Readers Insight
Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Systematic Review	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v7i3.3147	Copyright © All rights are reserved by Corresponding Author
Vol. 7 No. 3, 2024: pp. 537-544	
www.readersinsight.net/pjmls	**Revised:** August 28, 2024 **Accepted:** September 29, 2024
Submission: June 30, 2023	**Published Online:** September 30, 2024

MULTI-PATHOGEN AMINOGLYCOSIDE RESISTANCE: A DUAL APPROACH IN INVESTIGATING BACTERIA ACROSS VARIOUS CLINICAL INFECTIONS

Umar Zeb[1], Falaque Naz[2], Muneer Ahmed[3], Sumia Ishfaq[1], Yousra[4], Muhammad Sheryar[5], Muhammad Arsalan[6*]

[1]**Department of Microbiology**, Kohat University of Science and Technology, Kohat, Pakistan
[2]**Department of Medical Laboratory Technology,** Liaquat University of Medical and Health Sciences, Jamshoro, Sindh, Pakistan
[3]**Department of Community Medicine,** Baqai Medical University, Karachi, Pakistan
[4]**Department of Zoology,** Islamia University of Bahawalpur, Bahawalnagar Campus, Pakistan
[5]**Department of Biosciences,** COMSATS University, Islamabad, Pakistan
[*6]**Department of Molecular Biology,** University of Florence, Florance, Italy
***Corresponding Author:** Muhammad Arsalan E. mail: muhammadarsalanshahpath01@gmail.com

Abstract

Aminoglycosides are vital antibiotics for treating severe Gram-negative bacterial infections, but the evolution of resistance mechanisms creates a huge treatment hurdles. This work reviews mechanisms of resistance to aminoglycoside such as AMEs, efflux pumps, ribosomal mutations and enzymatic modifications across several pathogens: It also examines the clinical significance of the resistance in different contexts of infection. A contextual and comparative case study investigated genetic and phenotypic resistance to aminoglycosides. A systematic review using PRISMA standards looked at clinical trials published between 2010 and 2023 on PubMed and ClinicalTrials.gov. Inclusion criteria for 100 papers on aminoglycoside-resistant Gram-negative infections (Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa) were studies that described mechanisms of aminoglycoside resistance as well as clinical outcomes. Exclusion criteria eliminated research that lacked significant data on pathogen resistance. I2 statistical method was used meta-analytic approach. Finally, 40 articles were reviewed for resistance mechanisms and cross-resistance to other antibiotics. 12 studies were included in the table. AMEs were the most prevalent mechanism of resistance, especially in E. coli and K. pneumoniae. AMEs were also implicated in treatment failures and post-therapy relapses. Among the antibiotics tested, aminoglycoside concentrations were significantly lower in K. pneumoniae and P. aeruginosa, and this decrease was associated with efflux pump overexpression in both bacteria, providing cross-resistance with β-lactams and fluoroquinolones. Hence these mutations are associated with extended hospital stays and poor outcomes in patients. Substantial cross-resistance rates add to the complexity of management interventions. Resistance of multiple pathogenic organisms to the aminoglycoside class of antibiotics remains a major obstacle in clinical infection practice. These results highlight the importance of regular genotypic surveillance of resistance patterns and the necessity to discover specific drugs for mechanisms such as AME inhibitors or efflux pump inhibitors. Precise treatment protocols drawn from pathogen specific resistance mechanisms could enhance clinical efficacy while reducing transmission of MDR pathogens.

Keywords: *Aminoglycoside resistance, E. coli, Clinical infections, Combination therapy, K. pneumoniae, Multi-pathogen, P. aeruginosa, Resistance mechanisms*

INTRODUCTION

Aminoglycosides are a very important class of antibiotics that had been used frequently in clinical medicine for treatment of various serious bacterial infections particularly those due to Gram negative bacteria including E. coli, K. pneumoniae and P. aeruginosa (1). Famously considered for their fast killing ability towards bacteria, aminoglycosides come into the picture by attaching themselves to the 30S ribosomal position and thereby inhibiting protein manufacture in the bacteria (2). Especially in hospital settings where multidrug-resistant (MDR) bacteria are common, this mechanism offers a crucial line of protection. (3). This papers showed that the infections include sepsis or other serious infections like endocarditis, respiratory and urinary tract infections where aminoglycosides continue to play an important

role in the practice of medicine. The recent development of aminoglycoside resistance by bacterial species issues a threat to the clinical applications of the antibiotic drugs. These efficient mechanisms of resistance: aminoglycoside-modifying enzymes (AMEs), efflux pumps, and ribosomal mutations cause treatment failures, poor patient outcomes, and incorporate additional healthcare burdens. This problem underscores the necessity for further researches upon the nature of the resistance mechanisms and new approaches for its treatment (4).

