences USA, *112*(27), 8173–8180.
[20] Heath R. J., et al. (1999). A triclosan-resistant bacterial enzyme. Nature, *398*(6726), 383–384.
[21] Heath R. J., Rubin J. R., Holland D. R., Zhang E., Snow M. E., & Rock C. O. (1999). Mechanism of triclosan inhibition of bacterial fatty acid synthesis. Journal of Biological Chemistry, 274(16), 11110–11114.
[22] Latimer J., Forbes S., & McBain A. J. (2012). Attenuated virulence and biofilm formation in *Staphylococcus aureus*following sub-lethal exposure to triclosan. Antimicrobial Agents and Chemotherapy, 56(6), 3092–3100.
[23] Marcus E. A., Scott D. R., Lee J. Y., Reinscheid D., & Sachs G. (1996). Effects of environmental pH on the proton motive force of *Helicobacter pylori*. Gastroenterology, 111(4), 886–900.
[24] Hengge-Aronis R. (2002). Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. Microbiology and Molecular Biology Reviews, 66(3), 373–395.
[25] Guerzoni M. E., Lanciotti R., & Cocconcelli P. S. (2001). Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. Applied and Environmental Microbiology, 67(9), 4031–4036.
[26] Gunn J. S., Ryan S. S., & Alaniz R. C. (1998). Activation of the PhoPQ regulatory system in Salmonella leads to modification of lipid A with aminoarabinose and phosphoethanolamine, reducing binding of cationic antimicrobial peptides. Science, *279*(5349), 1001–1004.
[27] Nikaido H. (1998). Antibiotic resistance caused by Gram-negative multidrug efflux pumps. Clinical Infectious Diseases, *27*(Supplement 1), S32–S41.
[28] Hantke K. (2001). Bacterial zinc transporters and regulators. BioMetals, 14(3–4), 239–249.
[29] Ridlon J. M., Kang D. J., & Hylemon P. B. (2006). Bile salt biotransformations by human intestinal bacteria. Journal of Lipid Research, 47(2), 241–259.
[30] Morgun A., Dzutsev A., Dong X., et al. (2015). Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Cell, 162(1), 128–139.
[31] Caballero S., Kim S., Carter R. A., Leiner I., Sušac B., Miller L., Kim G. J., Ling L., Pamer E. G. (2017). Cooperation among commensal bacteria supports colonization resistance against pathogens. Cell Host & Microbe, 21(1), 56–72.