Journal of Physiology and Biology Sciences (JPBS)

E-ISSN: 3107-2283

journal homepage: https://physiobiojournal.com/

DOI: https://doi.org/10.69792/jpbs.2025.vol.2.3

Effects of Hydrogen Peroxide on Biofilm Formation and Antibiotic Susceptibility of Clinical Bacterial Isolates

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Received: 14/09/2025
Revised: 28/10/2025
Accepted: 07/11/2025
Published: 31/12/2025

ABSTRACT

Background: Reactive oxygen species (ROS) are products of aerobic metabolism that play dual roles as signaling molecules and as inducers of oxidative damage. Their impact on bacterial physiology, particularly biofilm formation and antibiotic resistance, is of growing clinical significance.

Objective: This study aimed to investigate the effects of hydrogen peroxide (H_2O_2)-induced oxidative stress on biofilm formation and antibiotic susceptibility among clinical bacterial isolates.

Methods: Seventy bacterial isolates were collected from clinical cases at Tikrit Teaching Hospital, Iraq, and identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus viridans*. Minimum inhibitory concentrations (MIC) and sub-MIC values of H_2O_2 were determined, followed by assessments of biofilm production (tube method) and antibiotic susceptibility (modified Bauer–Kirby disk diffusion) before and after H_2O_2 exposure.

Results: Sub-MIC values of H_2O_2 varied across species (30 mM for *E. coli*, 2 mM for K. pneumoniae, 70 mM for *S. aureus*, and 40 mM for *S. viridans*). Biofilm assays revealed significant modulation, with S. aureus losing its weak biofilm phenotype post-exposure, while *S. viridans* shifted from weak to strong biofilm production. Antibiotic susceptibility profiles demonstrated species-dependent alterations, including increased resistance to vancomycin and rifampin in *S. viridans*, linked to enhanced biofilm formation, and loss of resistance in other species. These changes are attributed to oxidative stress-induced genetic mutations, plasmid rearrangements, and quorum sensing regulation.

Conclusion: Sub-lethal oxidative stress induced by H_2O_2 profoundly modulates bacterial adaptive mechanisms, particularly biofilm formation and antibiotic resistance. These findings highlight the complex interplay between oxidative stress, microbial survival strategies, and antimicrobial efficacy, providing insights into the evolutionary dynamics of bacterial pathogenesis and potential therapeutic challenges.

KEYWORDS : Oxidative stress; Hydrogen peroxide; Biofilm; Antibiotic resistance; Clinical isolates.

INTRODUCTION

Oxygen is a fundamental element in energy production via oxidation of nutrients. However, its reduction is often incomplete even under normal conditions, leading to the formation of reactive intermediate species. These reactive species, known as free radicals, are byproducts of normal metabolic processes. Free radicals can attack and destroy cellular components, causing severe damage to genetic material and various cellular functions (Laylani *et al*., 2024; Manful *et al*., 2025).

Free radicals in the human body arise from both endogenous and exogenous sources and increase during illness, psychological or physical stress, and with aging . Cellular metabolism itself is considered an endogenous source of free radicals (Shawkat and Abd-alwahab,2024 ; Pooja *et al*., 2025). Aerobic bacteria utilize molecular oxygen (O_2) for respiration or nutrient oxidation to obtain energy. Reactive oxygen species (ROS) — potent forms of oxygen — are essential for many physiological processes in cells and organisms. However, an imbalance in favor

of elevated ROS levels over natural antioxidant defenses causes oxidative stress in humans (Rutherford, 2016; Pooja *et al*., 2025). Similarly, exposure of bacteria to ROS can damage various macromolecules within the cell, leading to mutations or cell death. On the other hand, free radicals can also function as signaling molecules, inducing coordinated responses in bacterial cells under oxidative stress conditions (Abd-alwahab *et al*., 2024) One study shows that oxidative stress caused by hydrogen peroxide and ferrous oxides affects bacterial virulence and antibiotic sensitivity. (Jasim and Al-juboory, 2023).

MATERIALS AND METHODS

Bacterial Isolates

Seventy bacterial isolates were collected from Tikrit Teaching Hospital (Salah al Din Governorate) from patients clinically diagnosed with wound infections, urinary tract infections, respiratory tract infections, skin infections, and other conditions, during the period from 15 August 2017 to 1 December 2017. These isolates were processed in the bacteriology laboratory, where they were identified as belonging to Gram-positive species (*Streptococcus viridans*, *Staphylococcus aureus*) and Gram-negative species (*Escherichia coli*, *Klebsiella pneumoniae*).

