INTERNATIONAL JOURNAL OF HEALTH & MEDICAL RESEARCH

ISSN(print): 2833-213X, ISSN(online): 2833-2148

Volume 03 Issue 09 September 2024

DOI : 10.58806/ijhmr.2024.v3i09n07

Page No. 687-694

Dissecting Molecular Pathways and Intricate Mechanisms Underpinning Antibiotic Resistance in Gram-Negative Bacteria: An Exhaustive and Integrative Review

Nawar Bahaa Abdulsahib[1], Yasser Ali Hussein[2], Bilal Husam Jasim[3]

[1,2,3] Biotechnology Branch, Departments of Applied Sciences, University of Technology - Iraq

ABSTRACT: Concern for antibiotic resistance in Gram negative bacteria is fast becoming a global issue due to the reduction of effectiveness of current drugs. This review deconstructs the material in a logical, categorized manner, examining the biochemistry of antibiotic resistance mechanisms such as efflux pumps, enzymatic degradation, genetic changes, and the regulation of those changes. Close associated gene transfer, especially the plasmids-mediated resistance, and how biofilms contribute to the survival of bacteria under antibiotic pressure. Present methods of detection and diagnosis are also described with emphasis on the importance of identifying a resistant strain quickly and accurately. Last, we discuss directions for ongoing and future progress in tackling antibiotic resistance, such as new treatments and public health directions. Based on these observations, it is the hope of this review to contribute to the existing literature and initiatives geared towards reversing the escalating problem of antibiotic resistance.

INTRODUCTION

Antibiotic resistance is one of the biggest threats confronting modern medicine, especially in light of emergent gram-negative bacteria, which are especially notorious for antibiotic resistance. These bacteria are known to contribute to a large extent to infections, particularly those prevalent in healthcare facilities and thereby compounding the rates of morbidity and mortality. The need to address this problem is significant especially given the emphasis given by the World Health Organization (WHO) as a leading global health concern (1-4).

Gram negative bacteria possess sticky outer membrane which prevents many antibiotics and hence are challenging to control. However, Gram-negative bacteria have so many developed strategies that can counteract the action of antibiotics. Antibiotic resistance mechanisms comprise efflux pump that eject antibiotics out of the cell, enzymes that inactivate or alter antibiotics, and changes to the target site that stop antibiotics from binding. Another challenge stems from genetic plasticity of these organisms in swiftly attaining and spreading resistance genes through processes known as Horizontal gene transfer (2, 3, 5, 6).

Elucidating the molecular basis and complex processes governing antibiotic resistance in gram negative bacteria is important in the formulation of therapeutic approaches. The current state of knowledge regarding this topic is comprehensively and systematically reviewed in this paper. We will discuss some of the biochemical and genetics aspects of resistance, how they are controlled by genetic networks and how antibiotic–resistant infections affect patient care (2, 7-10).

Furthermore, the current sources are reflective of both established resistance traits and newly discovered threats and additional resistance mechanisms that have become more recently recognized. We also review new horizons in the detection and diagnostic approaches which play a core role within the identify and manage the resistant infections at the early stage. In addition, we rely on the recent data concerning the approaches that are currently being investigated to address the issue of antibiotic resistance, including new antibiotics, combination therapies, phage therapy and vaccination (11-15).

This review aims to contribute to current knowledge and future conceptual developments on antibiotic resistance in gram negative bacteria by pointing out the extensive databases of research articles available regarding the topic and consolidating them as a central reference for researchers, clinicians, and policymakers. We aimed to determine what is known to date about antibiotic resistance, what remains unknown, where future research should be directed and how the identified problem could be addressed.

Mechanisms of Antibiotic Resistance

Knowledge on how Gram negative bacteria acquire and maintain their resistance to antibiotics is therefore vital in formulating strategies to counter the problem. These mechanisms are complex and frequently cooperate with one another to offer the essentional protection from a vast array of antibiotics. This section discusses antibiotic resistance in detail by looking at its main cause.

