ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2025, 10 (2): 342-351 doi: 10.28978/nesciences.1744935

Integrative Evaluation of adeA and adeS Efflux Gene Expression, Biofilm Production, and Antimicrobial Resistance in Clinical Isolates of Acinetobacter Baumannii

Inas Sattar Abd [1*], Halah Farazdaq [2], Akhlas Na'ama Khudair [3]

[1*] Lecture, Department of Microbiology, College of Science, Al-karkh University of Science, Bagdad, Iraq. E-mail: inasabd4@gmail.com

[2] Assistant Lecture, Department of Microbiology, College of Science, Al-karkh University of Science, Bagdad, Iraq. E-mail: halah.farazdaq@kus.edu.iq

[3] Assistant Lecture, Department of Microbiology, College of Science, Al-karkh University of Science, Bagdad, Iraq. E-mail: akhlasnaama@kus.edu.iq

Abstract

Acinetobacter baumannii has crystallized into a formidable adversary within clinical environments, primarily attributable to its adeptness at biofilm formation and its notable resistance to a spectrum of antibiotics. This study included 10 clinical isolates of A. baumannii, assessing their biofilm production capabilities, antibiotics resistance profiles, and expression levels of efflux pump genes (adeA besides adeS). Notably, only 10 isolates (33.3%) demonstrated quantifiable biofilm production, bifurcated into 5 categorized as moderate biofilm producers (optical density range: 0.638–0.845) and 5 as strong producers (optical density range: 0.980–1.67). Antibiotic susceptibility evaluations conducted via the VITEK 2 system illuminated that 80% of the isolates exhibited resistance to imipenem, while 60% displayed resistance to amikacin, contrastingly, merely 13.3% manifested resistance to colistin. Gene expression analysis unveiled a broad spectrum of fold changes in adeA expression (0.0089 to 9.11) and adeS (0.0161 to 28.73). Multidrug-resistant (MDR) isolates were characterized by a markedly higher median fold expression for adeS (13.4) in comparison to non-MDR counterparts (1.46), nevertheless this disparity did not influence statistical significance ($p > 0.05$). Likewise, a comparative analysis of biofilm phenotypes indicated that moderate biofilm producers exhibited marginally elevated median expression levels of adeA (3.7) relative to strong producers (1.4), yet once again, this difference lacked statistical significance. A resistance heatmap elucidated pronounced clustering of high resistance among MDR isolates, particularly regarding beta-lactams and aminoglycosides. These findings imply that while expression levels of adeA and *adeS* are indeed variable and sometimes heightened in MDR or biofilm-forming isolates, a consistent correlation remains elusive. This highpoints intricate in addition to multifaceted nature of

*Corresponding Author: Inas Sattar Abd, E-mail: inasabd4@gmail.com

antimicrobial resistance and biofilm regulation in A. baumannii, advocating for expansive molecular profiling to unearth more dependable resistance markers.

Keywords:

adeA, adeS, antimicrobial, biofilm, efflux, pumps.

Article history:

Received: 01/04/2025, Revised: 14/06/2025, Accepted: 18/07/2025, Available online: 30/08/2025

Introduction

Acinetobacter baumannii recently has became an adversary risk for healthcare environments due to its ability to persist once it establishes colonization on various surfaces (Nguyen & Joshi, 2021). A. baumannii not only survives in the extreme conditions but flourishes in clinical states that is characterized by extensive antibiotic usages (DeFlorio et al., 2021). Its ability to produce biofilm significantly enhances its resistance to immune responses and also to various medications which makes its infections persistent and challenging to manage (Almatroudi, 2025).

Biofilm is an extracellular matrix that effectively shields bacteria from the detrimental effects of antibiotics (Deshmukh-Reeves et al., 2025). Biofilms create a cohesive aggregates of bacteria to surfaces, and these aggregates ussually found on medical apparatus such as catheters and ventilators (Gedefie et al., 2021). Within these combinations of bacteria, bacteria are sheltered in self-produced extracellular matrix (Sharma & Subramanian, 2025). As a result, infections linked to biofilm formation frequently require long period of treatment plans, and in extreme cases, the total removal of compromised devices may be necessary (Haidar et al., 2024).