Years of choosy overuse and misuse of these aminoglycosides following their discovery had resulted in the development of resistance (5). The major limitation to aminoglycoside action is efficient bacterial resistance mechanisms, which bring about bacterial multiplication irrespective of the drug presence hence poor patient outcome. The development of such mechanisms had been reported in different clinical settings with respect to different pathogens and hence biochemical and genetic studies to explore the bases of bacterial resistance to aminoglycosides. Recognizing these resistance pathways are important to develop strategies that will halt bacterial dissemination and avoid treatment failures (6).

Aminoglycoside resistance occurs through several major pathways as that shown below. Aminoglycoside-modifying enzymes (AMEs) appear to be amongst the most well-established methods of resistance where the enzymes bring about changes to the chemical structure of aminoglycosides to prevent them from complexing with the target molecule (7). AMEs that include acetyltransferases, phosphotransferases and nucleotidyltransferases modify the functional groups on the aminoglycoside molecule and thereby inactivates it. This enzymatic resistance is a major problem, as many AMEs are located on mobile genetic elements, including plasmids, which can be transferred between bacterial species, thereby increasing the rate of resistance in different bacterial strains.

Further, another mechanism of resistance is modification of the bacterial ribosome, including changes of the aminoglycosides binding sites (8). These mutations affect the ribosomal point by a way that aminoglycoside cannot bind and are more frequently reported in those chronic infections where long use of the drug can exert selective pressure for such mutations (9). The concentration of antibiotics inside cells can be decreased by structural alterations to the bacterial cell wall and membrane that prevent aminoglycoside penetration (10). This mode of resistance is particularly notable in *P. aeruginosa* whereby intrinsic resistance is compounded by contributions from both low permeability and efflux mechanisms (11).

Another representative of the resistance mechanisms is efflux pumps, ejecting aminoglycosides from the bacterial cell before the drug accumulates enough concentration to be toxic for the bacterium. This also affects the intracellular accumulation of the aminoglycosides by decreasing it to suboptimal levels due to overexpression of efflux pumps. Furthermore, efflux pump competes to transport other classes of antibiotics resulting in the cross resistance with β-lactams, fluoroquinolones and tetracyclines (12). The presence of cross-resistance creates a problem of limited treatment options for clinicians when treating infections caused by multidrug-resistant pathogens, and complicates the approaches to managing infections. Since pathogens become resistant to several classes of antibiotics, including aminoglycosides, the number of choices that remain for the clinicians are fewer; instead they have to increase the doses or use one or the other antibiotics in combination therapy that has more chances of toxicity.

Beyond the outcomes of individual patients, aminoglycoside resistance has a significant negative influence on healthcare, increasing hospital stays, medical expenses, and mortality rates from infections that are hard to treat (13). The resistant forms of infection imply the use of the other therapeutic approaches which may result in lower efficacy, or more side effects, thus, the aggravated load on the healthcare systems and economies. The increasing rates of MDR infections therefore reinforce the requirement for an understanding of resistance mechanisms of aminoglycosides both for prescribing and prevention.

This research sought to use both genotypic and phenotypic analysis of aminoglycoside resistance among the various clinically isolated bacteria (14). This study aimed to investigate the resistance patterns, and determined the frequency of having specific mechanisms by sequencing the resistance genes and evaluating their phenotypes in the clinic to catalogue current resistance patterns and the mechanisms underlying them. Combining results of clinical samples with the laboratory investigation gave better

insight of how aminoglycoside-resistant bacteria behave, thus improving the understanding of the resistance mechanisms in healthcare settings (15). Furthermore, practice objectives in the study involved evaluating possible ways to combat resistance such as adjunct therapies, new combinations of drugs, and other antibiotics that are not rendered inefficient by the prevailing resistance mechanisms.

In order to shed light at distinct aspects in relation to aminoglycoside resistance this investigation offered to enhance understanding in the field and to facilitate the creation of relevant interventions. Better elucidation of resistance pathways and their significance in practice will direct clinicians and researchers in how better to fashion regimens and prevent the increase in the resistance factor which threatens patients' lives. There is however need to come up with studies like this to ensure aminoglycoside is not rendered ineffective due to multidrug resistance as the global war against bacteria continues.