Efflux Pumps and Their Roles

Efflux pumps are proteins imbedded in the cytoplasmic membrane that transport antibiotics and other detrimental substances out of the bacterial cell decreasing the intracellular concentration of the drug to a non lethal level. These pumps can be specific for a single sort of antibiotic or can discharge a number of sorts of antibiotics, hence being connected with multidrug resistance (MDR).The major families of efflux pumps are classified into (16-20).

- Resistance-nodulation-division (RND) family:

Most commonly observed in Gram-negative organisms, these efflux pumps are capable of ejecting several classes of antimicrobials such as β-lactam agents, tetracyclines, and fluoroquinolones.

- - Major Facilitator Superfamily (MFS): These pumps ordinarily transport a lesser variety of antibiotics, but they too play an essential role in clinical antimicrobial resistance.

ABC transporters utilize ATP hydrolysis to transport the antibiotics and other substrates out of the cell. Biological inactivation uses enzymes to degrade the antibiotic molecule so that it cannot work or to alter the antibiotic molecule so that it cannot work for the intended purpose.

THE KEY ENZYMES INVOLVED INCLUDE THE FOLLOWING (21-26):

- β-lactamases: These enzymes cleave the β-lactam ring found in penicillins, cephalosporins, and carbapenems, resulting in the modification of the antibiotic's function into an inactivated state. ESBLs and carbapenemases are of special interest due to the inclusion of a large number of antibiotics in their substrate profile.
- These enzymes either acetylate or phosphorylate or adenylate aminoglycoside antibiotics which prevents them to bind with their target site. Pharmacodynamic alterations Antibiotics exert their effects by binding to specific sites; therefore, changes to these sites may decrease a drug's binding affinity and lead to resistance. Examples include:

Penicillin-Binding Proteins (PBPs): They pointed out that changes in PBPs can adversely affect the effectiveness of β-lactam antibiotics.

Ribosomal Modifications: Ribosomal Modifications: Altered RRNA or ribosomal proteins also affect the resistance to macrolides, tetracyclines and aminoglycosides thoroughly.

- DNA Gyrase and Topoisomerase IV: Mutations in these enzymes can lead to fluoroquinolone resistance.

Reduced Permeability of Bacterial Membranes

Gram-negative bacteria possess an outer membrane that acts as an additional barrier to the entry of antibiotics. Modifications of membrane porins can further restrict drug penetration (27).

- Porin Loss or Modification: Reduction in the number or alteration of porins can decrease permeability and limit antibiotic access to intracellular targets (28).

Lipid A Modifications: Alterations in the lipid A component of lipopolysaccharides can affect membrane fluidity and antibiotic uptake (29).

Biofilm Formation

Biofilms are structured communities of bacteria encased in a self-produced extracellular matrix that adheres to the surface. Biofilm-associated bacteria exhibit an increased tolerance to antibiotics owing to multiple factors (30).

- Reduced Penetration: The extracellular matrix can impede the diffusion of antibiotics. Altered Microenvironment: Gradients of nutrients and oxygen within biofilms can create zones where bacteria are less metabolically active and more resistant to antibiotics (31).
- Enhanced Horizontal Gene Transfer: Close proximity of cells within biofilms facilitates exchange of resistance genes.

Table 1: Mechanisms of Antibiotic Resistance

Mechanism	Description	Examples
1. Efflux Pumps	Membrane proteins that expel antibiotics out of the cell, reducing intracellular concentrations.	- **RND family**: Expels β-lactams, tetracyclines, fluoroquinolones.
		- **MFS family**: Typically transports a narrower range of antibiotics.
		- **ABC transporters**: Utilize ATP hydrolysis to expel antibiotics.
2. Enzymatic Degradation	Enzymes that degrade or modify antibiotics, rendering them inactive.	- **β-Lactamases**: Hydrolyze the β-lactam ring in penicillins, cephalosporins, carbapenems.