Previous studies focused on clarifying the genetic frameworks that promote biofilm formation and antibiotic resistance in Acinetobacter baumannii (Dubois, 2023; Singh et al., 2022). AdeABC efflux pump system, a group of genes that enable bacterium to eliminate toxic substances, such as antibiotics, before causing cellular damage, is a crucial element in this context (Sharma et al., 2023). The resistance mechanism is dependent on essential genes like *adeA*, which is responsible of encoding a prtein that helps assemble the efflux pump, and adeS, which encode a protein that controls the timing and strength of pump activation. (Roemhild et al., 2022). Previous studies have showed the overexpression of these genes can lead to the bacterium acquiring multidrug resistance (Henderson et al., 2021).

Furthermore, this efflux system seems to have an influence on biofilm architecture along with drug resistance (Ramadan et al., 2024). Clinical isolates exhibiting elevated levels of adeA and adeS expression tend to generate more robust and resilient biofilms (AL-Azzawi et al., 2024). The precise mechanisms underlying this relationship remain somewhat elusive, yet it is plausible that the same signaling pathways driving antibiotic resistance may concurrently promote biofilm proliferation (Kaushik et al., 2022).

While the AdeABC efflux system is a well-documented contributor to resistance and is implicated in biofilm development, the consistency of this correlation across diverse clinical isolates remains debated. It is unclear whether the expression levels of regulatory genes like *adeA* and *adeS* can serve as reliable predictive markers for multidrug resistance or high biofilm-forming phenotypes (Kumar & Sunil, 2024). Consequently, this study purposed to investigate affiliation amid *adeA* and adeS expression, biofilm production, and antimicrobial resistance profiles in set of contemporary clinical isolates of A. baumannii from Baghdad. Our

goal was to determine if a direct, quantifiable link exists that could inform clinical predictions and treatment strategies.

Methods

Bacterial Isolates and Culture Conditions

For this study, 30 clinical *Acinetobacter baumannii* isolates were acquired from patient samples (respiratory, urinary, and wound) at hospitals in Baghdad, Iraq. The VITEK 2 Compact system (bioMérieux, France) was used for species confirmation of each isolate. Prior to experimental use, isolates were revived on nutrient agar from glycerol stocks that had been preserved at –80°C.

Quantification of Biofilm Formation

The evaluation for the ability for formation of biofilm was done via stain a crystal violet adapted for 96-well microtiter plates. After overnight cultures were diluted in sterile normal saline to turbidity of (0.5) McFarland standard, started by making bacterial suspensions. Then 180 of brain heart infusion broth (Oxoid, UK) was added to each well of the microtiter plates containing 1% glucose, into which inoculate 20 µL of a bacterial suspension. The plates were left to incubate aerobically for 24 hrs. at 37°C.

To get an estimation for the adhered biofilm, wells were washed three times with phosphate-buffered saline (PBS) to become rid of cells that weren't attached. Then the rest of biofilm were fixed with methanol, dyed them with a 1% crystal violet solution, and then washed them again (Verma & Nair, 2025). After allowing the plates to air-dry, the bounded stain were resolubilized by adding 95% ethanol and optical density (OD) at 630 nm recorded using a microplate reader. An isolate's biofilm production was regarded for instance non-biofilm, weak, moderate, or strong based on its OD measurement relative to a calculated cutoff value (ODc), distinct as mean OD of control wells plus three standard deviations (Nguyen & Joshi, 2021).

From the initial thirty clinical isolates screened, only ten demonstrated quantifiable biofilm formation (OD > ODc) under these in vitro conditions. These ten biofilm-producing isolates were subsequently used for all further analyses, including antimicrobial susceptibility testing and gene expression studies (Kapoor et al., 2025).