Identification of Test Isolates

The bacterial species under study were identified using standard diagnostic tests.

Antibiotic Susceptibility Test

The antibiotic susceptibility test was performed using the modified Bauer–Kirby disk diffusion method (Bauer *et al*., 1966) as recommended by the World Health Organization.

Biofilm Formation Test

The tube method (TM) was used to determine the ability of the bacteria to form biofilm, as described by Christensen et al. (1985). For each isolate, bacterial cells were cultured in test tubes containing appropriate medium. After incubation, tubes were stained and examined for the presence of a biofilm layer on the tube walls, indicating biofilm formation.

Determination of MIC and Sub-MIC of Hydrogen Peroxide

The minimum inhibitory concentration (MIC) of hydrogen peroxide (H_2O_2) was determined for the bacterial species under study. A series of H_2O_2 dilutions were prepared (2, 2.5, 5, 7.5, 10, 20, 30, 40, 50, 60, 70, 80, 90 mM). The MIC was defined as the lowest H_2O_2 concentration that completely inhibited bacterial growth, whereas the sub-MIC was defined as the H_2O_2 concentration just below the MIC at which visible bacterial growth first appeared (Collee *et al.,* 1996).

RESULTS AND DISCUSSION

The studied isolates were identified by a series of biochemical tests. *E. coli* and *K. pneumoniae* were found to be Gram-negative, whereas *Streptococcus viridans* and *Staphylococcus aureus* were Gram-positive. The oxidase test was negative for *E. coli*, *K. pneumoniae*, and *S. aureus*, but positive for *S. viridans*. In contrast, the catalase test was positive for *E. coli*, *K. pneumoniae*, and *S. aureus*, and negative for *S. viridans*. The β-lactamase test (β-lactam test) was negative for *E. coli* and *S. viridans*, but positive for *K. pneumoniae* and *S. aureus*. All three of *E. coli*, *K. pneumoniae*, and *S. viridans* were negative for the gelatin hydrolysis and coagulase tests, whereas *S. aureus* was positive for coagulase. In the motility and indole production tests, *E. coli* was the only strain that was positive for indole production and was motile; all other species were non-motile and indole-negative. In the methyl red test, *E. coli* and *S. aureus* were positive while *K. pneumoniae* and *S. viridans* were negative. Conversely, in the Voges-Proskauer and citrate tests, *K. pneumoniae* and *S. viridans* were positive, whereas *E. coli* and *S. aureus* were negative. Finally, *E. coli* was urease-negative, while the remaining species (*K. pneumoniae*, *S. aureus*, and *S. viridans*) were urease-positive. These results (Gram staining and biochemical tests) confirmed the identification of the bacterial species under study.

MIC and Sub-MIC of H_2O_2

The MIC and sub-MIC of hydrogen peroxide were determined by serial dilution(Collee *et al*., 1996). The results showed that the sub-MIC values of H_2O_2 for *K. pneumoniae*, *E. coli*, *S. viridans*, and *S. aureus* were 2, 30, 40, and 70 mM, respectively.

Biofilm Formation Assay

Bacterial biofilm formation occurs in five stages: (1) initial attachment of cells to each other or to a surface, (2) secretion of extracellular polymeric substances (especially polysaccharides), (3) early biofilm development, (4) maturation of the biofilm, and (5) formation of a mature biofilm (Siddhardha *et al*., 2020). Biofilm development is considered a defense mechanism that bacteria acquire or evolve to overcome external stresses, antibiotics, and host immune defenses (Fux *et al*., 2005).

Table 1. Biofilm formation by the studied bacterial species before and after exposure to hydrogen peroxide (H_2O_2) at the sub-MIC concentration.

Bacterial species	Before exposure	After exposure
E. coli	– (Absent)	– (Absent)
K. pneumoniae	– (Absent)	– (Absent)
S. aureus	+ (Weak)	– (Absent)
S. viridans	+ (Weak)	+++ (Strong)

(–) indicates absence of biofilm formation (Absent).
(+) indicates weak biofilm formation (Weak).
(++) indicates moderate biofilm formation (Moderate).
(+++) indicates strong biofilm formation (Strong).