		- **Aminoglycoside-modifying enzymes**: Acetylate, phosphorylate, or adenylate aminoglycosides.
3. Alterations in Target Sites	Mutations or modifications in antibiotic target sites, reducing drug binding affinity.	- **PBPs (Penicillin-Binding Proteins)**: Alterations reduce β-lactam efficacy.
		- **Ribosomal Modifications**: Changes in ribosomal RNA/proteins confer resistance to macrolides, tetracyclines.
		- **DNA Gyrase and Topoisomerase IV**: Mutations lead to fluoroquinolone resistance.
4. Reduced Permeability of Membranes	Modifications in membrane porins and lipid A affect antibiotic uptake and penetration.	- **Porin Loss/Modification**: Reduced number or alterations decrease antibiotic permeability.
		- **Lipid A Modifications**: Alterations affect membrane fluidity and antibiotic uptake.
5. Biofilm Formation	Structured bacterial communities encased in an extracellular matrix, increasing tolerance to antibiotics.	- **Reduced Penetration**: Matrix impedes antibiotic diffusion.
		- **Altered Microenvironment**: Gradients create zones of low metabolic activity and increased resistance.
		- **Enhanced Horizontal Gene Transfer**: Close cell proximity facilitates resistance gene exchange.

GENETIC BASIS OF RESISTANCE

The genetic basis of antibiotic resistance in Gram-negative bacteria is complex and multifactorial. It involves the acquisition of resistance genes through various mechanisms, and the subsequent expression and regulation of these genes.

1. Horizontal Gene Transfer (HGT)

Horizontal gene transfer is the primary method by which bacteria acquire antibiotic-resistance genes from other organisms. There are three main modes of HGT (32, 33).

a. Conjugation

Conjugation involves direct transfer of DNA from one bacterium to another through physical contact, which is typically mediated by a pilus. This process often involves plasmids, which are small circular DNA molecules that can carry multiple resistance genes. Conjugation can rapidly spread resistance among bacterial populations.

b. Transformation

Transformation is the uptake of naked DNA from the environment by bacteria. This DNA may originate from lysed cells and may include antibiotic resistance genes. Once inside the recipient cell, this DNA can be integrated into the bacterial genome or can be maintained as a plasmid.

c. Transduction

Transduction occurs when bacteriophages (viruses that infect bacteria) transfer DNA between them. During infection, bacteriophages can accidentally incorporate fragments of bacterial DNA, including resistance genes, and transfer them to new host cells during subsequent infections.

2. Plasmid-Mediated Resistance

Plasmids play a crucial role in ARG dissemination of antibiotic resistance genes. These extrachromosomal DNA elements can replicate independently of the bacterial chromosome and often carry multiple resistance genes, thereby conferring multidrug resistance. Plasmids can be transferred between bacteria via conjugation, making them potent vectors for the spread of resistance (34).

3. Chromosomal Mutations and Adaptations (35, 36)

Mutations in the chromosomal DNA can also confer antibiotic resistance. These mutations can arise spontaneously or can be induced by exposure to antibiotics and other stressors. Key examples include:

- Point Mutations: Single nucleotide changes in genes encoding antibiotic targets (e.g., DNA gyrase and ribosomal proteins) can reduce drug binding and confer resistance.
- Gene Amplification: Duplication of resistance genes can lead to increased expression and higher levels of resistance.
- Regulatory Mutations: Changes in regulatory genes or promoter regions can alter the expression of resistance genes and enhance bacterial survival under antibiotic pressure.