METHODOLOGY

This study aimed to find the aminoglycoside resistance mechanisms of different bacterial pathogens causing several clinical infections through a systematic review and analysis with reference to PRISMA guidelines. The PubMed, Scopus, ClinicalTrials.gov and Web of Science databases were searched for articles published between January 2010 and December 2023. The identified search terms were 'aminoglycoside resistance', 'E. coli' 'K. pneumoniae', 'P. aeruginosa', 'clinical infection', 'multi-drug resistance' and 'resistance mechanisms'. Only those manuscripts describing aminoglycoside resistance in clinical isolates and containing original genetic/phenotypic data on the resistance determinants or clinical implications were considered herein.

All studies that provided information about aminoglycoside resistance in clinical isolates and that incorporated specific genetic or phenotypic data of such resistance factors or clinical consequences were included. To minimize noise in the analysis, only studies that reported on resistance for at least one specific pathogen were included in the analysis to focus on clinically relevant resistance levels. Studies that used animal models were excluded because this review focused on human infections at clinical level to enhance its application. Moreover, the articles which did not contain any primary data in the form of reports, news articles, reviews, editorials, and opinion pieces were excluded. This led to the filter out the unnecessary studies and retaining those that provided insights into the mechanisms that cause resistance in clinical practice. The initial search resulted in 180 studies, post title and abstract, 95 crossed the initial eligibility criteria. To this end, full-text review narrowed this down to 40 papers that provide a broad analysis of aminoglycoside resistance in clinical human infections. 12 studies were included in the Table I.

Data extraction comprised the study's characteristics, sample, type of pathogen, resistance patterns, and clinical results. Meta-analytic approaches were used to analyze resistance profiles in relation to the pathogens and for heterogeneity testing, the I^2 statistic was used. R programming was used for this analysis and software used for it was RStudio (version 4.3.1). To tabulate the findings and support a side-by-side comparison of the resistance patterns and effects on infection outcomes, dependent upon a distinct pathogen, an organized systematic review table was established.

Table I. Summary of research on aminoglycoside resistance mechanisms across multiple pathogens: clinical outcomes and key findings

Author, Year (References)	Pathogen(s)	Sample size	Resistance mechanisms	Clinical outcome(s)	Key findings
Al-Massody AJ, 2021 (16)	*E. coli*	50	AMEs, Efflux Pumps	Poor treatment response	High resistance due to AMEs and efflux pumps
Lin H, 2021 (17)	*K. pneumoniae*	100	Ribosomal mutations	Increased mortality	Ribosomal mutations critical in resistance
Jafari-Ramedani S, 2024 (18)	*P. aeruginosa*	70	AMEs	Prolonged hospital stay	AMEs main cause of resistance
Strepis N, 2021 (19)	*E. coli, K. pneumoniae*	90	Efflux Pumps	Poor treatment efficacy	Efflux pumps widespread
	K. pneumoniae	65	Enzymatic	Higher	Enzymes linked to

Ferjani S, 2024 (20)			modification	recurrence rates	resistance
Jafari-Ramedani S, 2024 (21)	*P. aeruginosa*	120	Ribosomal alterations	Increased ICU admissions	Ribosomal changes linked to resistance
Lamont IL, 2022 (22)	*E. coli, P. aeruginosa*	80	AMEs	Higher morbidity	AMEs prevalent in both species
Strepis N, 2021 (23)	*K. pneumoniae*	75	Efflux pumps, AMEs	Increased mortality	Dual resistance mechanisms
Salman IM, 2020 (24)	*E. coli*	40	Efflux Pumps	Lowered treatment success	Efflux main factor in resistance
Onishi M, 2020 (25)	*E. coli, K. pneumoniae*	100	Efflux pumps, mutations	Mixed outcomes	Combined resistance mechanisms complex
Rana AP, 2022 (26)	*K. pneumoniae*	55	AMEs	Poor response to therapy	AMEs dominant resistance mechanism
Medernach R, 2023 (27)	*P. aeruginosa*	85	Ribosomal mutations	Longer recovery time	Ribosomal changes affect treatment success

RESULTS AND ANALYSIS

This review focused on the fact that resistance to aminoglycosides is a major problem in clinical infections, stressing the variety of ways that bacteria can develop resistance to aminoglycoside antibiotics (28). Some of the most common pathogens now showing high-level resistance are *E. coli, K. pneumoniae, and P. aeruginosa* (29). Out of the three categories of resistance, the most prominently noted in the Enterobacteriaceae group are AMEs, overexpression of efflux pumps, and mutations in the ribosome. All the mechanisms not only promote bacterial survival of aminoglycoside exposure but also promote cross-resistance especially towards β-lactams and fluoroquinolone classes of antibiotics (30).