As shown in Table 1, *S. aureus* lost its biofilm-forming ability after H_2O_2 treatment (from weak biofilm to none), whereas *S. viridans* showed a marked increase (from weak to strong biofilm formation). The variability in biofilm production among the tested bacteria may be explained by quorum sensing (QS) mechanisms. QS regulates many bacterial functions, including the production of rhamnolipids involved in stress responses. These rhamnolipids form a foam layer on the biofilm surface that limits oxygen penetration, thereby protecting the cells from toxic oxygen species (Pacheco et al., 2012; Malešević et al., 2019). QS also controls the expression of antioxidant enzymes (da Cruz Nizer et al., 2021); thus, only cells with active QS systems can survive and respond effectively to oxidative stress (Zhang *et al*.,2025). Mutations induced by oxidative stress may further alter biofilm formation. If such mutations increase biofilm production, this can enhance bacterial survival (Häussler and Becker, 2008), as seen with *S. viridans* becoming a strong biofilm producer after treatment. Conversely, mutations that disrupt biofilm formation can reduce survival, as observed with *S. aureus*. Additionally, the reactive oxygen species generated by H_2O_2 treatment can affect the production of exopolysaccharides (a key biofilm component), potentially weakening or enhancing biofilm production (Seder *et al*., 2023).

Antibiotic Susceptibility Before and After H_2O_2 Treatment

The antibiotic susceptibility of the bacterial isolates was tested before and after treatment with H_2O_2 at the sub-MIC level. The results (summarized in Table 2) indicate that H_2O_2 treatment altered the sensitivity of some bacterial species to certain antibiotics, while others showed no change. Importantly, all susceptibility readings remained within the normal ranges according to international standards (Vandepitte *et a*l., 1991; NCCLS, 2023).

The changes that observed in antibiotic susceptibility after H_2O_2 treatment can be explained by multiple factors. Hydrogen peroxide generates reactive oxygen species, especially the superoxide anion (O_2^-) and the hydroxyl radical (OH·), which react with cellular DNA and proteins, causing oxidation and damage (Ferdinandy, 2006). These reactive species can induce mutations in plasmids (mobile genetic elements carrying antibiotic resistance genes) (Flannagan *et al*., 2003; Sinha *et al*., 2011). Plasmids play a key role in the development and transfer of antibiotic resistance among bacteria (Kenneth *et al*., 2018). Thus, mutations in plasmids might lead to the loss of resistance genes, converting resistant bacteria into sensitive ones, or vice versa.

Table 2. Antibiotic susceptibility of the Gram-positive and Gram-negative bacteria under study to the tested antibiotics, before and after treatment with hydrogen peroxide (H_2O_2) at the sub-MIC level.

Antibiotics	Species							
	St. viridans		***S. aureus***		***E. coli***		***K. pneumoniae***	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Amikacin	S	S	S	R	S	R	S	I
AK(10 mcg)	20	18	24	13	18	13	19	16
Tetracycline	S	S	I	R	R	R	S	S
TE(30 mcg)	29	23	18	0	0	0	22	19
Penicillin G	S	S	R	R	R	R	R	R
P(10 Units)	35	29	0	0	9	0	0	0
Vancomycin	S	R	R	R	R	R	R	S
VA(30 mcg)	42	13	9	9	10	9	14	17
Gentamicin	I	S	S	R	S	I	R	R
Cn(10 mcg)	13	18	21	9	18	13	8	0
Rifampin	S	R	R	R	R	R	R	R
RA(5 mcg)	24	14	0	10	9	0	0	11
Kanamycin	S	I	S	I	S	I	I	R
K(30 mcg)	25	17	24	17	19	14	14	12

(Legend: S = Sensitive; R = Resistant; I = Intermediate.)

Also, vancomycin resistance is often encoded by the VanA gene on a plasmid (Jacobs, 2005). A mutation in this plasmid could disable the VanA gene, rendering *S. viridans* sensitive to vancomycin. Conversely, H_2O_2 may activate transposable elements in bacteria, such as the transposon Tn1546 carrying VanA (Srinivasan et al., 2002). The VanA-encoded enzyme modifies the D-Ala-D-Ala terminus of peptidoglycan precursors, leading to a thicker cell wall with reduced cross-linking. This modification results in the accumulation of altered peptide termini that act as false binding sites for vancomycin, preventing the antibiotic from binding effectively (Moller *et al*., 2023). This mechanism explains the development of vancomycin resistance observed in the treated bacteria.