4. Integrons and Gene Cassettes
   Integrons are genetic elements that can capture and express genes, including antibiotic resistance genes, via site-specific recombination. They consist of an integrase gene, attachment site, and promoter. Integrons can incorporate gene cassettes, which are mobile DNA elements containing a single gene and recombination site. This mechanism allows for rapid acquisition and expression of multiple resistance genes (37).
5. Mobile Genetic Elements
   Mobile genetic elements, such as transposons and insertion sequences, can move within and between genomes, contributing to the spread of resistance genes. Transposons often carry antibiotic resistance genes and can be inserted into plasmids or chromosomal DNA to facilitate horizontal gene transfer (38).
6. CRISPR-Cas Systems
   Some bacteria possess CRISPR-Cas systems that provide adaptive immunity against foreign genetic elements, including plasmids and phages that carry antibiotic resistance genes. Although CRISPR-Cas systems can limit the acquisition of new resistance genes, their role in mediating existing resistance is an area of ongoing research (39).

Table 2: Genetic Basis of Resistance

Mechanism	Description	Examples
1. Horizontal Gene Transfer (HGT)	Transfer of genetic material between bacteria, leading to the acquisition of resistance genes.	
a. Conjugation	Direct transfer of DNA through a pilus from one bacterium to another; often involves plasmids.	- Spread of multidrug resistance plasmids.
b. Transformation	Uptake of naked DNA from the environment by a bacterium.	- Integration of resistance genes from lysed cells.
c. Transduction	Transfer of DNA between bacteria via bacteriophages.	- Bacteriophage-mediated transfer of resistance genes.
2. Plasmid-Mediated Resistance	Plasmids carrying multiple resistance genes replicate independently and can be transferred between bacteria.	- Rapid spread of resistance through bacterial populations.
3. Chromosomal Mutations and Adaptations	Mutations in chromosomal DNA that confer resistance; can arise spontaneously or due to antibiotic pressure.	- **Point Mutations**: Changes in genes encoding antibiotic targets (e.g., DNA gyrase).
		- **Gene Amplification**: Duplication of resistance genes increasing expression levels.
		- **Regulatory Mutations**: Altered expression of resistance genes via changes in regulatory regions.
4. Integrons and Gene Cassettes	Integrons capture and express genes, including resistance genes, through site-specific recombination.	- Incorporation of multiple resistance gene cassettes into integrons.
5. Mobile Genetic Elements	Transposons and insertion sequences that can move within and between genomes, spreading resistance genes.	- Insertion of transposons carrying resistance genes into plasmids or chromosomal DNA.
6. CRISPR-Cas Systems	Adaptive immunity systems in bacteria that can limit the acquisition of new resistance genes.	- Restriction of plasmid and phage-borne resistance gene acquisition by CRISPR-Cas systems.

Table 3: Regulatory Networks and Signal Transduction

Mechanism	Description	Examples

1. Quorum Sensing	Cell-to-cell communication mechanism that bacteria use to coordinate group behaviors, including biofilm formation and virulence.	- **Pseudomonas aeruginosa**: Uses quorum sensing to regulate the production of virulence factors and biofilm formation.
2. Two-Component Regulatory Systems	Systems consisting of a sensor kinase and a response regulator that help bacteria sense and respond to environmental changes.	- **EnvZ/OmpR system in E. coli**: Regulates expression of outer membrane proteins in response to osmolarity changes.
3. Stress Response Pathways	Cellular pathways activated in response to stress conditions such as antibiotic exposure, leading to adaptive resistance mechanisms.	- **SOS Response**: Stimulated by DNA damage resulting in the production of DNA repair enzymes and infra mutagenesis.
4. Global Regulatory Networks	Networks that coordinate the expression of multiple genes in response to various environmental stimuli, enhancing survival and resistance.	- **MarA, SoxS, and Rob Regulons**: The following pathways are influenced: antibiotic resistance genes, oxidative stress, efflux pumps in E.coli.
5. Biofilm Formation Regulation	Specific regulatory pathways that control the development and maintenance of biofilms, which protect bacteria from antibiotics.	- **c-di-GMP Signaling**: Cyclic di-GMP is a second messenger for controlling biofilm development and integrity in numerous bacteria.
6. Small RNAs (sRNAs)	Regulatory RNAs that modulate gene expression post-transcriptionally, influencing resistance mechanisms.	- **MicF RNA in E. coli**: Inhibits translation of OmpF porin, reducing antibiotic uptake.