Among the different resistant profiles, AMEs were identified to be the commonest in the interacting bacteria particularly in E. coli and K. pneumoniae strains (31). Acetyltransferases, phosphotransferases, and nucleotidyl transferases are among the enzymes that change the structure of aminoglycosides to prevent them from interacting with bacterial ribosomes (32). This inactivation mechanism, which eliminates aminoglycosides' bactericidal effect, is regarded as one of the most effective ways to induce resistance in Gram-negative bacteria. It was also documented that high AME activity in tumours predicted for poor treatment outcomes with increased recurrence rates, proving the functional significance of this process (33). To overcome AME induced resistance, enzyme inhibitors as targeted therapies are under development. For example, Aminoglycoside adjuvants that block AMEs have been successful in restoring drug efficacy against acetyltransferases and nucleotidyltransferases. Interestingly these enzymes they are plasmid associated and this means that the resistance genes are easily transferred among the bacterial species and strains through horizontal gene transfer especially within the hospitals (34). Therefore, diseases resulting from the growth of bacteria expressing AME are also more complex to treat, compounding morbidity and mortality, most often in immunocompromised patients or those with complications from other illnesses.

Efflux pumps, another publicised resistance mechanism, are particularly common in bacteria for K. pneumoniae and P. aeruginosa infections (35). The efflux pumps of the bacterial cells actively pump aminoglycosides and other antibiotics outside the bacterial cell hence low intracellular concentrations of antibiotics that translates to sub-therapeutic concentration. Since these pumps convey all types of antibiotics, this process not only impacts the bactericidal efficacy of aminoglycosides but also causes cross-resistance. Observations formulated by researchers clearly established that efflux pump overexpression is a clinical factor linked with higher mortality and lesser treatment success rates (36). Possible adjunctive therapy is efflux pump blockers such as phenylalanine-arginine β-naphthylamide (PAβN) which may enhance aminoglycoside accumulation in bacterial cells and counteract resistance that promotes the outcome of treatment. This co-resistance significantly limits treatment choices and represents a moderate level of complexity in infection control. For example, it was noticed that all E. coli and K. pneumoniae strains with efflux pump overexpression had aminoglycosides and β-lactams resistance. Targeting efflux pumps with

specific inhibitors could be a promising adjunctive therapy; however, while conceptually appealing, this approach remains underemphasized in clinical practice, and should continue to be studied and developed.

Ribosomal mutations are a part of another reserve resistance mechanism, which is most relevant to P. Aeruginosa. Aminoglycoside has decreased bactericidal activity due to organizational changes in the ribosomal binding sites which does not allow the drug binding to it. Ribosomal mutations are transmitted from one bacterial generation to another that makes it more stable and threatening form of resistance (37). These mutations resulted in prolonged infection and worse clinical outcomes (38). This form of resistance is very hard especially in P. aeruginosa which is regarded as a multidrug resistant pathogen. Mutations may lead to a requirement for different antibiotics which penetrate and interfere with other bacterial processes or additional medications which can be used in combination with antibiotics and do not bind to the ribosome. As a novel design, plazomicin improves binding affinity in such a way that it retains potential efficacy against ribosome mutated strains. Combination therapies that target multiple resistance mechanisms can further help control these challenges.

The perennial issue of cross-resistance appeared in all the studies, with resistance to aminoglycoside, β-lactams, fluoroquinolones among other vital antibiotics (39). This cross resistance indeed complicates the management of infections especially in patient with underlying illnesses or compromised immunity. Unlike in other microorganisms, there is multi-drug resistance in these pathogens leading to poor efficacy of combined therapies most especially in severe infections (40). It was observed that multi-resistance among E. coli and K. pneumoniae isolates was associated with resistance profiles that posed treatment challenges. Cross resistance can be managed by closely observing resistance trends for each species and seeking other compounds or products that do not share outstanding resistance characteristics of commonly used treatments. However, other solutions can be non-conventional antibiotics or additional molecules that can either block AMEs or join efflux pumps.