Oxidative damage can also cause mutations in chromosomal genes. For instance, H_2O_2 can induce DNA base modifications and miscoding errors (da Cruz Nizer *et al*., 2021). Mutations in the 16S rRNA gene (rrs) can reduce the binding affinity of aminoglycosides, such as amikacin, to the bacterial ribosome (Ahmad and Mokaddas, 2014). Likewise, mutations in the rpoB gene (encoding the β-subunit of RNA polymerase) can reduce the binding of rifampin to its target (Campbell *et al*., 2001; Feklistov *et al*., 2008). Rifampin normally inhibits RNA synthesis by binding to the RNA polymerase (Scheinfeld, 2016), so changes in this target can lead to rifampin resistance. Furthermore, H_2O_2-induced damage may create mutations in bacterial genes encoding aminoglycoside-modifying enzymes, such as aminoglycoside acetyltransferases (AAC), phosphotransferases (APH), and nucleotidyltransferases (ADD) (Wang *et al*., 2022). These enzymes typically inactivate aminoglycoside antibiotics; mutations might disrupt their function, affecting antibiotic binding to the ribosome and leading to resistance to drugs like amikacin, gentamicin, and kanamycin. Similarly, oxidative stress may cause mutations in genes encoding ribosomal proteins or associated factors that affect tetracycline binding. Such mutations can alter the ribosomal structure (for example, blocking the tetracycline binding site or displacing tetracycline) (Markley and Wencewicz, 2018; Sheykhsarana et al., 2019), resulting in tetracycline resistance. On the other hand, the lack of change in β-lactam antibiotic susceptibility (e.g., penicillin G) can be attributed to their mechanism of action. β-lactams target penicillin-binding proteins (PBPs) in the bacterial cell wall (Zabiszak *et al.,* 2023). H_2O_2-induced damage may not significantly affect these PBPs or β-lactamase enzymes (da Cruz Nizer *et al.,* 2021), so the susceptibility to β-lactams remains unchanged. Another factor is the effect of H_2O_2 on the bacterial cell envelope. Oxidative damage can create pores and reduce membrane integrity (Imlay, 2013), increasing permeability. This would allow β-lactam antibiotics easier access to their target, the transpeptidase enzyme involved in peptidoglycan synthesis. Penicillins bind to the active site serine of transpeptidase, inhibiting cell wall cross-linking and thus cell wall synthesis (Sauvage and Terrak, 2016) . Increased membrane permeability could enhance antibiotic efficacy and reduce

observed resistance. Finally, the formation of biofilms can impact antibiotic resistance. Biofilms act as physical barriers that impede antibiotic penetration (Chen et al., 2023; Wang *et al*., 2025). In this study, *S. viridans* exhibited increased biofilm formation after H_2O_2 treatment, which was associated with a shift from vancomycin sensitivity to resistance (and increased rifampin resistance). In contrast, bacteria that showed reduced biofilm formation after treatment tended to exhibit decreased resistance (i.e., increased sensitivity) to antibiotics.

CONCLUSIONS

Acknowledgments

The authors acknowledge the valuable contributions of researchers and clinicians whose work has advanced our understanding of neuroplasticity in stroke recovery. No specific funding was received for this review.

REFERENCES

Abd-alwahab, W.I.A., Al-Assie, R. J.J, Kamil Azeez, A., & Kamil Ghadir, G. (2024). The effect of carotenoids of Rhodotorula glutinis and probiotic of Lactobacillus acidophilus on some physiological and histological variables of the pancreas and liver in male rats exposed to ultraviolet radiation. *Tikrit Journal for Agricultural Sciences*, *24*(2), 235-245.

Ahmad, S. & Mokaddas, E. (2014). Current status and future trends in the diagnosis and treatment of drug-susceptible and multidrug-resistant tuberculosis. *Journal of Infection and Public Health*, 7(2), 75–91.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493–496.

Campbell, E. A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A. & Darst, S. A. (2001). Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell*, 104(6), 901–912.