Table 4: Detection and Diagnostic Techniques

Technique	Description	Examples
1. Molecular and Genomic Methods	Methods that identify DNA sequences responsible for antibiotic resistance.	- **Polymerase Chain Reaction (PCR)**: THIS increases the amount of specific DNA sequences to obtain detectable resistance genes.
		- **Whole Genome Sequencing (WGS)**: Is useful to get a general picture of all the genetic parts of a bacterial genome and possible resistance genes.
		- **Quantitative PCR (qPCR)**: Estimates the amount of target DNA useful in determining the load of resistant bacteria in a population.
2. Phenotypic Assays	Those that involve challenges of bacterial growth, or their ability to survive in the presence of an antibiotic agent, to establish resistance.	- **Disk Diffusion (Kirby-Bauer) Test**: Meaning, the best measures the zone of inhibition to monitor susceptibilities or resistance.
		- **Broth Microdilution**: Defines a minimum inhibitory concentration (MIC) of antibiotics needed for bacterial growth to be negatively affected.
		- **E-test (Epsilometer Test)**: It's a hybrid of the disk diffusion technique and the MIC determination CARBA method.
3. Rapid Diagnostic Tools	Methods aimed at rapid detection of resistant bacteria and resistance genes; can be used at the point of care.	- **Lateral Flow Assays**: Today diagnostic test include: Rapid, easy to perform tests that aim at detecting one specific antigen or a resistance protein.
		- **Loop-Mediated Isothermal Amplification (LAMP)**: A DNA amplification technique that can be accomplished in a constant temperature without needing extensive temperature cycling.
		- **CRISPR-based Diagnostics**: Utilizes CRISPR-Cas systems for highly specific detection of genetic sequences associated with resistance.

4. Automated Systems	Multi-Diagnostics systems that consist of several diagnostic technologies for rapid throughput and subsequent analysis.	- **VITEK 2 System**: Automated system for both identification and antimicrobial susceptibility testing of bacteria.
		- **MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry)**: Identifies bacteria based on protein fingerprinting.
5. Biosensors	Technologies that employ biological reagents to identify antibiotic resistance or antibiotic resistance genes.	- **Electrochemical Biosensors**: Detect resistance genes through changes in electrical signals upon binding target DNA/RNA.
		- **Optical Biosensors**: Use light-based techniques to detect interactions between biological molecules and target resistance markers.

Table 5: Conclusion and Future Perspectives

Aspect	Description	Details
Summary of Key Findings	Recapitulation of the main points discussed in the review.	- Comprehensive understanding of the mechanisms, genetic basis, regulatory networks, and detection techniques of antibiotic resistance in Gram-negative bacteria.
Gaps in Current Research	Identification of areas needing further investigation.	- Limited knowledge on the impact of environmental factors on resistance development.
		- Need for more studies on the role of CRISPR-Cas systems in resistance mitigation.
		- Insufficient understanding of biofilm-specific resistance mechanisms.
Future Directions	Potential avenues for further research and development.	- Development of novel antibiotics targeting unique bacterial structures or functions.
		- Exploration of combination therapies to counteract multiple resistance mechanisms simultaneously.
		- Investigation into non-traditional therapies such as phage therapy and antimicrobial peptides.
Innovative Strategies	Emerging approaches to combat antibiotic resistance.	- Utilization of machine learning and AI for predicting resistance patterns and designing new drugs.
		- Advancements in rapid diagnostic tools for early detection and appropriate treatment of resistant infections.
		- The application of CRISPR technologies for targeting and selectively eliminating or deactivating specific resistance genes.
Policy and Public Health Initiatives	Recommendations for policy changes and public health strategies.	- Enhancing antibiotic stewardship programs that will work to reduce the overuse of antibiotics.
		- Improving global function for tracking the trends and outbreaks of the resistance.
		- Advertising crusade on correct and prudent utilization of antibiotics.
Collaborative Efforts	Importance of multidisciplinary and global collaborations.	- Promoting academic-industrial and governmental research collaborations.
		- Building international partnership to address the increased global trends in antibiotic resistance.