RNA Extraction and cDNA Synthesis

Total RNA were extracted from isolates of A. baumannii using Quick-RNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to manufacturer’s protocol. Bacterial pellets were homogenized in ZR BashingBead™ Lysis Tubes. After centrifugation, the resulting lysates were passed through Zymo-Spin™ columns to purify the RNA. A NanoDrop™

Spectrophotometer was used to measure quantity besides purity of extracted RNA.

Conversion of RNA to cDNA performed by using Kit PrimeScript™ RT Reagent (TAKARA, Japan) in final reaction volume of 10 µL (Ansari & Parmar, 2024). Respectively reaction contained 1 µg of total RNA, random hexamers, besides reverse transcriptase. Then reaction tubes placed in a thermal cycler machine which programmed previously to incubate tubes at temperature 37 C for 15 min then for enzyme inavtivation temperature increases to 85 C for 5 seconds.

Real-Time PCR (qPCR) Analysis

For estimation of the targeted genes expression, qRT-PCR was used. And inorder to achieve this effeciently, a commerial kit KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems, USA) has been used in RT-PCR reaction. For the final volume of each tube 20 µL reaction mixture was sat up and containing the following additives, 10.0 µL of 2× Master Mix, 0.4 µL of both forward and reverse primer with final concentration of 0.2 µM, 3 µL of cDNA template, besides 16.2 µL of nuclease-free water. Primers and respective annealing temperatures used in this study are detailed in Table (1):

Table 1. Primers used for qPCR analysis

Primer	Sequence	Length (bp)	GC Content (%)	Tm (°C)	Reference
adeA F	TTGATCGTGCTTCTATTCCTCAAG	24	41.7	68	(Sun et al., 2012)
adeA R	GGCTCGCCACTGATATTACGTT	22	50	66	
adeS F	TTCAACAAGAAGATTGACC	19	36.8	52	
adeS R	CTTGCTCAATACGG	14	50	42	
16S rRNA F	CCACACTGGGACTGAGACAC	20	60	64	(Al-Imam et al., 2024)
16S rRNA R	CCACTCCCGCTAACGTTCTT	20	55	62	

The thermal cycling process was directed under the following thermal conditions and timing: an initial denaturation at 94°C for 5 minutes, hounded by 40 cycles of 94°C for 30 sec., a primer-specific annealing phase (see Table 1) for 30 sec., besides extension at 72°C for 45 sec. The final extension was performed in 72°C for 5 minutes. Using melt curve analysis, the specificity of amplification was confirmed. The 2–ΔΔCt technique was used to quantify relation gene expression, and the endogenous reference gene was 16S rRNA.

Antimicrobial Susceptibility Testing

GN-specific AST cards and the VITEK 2 Compact system were used to measure antimicrobial susceptibility. Colonies from a new culture were suspended in sterile saline to turbidity that matched the 0.5 McFarland standard in order to create inocula. According to criteria of Clinical and Laboratory Standards Institute (CLSI), this system automatically determined Minimum Inhibitory Concentrations (MICs) via analyzing growth kinetics. The findings were then interpreted as susceptible, intermediate, or resistant.

Results

Out of thirty clinical *A. baumannii* isolates, ten exhibited measurable biofilm formation based on the microtiter plate assay. Among these, five were classified as moderate biofilm producers and five as strong producers. As illustrated in Figure (1), the mean OD_{630} for the strong producers was significantly (p-value = 0.008) higher than that of the moderate group (mean ± SE: 1.30 ± 0.10 vs. 0.76 ± 0.03, respectively).