Chen, W., Xu, Z., Li, C., Wang, C., Wang, M., Liang, J. & Wei, P. (2023). Investigation of biofilm formation and associated genes in multidrug-resistant *Salmonella pullorum* in China (2018–2022). *Frontiers in Veterinary Science*, 10, 1248584.

Christensen, G. D., Simpson, W. A., Bisno, A. L. & Beachey, E. H. (1985). Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infection and Immunity*, 37, 318–326.

Collee, J. G., Marmion, B. P., Fraser, A. G. & Simmons, A. (1996). *Mackie & McCartney Practical Medical Microbiology* (14th ed.). Churchill Livingstone, New York.

da Cruz Nizer, W. S., Inkovskiy, V., Versey, Z., Strempel, N., Cassol, E. & Overhage, J. (2021). Oxidative stress response in *Pseudomonas aeruginosa*. *Pathogens*, 10(9), 1187.

Feklistov, A., Mekler, V., Jiang, Q., Westblade, L. F., Irschik, H., Jansen, R., Mustaev, A., Darst, S. A. & Ebright, R. H. (2008). Rifamycins do not function by allosteric modulation of binding of Mg^{2+} to the RNA polymerase active center. *Proceedings of the National Academy of Sciences of the USA*, 105(39), 14820–14825.

Ferdinandy, P. (2006). Peroxynitrite: Just an oxidative/nitrosative stressor or a physiological regulator as well. *British Journal of Pharmacology*, 148, 1–3.

Flannagan, S. E., Chow, J. W., Donabedian, S. M., Brown, W. J., Perri, M. B., Zervos, M. J., Ozawa, Y. & Clewell, D. B. (2003). Plasmid content of a vancomycin-resistant *Enterococcus faecalis* isolate from a patient also colonized by *S. aureus* with a VanA phenotype. *Antimicrobial Agents and Chemotherapy*, 47(12), 3954–3959.

Fux, C. A., Costerton, J. W., Stewart, P. S. & Stoodley, P. (2005). Survival strategies of infectious biofilms. *Trends in Microbiology*, 13, 34–40.

Häussler, S. & Becker, T. (2008). The pseudomonas quinolone signal (PQS) balances life and death in *Pseudomonas aeruginosa* populations. *PLoS Pathogens*, 4(9), e1000166.

Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nature Reviews Microbiology*, 11, 443–454.

Jacobs, M. (2005). Epidemiology and clinical implications of glycopeptide-resistant *Staphylococcus aureus*. *Infection and Disease*, 7, 1023–1028.

Jasim, R. J., & Al-juboory, Y. H. O. (2023). Effects of hydrogen peroxide and ferrous ions on the ability of Pseudomonas aeruginosa isolates to adhere to host cells in vitro. *African Journal of Biological Sciences*, 5(4), 15-22

Kenneth, J. R., Nafees, A., Alspaugh, J. A., Drew, W. L., Lagunoff, M., Pottinger, P., Reller, L. B. & Reller, M. E. (2018). *Sherris Medical Microbiology* (7th ed.). McGraw-Hill Education, New York, p. 1054.

Laylani, L. S., Abd-Alwahab, W. I. A., Ahmad, H. S., & Mustafa, M. A. (2024). The effect of carotenoids of Rhodotorula glutinis and probiotic of Lactobacillus acido-philus on physiological and histological variables of the kidney in male rats exposed to ultraviolet radiation. *Journal of Animal Health and Production*, *12*, s1.

Malešević, M., Di Lorenzo, F., Filipić, B., Stanisavljević, N., Novović, K., Senerović, L. & Jovčić, B. (2019). *Pseudomonas aeruginosa* quorum sensing inhibition by clinical isolate *Delftia tsuruhatensis* 11304: involvement of N-octadecanoylhomoserine lactones. *Scientific Reports*, 9(1), 16465.

Manful, C. F., Fordjour, E., Subramaniam, D., Sey, A. A., Abbey, L., & Thomas, R. (2025). Antioxidants and reactive oxygen species: shaping human health and disease outcomes. *International Journal of Molecular Sciences*, *26*(15), 7520.

Markley, J. L. & Wencewicz, T. A. (2018). Tetracycline-inactivating enzymes. *Frontiers in Microbiology*, 9, 1–17.