CONCLUSION

Gram negative bacterial resistance to antibiotics is acknowledged as a major public health challenge globally due to factors such as efflux pumps, enzymatic inactivation, and horizontal gene transfer. Recognizing these issues is critical for designing specific approaches as well as novel treatments to address the disease. Promising diagnostics and interventions are paramount in addressing

the current increased prevalence of resistance. Integrated and interdisciplinary approaches will improve our capacity to maintain antibiotic efficacy. More work is required to bring understanding to this ever present issue in modern medicine.

REFERENCES

1) Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. Pathogens. 2021;10(10):1310.
2) Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. Pharmaceuticals (Basel). 2023;16(11).
3) Kamble SA, Barale SS, Mohammed AA, Paymal SB, Naik NM, Sonawane KD. Structural insights into the potential binding sites of Cathepsin D using molecular modelling techniques. Amino Acids. 2024;56(1):33.
4) Dhale PC, Mohammed AA, Al-Shimary AA, Shaikh AB, Kamble AA, Gaikwad SH, et al. Exploring Triazole-Based Co (Ii), Ni (Ii) and Cu (Ii) Complexes as Biologically Potent Molecules: Chemical Synthesis, Structural Elucidation and Molecular Docking Studies. Ni (Ii) and Cu (Ii) Complexes as Biologically Potent Molecules: Chemical Synthesis, Structural Elucidation and Molecular Docking Studies.
5) Maher C, Hassan KA. The Gram-negative permeability barrier: tipping the balance of the in and the out. mBio. 2023;14(6):e0120523.
6) Jasim B, Ali E. Isolation, extraction, purification, and characterization of fibrinolytic enzyme from Pseudomonas aeruginosa and estimation of the molecular weight of the enzyme. Archives of Razi Institute. 2021;76(4):809-20.
7) Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74(3):417-33.
8) Haider J, Jasim H. Bioinformatics Analysis of BMP15 Gene and Bone Morphological Protein-15 Sequence. 2024.
9) Khamees HH, Mohammed AA, Hussein SAM, Ahmed MA, Raoof ASM. In-Silico study of Destabilizing Alzheimer's Aβ42 Protofibrils with Curcumin. International Journal of Medical Science and Dental Health. 2024;10(05):76-84.
10) Gavali LV, Mohammed AA, Al-Ogaili MJ, Gaikwad SH, Kulkarni M, Das R, et al. Novel terephthalaldehyde bis (thiosemicarbazone) Schiff base ligand and its transition metal complexes as antibacterial Agents: Synthesis, characterization and biological investigations. Results in Chemistry. 2024;7:101316.
11) Yamin D, Uskoković V, Wakil AM, Goni MD, Shamsuddin SH, Mustafa FH, et al. Current and Future Technologies for the Detection of Antibiotic-Resistant Bacteria. Diagnostics. 2023;13(20):3246.
12) Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MTS, Chinedu Ikem J, Victor Chigozie U, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022;36(9):e24655.
13) MOHAMMED M, Mousa D, Tareq Jafaar Al-Jindeel H, Al-Karawi S. Latent and reactivation Cytomegalovirus (CMV) infection can cause severe fetal sequelae despite pre-conceptional immunity.
14) Jasim B, Ali E. Enhanced production of fibrinolytic enzyme from Pseudomonas aeruginosa by optimization media components. Journal of Applied Sciences and Nanotechnology. 2021;1(2):58-65.
15) Mohammed AA, Mahmoud HQ, Mhana RS. ADVANCES IN THE DIAGNOSIS AND MANAGEMENT OF BREAST CANCER: A SYSTEMATIC REVIEW. World. 2023;2(6).
16) Huang L, Wu C, Gao H, Xu C, Dai M, Huang L, et al. Bacterial Multidrug Efflux Pumps at the Frontline of Antimicrobial Resistance: An Overview. Antibiotics (Basel). 2022;11(4).
17) Gaurav A, Bakht P, Saini M, Pandey S, Pathania R. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. Microbiology (Reading). 2023;169(5).
18) Al-Karawi AS, Abid FM, Mustafa A, Abdulla M. Revealing the Urinary Microbiota in Prostate Cancer: A Comprehensive Review Unveiling Insights into Pathogenesis and Clinical Application. Al-Salam Journal for Medical Science. 2024;3(1):45-54.
19) Mohammed M, Al-Saadi MS, Al-Karawi AS. An examination of the seroprevalence of torch infections and their correlation with adverse reproductive outcomes in females exhibiting a bad obstetric history.
20) Al-Azzawi MA, Maftool AJ, Al-Shimary AA, Mohammed AA. A Comprehensive Review of Vitamin D3: Metabolism, Functions, and Clinical Implications. International Journal of Medical Science and Dental Health. 2023;9(12):37-46.
21) Bonomo RA. β-Lactamases: A Focus on Current Challenges. Cold Spring Harb Perspect Med. 2017;7(1).
22) Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016;6(6).
23) Egorov AM, Ulyashova MM, Rubtsova MY. Bacterial Enzymes and Antibiotic Resistance. Acta Naturae. 2018;10(4):33-48.
24) Bahr G, González LJ, Vila AJ. Metallo-β-lactamases in the Age of Multidrug Resistance: From Structure and Mechanism to Evolution, Dissemination, and Inhibitor Design. Chem Rev. 2021;121(13):7957-8094.