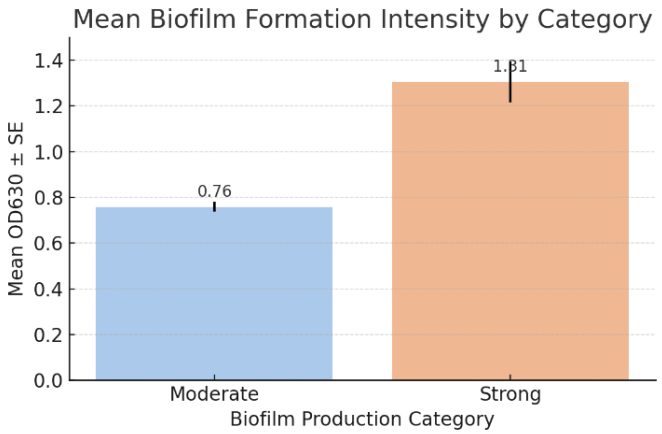

Figure 1. Mean ± S.E. of the OD for biofilm production isolates

Antimicrobial susceptibility testing of the ten Acinetobacter baumannii isolates revealed high levels of multidrug resistance. All isolates (100%) were resistant to imipenem, and 90% showed resistance to trimethoprim/sulfamethoxazole. Gentamicin and amikacin resistance were observed in 80% and 70% of isolates, respectively. Resistance to tobramycin was noted in 60% of cases, while only 50% were resistant to meropenem. In contrast, ciprofloxacin exhibited the lowest resistance rate, with 40% of isolates being resistant and several exhibiting either sensitivity or intermediate susceptibility. These findings demonstrate the widespread resistance of clinical A. baumannii isolates to multiple antibiotic classes, which is consistent with their classification as multidrug-resistant pathogens.

A detailed visualization of resistance levels across antibiotics and isolates is provided in the heatmap (Figure 2). The heatmap illustrates individual susceptibility patterns, where resistance (R), intermediate susceptibility (I), and sensitivity (S) are numerically encoded as 1, 0.5, and 0, respectively.

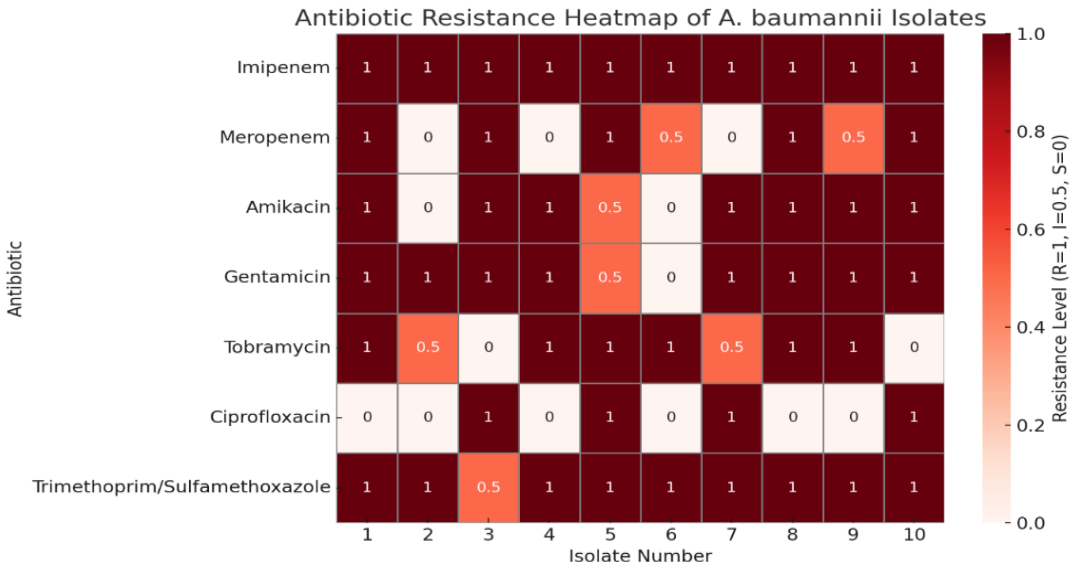

Figure 2. Antibiotic resistance heatmap of ten clincal isolates of Acinetobacter baumannii.. Each cell represents the resistance interpretation of a given isolate to a specific antibiotic, encoded as: Resistant (R = 1), Intermediate (I = 0.5), and Sensitive (S = 0). Darker shades indicate higher levels of resistance. The heatmap highlights widespread resistance to imipenem, trimethoprim/sulfamethoxazole, and aminoglycosides.