Moller, A. G., Petit III, R. A., Davis, M. H. & Read, T. D. (2023). Development of an amplicon nanopore sequencing strategy for detection of mutations conferring intermediate resistance to vancomycin in *Staphylococcus aureus* strains. *Microbiology Spectrum*, 11(1), e02722-e02728.

National Committee for Clinical Laboratory Standards (NCCLS). (2023). *Performance Standards for Antimicrobial Susceptibility Testing* (33rd ed.), NCCLS document M100, USA.

Pacheco, G. J., Reis, R. S., Fernandes, A. C. L. B., da Rocha, S. L. G., Pereira, M. D., Perales, J. & Freire, D. M. G. (2012). Rhamnolipid production: effect of oxidative stress on virulence factors and proteome of *Pseudomonas aeruginosa* PA1. *Applied Microbiology and Biotechnology*, 95(6), 1519–1529.

Pooja, G., Shweta, S., & Patel, P. (2025). Oxidative stress and free radicals in disease pathogenesis: a review. *Discover Medicine*, *2*(1), 104.

Rutherford, M. (2016). *Free Radicals & Health*. Nova Science Publishers, New York.

Sauvage, E. & Terrak, M. (2016). Glycosyltransferases and transpeptidases/penicillin-binding proteins: valuable targets for new antibacterials. *Antibiotics*, 5(12), 1–17.

Shawkat, M. A., & Abd-alwahab, W. I. (2024). Evaluation of the effect of the pesticides glyphosate and chlorpyrifos on the activity of some neuronal enzymes using milk and turmeric in laboratory albino Wistar rats. Journal of Applied and Natural Science, 16(4), 1662-1668.

Scheinfeld, N. (2016). Why rifampin (rifampicin) is a key component in the antibiotic treatment of hidradenitis suppurativa: a review of rifampin's effects on bacteria, bacterial biofilms, and the human immune system. *Dermatology Online Journal*, 22(6), 2–9.

Seder, N., Rayyan, W. A., O'la Al-Fawares, M. H. & Bakar, A. (2023). *Pseudomonas aeruginosa* virulence factors and antivirulence mechanisms to combat drug resistance: a systematic review. *Mortality*, 10, 11.

Sheykhsarana, E., Baghi, H. B., Sorousha, M. H. & Ghotaslou, R. (2019). An overview of tetracyclines and related resistance mechanisms. *Reviews in Medical Microbiology*, 30(1), 69–75.

Siddhardha, B., Dyavaiah, M. & Syed, A. (2020). *Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery* (1st ed.). Springer Nature Singapore, p. 684.

Sinha, V., Mishra, R., Kumar, A., Kannan, A. & Upreti, R. K. (2011). Amplification of arsH gene in *Lactobacillus acidophilus* resistant to arsenite. *Biotechnology*, 10, 101–107.

Srinivasan, A., Dick, J. D. & Perl, T. M. (2002). Vancomycin resistance in streptococci. *Clinical Microbiology Reviews*, 15(3), 430–438.

Vandepitte, J., Engbaek, K., Piot, P. & Heuck, C. C. (1991). *Basic Laboratory Procedures in Clinical Bacteriology*. World Health Organization, Geneva, p. 121.

Wang, D., Wang, L., Liu, Q., & Zhao, Y. (2025). Virulence factors in biofilm formation and therapeutic strategies for Staphylococcus aureus: A review. *Animals and Zoonoses*, *1*(2), 188-202.

Wang, N., Luo, J., Deng, F., Huang, Y. & Zhou, H. (2022). Antibiotic combination therapy: a strategy to overcome bacterial resistance to aminoglycoside antibiotics. *Frontiers in Pharmacology*, 13, 839808.

Zabiszak, M., Frymus, J., Ogawa, K., Skrobańska, M., Nowak, M., Jastrząb, R. & Kaczmarek, M. T. (2023). Complexes of β-lactam antibiotics and their Schiff-base derivatives as a weapon in the fight against bacterial resistance. *Coordination Chemistry Reviews*, 493, 215326.

Zhang, X., Zhang, D., Zhou, D., Zheng, S., Li, S., Hou, Q., ... & Han, H. (2025). A comprehensive review of the pathogenic mechanisms of Pseudomonas aeruginosa: Synergistic effects of virulence factors, quorum sensing, and biofilm formation. *Frontiers in Microbiology*, *16*, 1619626.