25) Al-Karawi AS, Kadhim AA, Kadum MM. Recent advances in tuberculosis: A comprehensive review of emerging trends in pathogenesis, diagnostics, treatment, and prevention.
26) Al-Karawi AS, Rasool KH, Atoom AM, Kadhim AS. Correlation between H. pylori infection and serum levels of inflammatory markers: A retrospective study. Al-Salam Journal for Medical Science. 2023;2(2):20-4.
27) Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794(5):808-16.
28) Fernández L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev. 2012;25(4):661-81.
29) Needham BD, Trent MS. Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. Nat Rev Microbiol. 2013;11(7):467-81.
30) Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023;13:1137947.
31) Mirghani R, Saba T, Khaliq H, Mitchell J, Do L, Chambi L, et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. AIMS Microbiol. 2022;8(3):239-77.
32) Michaelis C, Grohmann E. Horizontal Gene Transfer of Antibiotic Resistance Genes in Biofilms. Antibiotics (Basel). 2023;12(2).
33) Al-Karawi AS, Kadhim AS. Exploring the role of autoantibodies in Iraqi females with polycystic ovary syndrome. 2024.
34) Vrancianu CO, Popa LI, Bleotu C, Chifiriuc MC. Targeting Plasmids to Limit Acquisition and Transmission of Antimicrobial Resistance. Front Microbiol. 2020;11:761.
35) Coculescu BI. Antimicrobial resistance induced by genetic changes. J Med Life. 2009;2(2):114-23.
36) Kadhim AS, Al-Karawi AS. A Clinical Study on the Association Between Bacterial Infection and Inflammatory Cytokines in the Wounds of Burn Injury Patients.
37) Gillings MR. Integrons: past, present, and future. Microbiol Mol Biol Rev. 2014;78(2):257-77.
38) Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. Clin Microbiol Rev. 2018;31(4).
39) Tao S, Chen H, Li N, Liang W. The Application of the CRISPR-Cas System in Antibiotic Resistance. Infect Drug Resist. 2022;15:4155-68.