To Investigate the potential association between efflux pump gene expression besides multidrug resistance (MDR) in *Acinetobacter baumannii*, we compared the fold change of adeA and adeS expression levels between MDR and non-MDR isolates. Although isolates classified as MDR generally exhibited higher median expression levels of both genes, the difference was not statistically significant.

Specifically, the median fold change of adeA in MDR isolates was higher than in non-MDR isolates; however, Mann–Whitney U test produced p-value of 0.222, designating no significant difference between groups. A similar trend was observed for adeS expression, where the p-value was also 0.222, failing to reach statistical significance. These outcomes submit that while efflux pump overexpression is common in *A. baumannii*, it may not independently predict MDR phenotype within the small sample size analyzed.

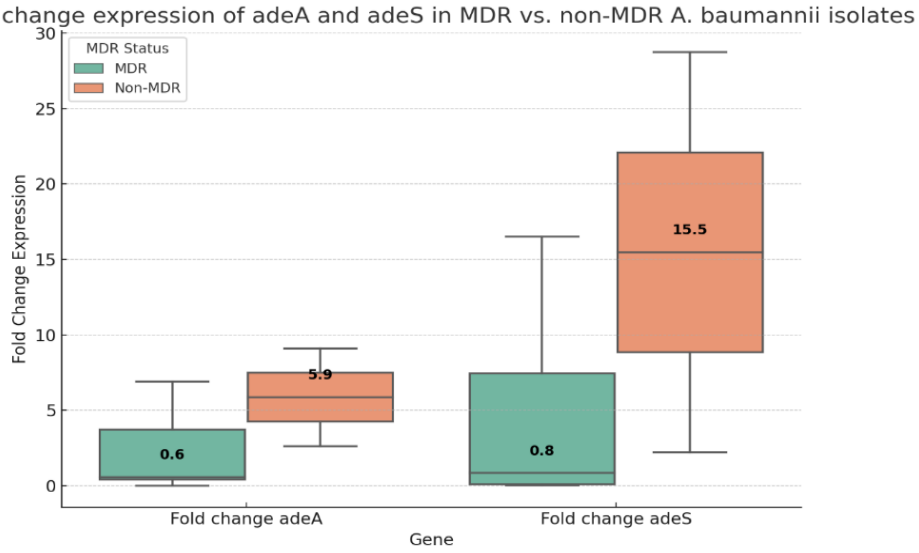

Figure 3. The boxplot showing fold change expression of *adeA* and *adeS* in MDR vs. non-MDR *Acinetobacter baumannii* isolates.

To explore whether efflux pump gene expression correlates with biofilm production, we compared the fold change expression of *adeA* and *adeS* between isolates categorized as moderate and strong biofilm producers. Although the mean expression levels of both genes were numerically higher in strong biofilm producers, no statistically significant differences were found.

Specifically, Mann–Whitney U test generated p-values of 0.730 for adeA and 0.905 for *adeS*, indicating that variation in gene expression does not clearly distinguish between moderate and strong biofilm-forming phenotypes within this group of isolates.

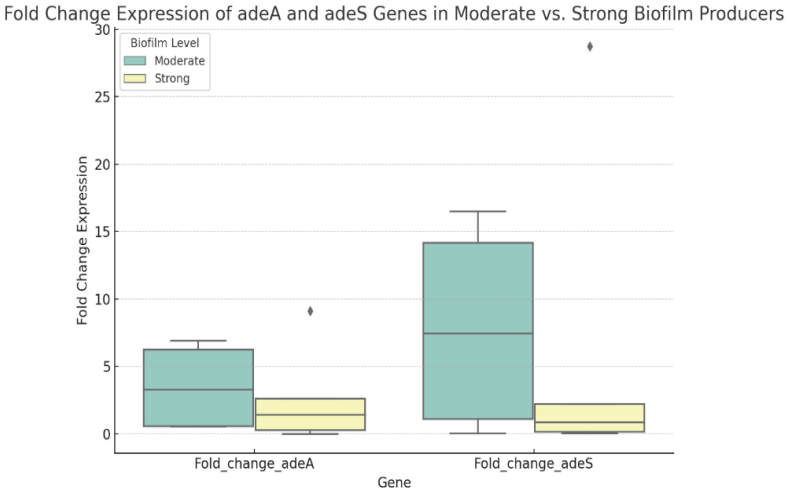

Figure 4. Fold change expression of *adeA* and *adeS* genes in moderate versus strong biofilm-producing *A. baumannii* isolates.

Discussion

This study propositions new visions into complex interplay amid antimicrobial resistance, biofilm formation, and efflux pump gene expression in clinical isolates of Acinetobacter baumannii. Although several multidrug-resistant (MDR) and strong biofilm-producing isolates demonstrated elevated fold changes in adeA and adeS expression, these increases did not reach statistical significance. These findings suggest a possible association between efflux pump activity and resistance or biofilm intensity, but they stop short of establishing a definitive link, likely due to biological variability and the limited sample size.

Previous research has highlighted the role of AdeABC efflux system in promoting in cooperation drug resistance and biofilm formation. Ruzin et al. conveyed that overexpression of adeA and adeS was allied with diminished susceptibility to aminoglycosides, fluoroquinolones, and tigecycline (Ruzin et al., 2007). Similarly, Yoon and colleagues observed that high expression of AdeABC components corresponded with the development of thicker, more resilient biofilms, especially under antibiotic stress (Yoon et al., 2013). The current study aligns with these observations to some extent, as isolates with strong biofilm formation and MDR status tended to show higher gene expression levels. However, the lack of statistical significance suggests that efflux pump expression alone may not fully explain the phenotypic outcomes observed.

Contradictory findings in the literature further emphasize the complexity of this relationship. Marchand et al. found no consistent correlation between AdeABC expression and biofilm development, pointing instead to the potential influence of other regulatory pathways or surface-associated proteins (Marchand et al., 2004). Likewise, Kuo et al. described isolates capable of forming substantial biofilms despite the absence of notable efflux pump overexpression, reinforcing the view that biofilm formation is governed by multiple, interrelated mechanisms.

In our assessment of biofilm production, only 10 of the 30 isolates demonstrated measurable biofilm formation, with most classified as moderate producers. This proportion is proportionate with outcomes of Pour et al., who noted that only subset of clinical A. baumannii isolates form significant biofilms under in vitro conditions (Kuo et al., 2012). The variability observed in biofilm strength may be shaped by environmental adaptation, differences in surface adhesins, or gene regulation beyond the AdeABC system (Livak & Schmittgen, 2001).

Antibiotic resistance profiling through the VITEK 2 platform revealed a high rate of resistance to carbapenems and aminoglycosides, in keeping with global surveillance data on A. baumannii. Yet, no statistically significant association emerged between resistance patterns and the expression levels of adeA or adeS (Peleg et al., 2008). This indicates that while efflux systems contribute to resistance, they likely act in synergy with other mechanisms, for instance production of β-lactamase, alteration of outer membrane porin, and modifications in target site.

The results of none-significant correlation between *adeA*/*adeS* genes expression, MDR, and biofilm production showed a pivotal findings (Peleg et al., 2008). This suggest tht inaddition of the importance of those genes expression on the overall resistance profile but their expression is not the standalone predictors and there are other mechanisms likely play a dominant role. As an example of other possible pathways are β-lactamase production or outer membrane porin alterations that can caused the antibiotic resistance in our samples (Pour et al., 2020).

Overall, our findings reflect the multifactorial nature of both resistance and biofilm development in A. baumannii. While adeA and adeS expression may contribute to these traits, they are not sufficient as standalone indicators. Future investigations using larger sample sizes, complemented by functional assays such as gene knockouts or efflux inhibition studies are necessary to clarify the specific contributions of these genes. Importantly, these data support the view that targeting efflux systems in isolation is unlikely to be an effective therapeutic strategy. A more comprehensive approach, one that addresses both genetic regulation and the physical architecture of biofilms, may offer greater promise in treating persistent Acinetobacter baumannii infections in clinical settings.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors' contributions are equal for the preparation of research in the manuscript.

References

AL-Azzawi, M. K., Hasan, N. A., & Barrak, M. M. (2024). A review of the development of an understanding of antibiotic interactions, from mechanisms of action to novel resistance and the search for natural alternatives. *Journal of Medical Genetics and Clinical Biology*, *1*(6), 78-102.

Al-Imam, M. J., Abbood, A. S., Faisal, A. J., & Abbas, M. S. (2024). Evaluation of the effect of Cefotaxime on gene expression of *Eno* in *Pseudomonas aeruginosa*. *Pakistan Journal of Life and Social Sciences*, *22*(1), 2262–2268.

Almatroudi, A. (2025). Biofilm resilience: Molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology*, *14*(2), 165. https://doi.org/10.3390/biology14020165

Ansari, H., & Parmar, J. (2024). Tracing Human Evolution through Ancient DNA: Insights from Paleogenomic Studies. *Progression journal of Human Demography and Anthropology*, 13-16.

DeFlorio, W., Liu, S., White, A. R., Taylor, T. M., Cisneros-Zevallos, L., Min, Y., & Scholar, E. M. (2021). Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and

cross-contamination of food contact surfaces by bacteria. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 3093-3134. https://doi.org/10.1111/1541-4337.12750

Deshmukh-Reeves, E., Ryan, F., & Gourlay, C. W. (2025). Formation and Prevention of Biofilms on Airway Management Devices. In *Fungal Biofilms* (pp. 55-80). Cham: Springer Nature Switzerland.

Dubois, T. (2023). A Hybrid Genetic Algorithm for Multi-Objective Job Shop Scheduling in Manufacturing Systems. *International Academic Journal of Science and Engineering, 10*(3), 35–39. https://doi.org/10.71086/IAJSE/V10I3/IAJSE1030

Gedefie, A., Demsis, W., Ashagrie, M., Kassa, Y., Tesfaye, M., Tilahun, M., ... & Sahle, Z. (2021). Acinetobacter baumannii biofilm formation and its role in disease pathogenesis: a review. *Infection and drug resistance*, 3711-3719.

Haidar, A., Muazzam, A., Nadeem, A., Atique, R., Saeed, H. A., Naveed, A., ... & Samad, A. (2024). Biofilm formation and antibiotic resistance in Pseudomonas aeruginosa. *The Microbe*, *3*, 100078.

Henderson, P. J., Maher, C., Elbourne, L. D., Eijkelkamp, B. A., Paulsen, I. T., & Hassan, K. A. (2021). Physiological functions of bacterial "multidrug" efflux pumps. *Chemical reviews*, *121*(9), 5417-5478.

Kapoor, T., Kansal, A., Mohamed Jaffar, A., Venkatesan, D., Sarmah, R. G., Renuka Jyothi, R., & Verma, S. (2025). Nutritional innovations in aquafeed for sustainable and eco-friendly fish farming. *International Journal of Aquatic Research and Environmental Studies, 5*(1), 685–695. https://doi.org/10.70102/IJARES/V5I1/5-1-61

Kaushik, V., Tiwari, M., Joshi, R., & Tiwari, V. (2022). Therapeutic strategies against potential antibiofilm targets of multidrug-resistant Acinetobacter baumannii. *Journal of cellular physiology*, *237*(4), 2045-2063.

Kumar, R. B., & Sunil, K. (2024). Biotechnological Approaches to Develop Personalized Medicines for Rare Genetic Disorders. *Clinical Journal for Medicine, Health and Pharmacy*, *2*(2), 20-28.

Kuo, S. C., Chang, S. C., Wang, H. Y., Lai, J. F., Chen, P. C., Shiau, Y. R., ... & TSAR Hospitals. (2012). Emergence of extensively drug-resistant Acinetobacter baumannii complex over 10 years: nationwide data from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program. *BMC infectious diseases*, *12*(1), 200. https://doi.org/10.1186/1471-2334-12-200

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods*, *25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Marchand, I., Damier-Piolle, L., Courvalin, P., & Lambert, T. (2004). Expression of the RND-type efflux pump AdeABC in Acinetobacter baumannii is regulated by the AdeRS two-component system. *Antimicrobial agents and chemotherapy*, *48*(9), 3298-3304. https://doi.org/10.1128/aac.48.9.3298-3304.2004

Nguyen, M., & Joshi, S. G. (2021). Carbapenem resistance in Acinetobacter baumannii, and their importance in hospital-acquired infections: a scientific review. *Journal of applied microbiology*, *131*(6), 2715-2738.

Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). Acinetobacter baumannii: emergence of a successful pathogen. *Clinical microbiology reviews*, *21*(3), 538-582. https://doi.org/10.1128/cmr.00058-07

Pour N., Gholami M., Hashemi A., & Goudarzi H. (2020). Detection of biofilm formation in *Acinetobacter baumannii* and its association with antibiotic resistance and genetic markers. *Journal of Infection in Developing Countries.14*(7):804–811.

Ramadan, A. E., Elgazar, A. S., Amin, N. A., Allam, A. H., Elmahdy, M. A., Eldeen, N. A., ... & Shaker, D. A. (2024). Efflux pumps encoding genes (adeA and adeS) in relation to antibiotic resistance pattern in Acinetobacter baumannii strains isolated from Benha university hospital. *The Egyptian Journal of Chest Diseases and Tuberculosis*, *73*(3), 241-247.

Roemhild, R., Bollenbach, T., & Andersson, D. I. (2022). The physiology and genetics of bacterial responses to antibiotic combinations. *Nature Reviews Microbiology*, *20*(8), 478-490.

Ruzin, A., Keeney, D., & Bradford, P. A. (2007). AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in Acinetobacter calcoaceticus–Acinetobacter baumannii complex. *Journal of Antimicrobial Chemotherapy*, *59*(5), 1001-1004. https://doi.org/10.1093/jac/dkm058

Sharma, P., & Subramanian, K. (2025). Molecular Mechanisms of Antibiotic Resistance in Bacteria. *In Medxplore: Frontiers in Medical Science (pp. 19-36). Periodic Series in Multidisciplinary Studies.*

Sharma, S., Kaushik, V., Kulshrestha, M., & Tiwari, V. (2023). Different efflux pump systems in Acinetobacter baumannii and their role in multidrug resistance. *Advances in Microbiology, Infectious Diseases and Public Health: Volume 17*, 155-168.

Singh, A., Amod, A., Pandey, P., Bose, P., Pingali, M. S., Shivalkar, S., ... & Samanta, S. K. (2022). Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. *Biomedical Materials*, *17*(2), 022003.

Sun, J. R., Perng, C. L., Chan, M. C., Morita, Y., Lin, J. C., Su, C. M., ... & Chiueh, T. S. (2012). A truncated AdeS kinase protein generated by IS Aba1 insertion correlates with tigecycline resistance in Acinetobacter baumannii. *PLoS One*, *7*(11), e49534. https://doi.org/10.1371/journal.pone.0049534

Verma, A., & Nair, R. (2025). Chromatographic Methods for the Separation of Naturally Occurring Bioactive Compounds and Their Applications in Industry. *Engineering Perspectives in Filtration and Separation*, 18-24.

Yoon, E. J., Courvalin, P., & Grillot-Courvalin, C. (2013). RND-type efflux pumps in multidrug-resistant clinical isolates of Acinetobacter baumannii: major role for AdeABC overexpression and AdeRS mutations. *Antimicrobial agents and chemotherapy*, *57*(7), 2989-2995. https://doi.org/10.1128/aac.02556-12