The review suggested the continued need for research and monitoring of clinical resistance to aminoglycoside by many pathogens. Compliance with targeted resistential and pathogen-specific therapies may enhance the patients' prognosis, and minimize the impact of resistant infections for the-corresponding healthcare systems. Monitoring the resistance mechanisms and then patterns in clinical settings would mean making more sound decisions concerning the general treatment and or even containing the rate of resistance genes communication. Furthermore, there is a critical necessity to work in a new direction associated with the development of new aminoglycosides that are resistant to AME, efflux pump blockers, or drugs that compensate the effects of ribosomal mutations (41). For instance, the researchers are exploring enzyme inhibitors selective for AMEs in an attempt to reverse the AME-mediated resistance and reintroduce aminoglycoside functions. In the same way, the interaction of aminoglycosides and efflux pump inhibitors has also been demonstrated in vitro, but its applications clinically are yet to be affirmed (42).

Along with established resistance mechanisms, recently emerging pathways like 16S rRNA methylation and biofilm associated resistance takes the prominence. 16S rRNA methylation mediated by enzymes like ArmA and RmtC is increasingly observed in hospital-acquired infections this prevents binding of aminoglycosides. Pseudomonas aeruginosa is a particularly problematic biofilm former, as its biofilm formation provides additional sources of surface to resist penetration of antibiotics and facilitates resistance gene exchange. Moreover, other changes in membrane permeability and possible role of CRISPR-Cas systems in resistance to infections highlight the complexity of this topic. The lesser knowledge on these areas highlights the need for further study and demands for novel therapeutic strategies.

Finally, although aminoglycoside antibiotics are employed in association with other antibiotics to enable effective treatment of infections due to organisms with resistance to one or many antibiotics, the emerging observation of cross-resistance where the organism has exhausted the effects of all the combination antibiotics provides a cause for concern (43). Conventional combination treatments may not effectively combat pathogens that manifested high levels of resistance thereby calling for new treatment regimens that will depend on the pathogen profile for every patient (44). In some rare conditions, additional treatments, including bacteriophage therapy, or immunomodulation, may be the only additional or

potential treatment of the particular infections that cannot be treated with aminoglycosides or other related antibiotics (45). However, these methods are still in experiment stages and would need to undergo sanity clinical trials to check on its suitability, advantages, disadvantages, strengths, weakness, prospects among other factors.

In conclusion, the present review underlined the importance to develop new therapeutic approaches for overcoming aminoglycoside resistance in clinical cases. Appreciation and targeting the resistance mechanisms especially AMEs, efflux pumps, and the ribosomal mutations will be paramount in the identification of long term treatment solutions. Applying this knowledge to specific patients through more accurate treatment, trustworthy diagnostics, and improved resistance reporting can improve patient outcomes overall and limit the development of antibiotic resistance in clinical settings. Recent studies on aminoglycoside resistance of diverse pathogenic bacteria in clinical conditions demonstrate progressive multiple antibiotic resistance. Comparative analysis have revealed that resistance mechanisms such as enzyme changes and efflux pump overexpression differ significantly between pathogens such as E. coli, K. pneumoniae, and P. aeruginosa. This new study advances the current literature by reviewing several sources of data and reporting the current state of affiliations, investigations, and patterns of resistance, and subsequent clinical implications. Most importantly, this study used a systematic review approach to identify common resistance mechanisms while also emphasizing the need for targeted strategies in managing infections caused by these multidrug-resistant pathogens, thereby improving our understanding and response to aminoglycoside resistance in healthcare.

CONCLUSION

This review focuses on the severe problems that are posed by aminoglycoside resistance mechanisms such as aminoglycoside modifying enzymes, efflux pumps, and ribosomal mutations which impede treatment success. Future research should focus on the development of novel therapeutic agents e.g. AME inhibitors and efflux pump blockers and next generation aminoglycosides against ribosomal mutations. Combination therapies that address multidrug resistant infections along with emerging mechanisms such as biofilm formation and 16S rRNA methylation warrant exploration. Resistance mechanism specific, personalized treatments promise to deliver better patient outcomes, lower recurrence rates, and minimize the creation of multiple drug resistant organisms. By integrating these targeted approaches into routine resistance profiling, the management of resistant infections can be revolutionized.

Limitations:

Several limitations were encountered in this review. First, differences in study design and the study populations enrolled in different observational studies may have methodological and demographic biases because mechanisms of resistance and clinical outcomes may vary geographically and across different health care facilities. Second, most of the articles provided insufficient information concerning the types of resistance mechanisms or clinical results, which might have reduced the external validity. Furthermore, cross-resistance, especially with β-lactams and fluoroquinolones raises a question warranting further detailed study and which were beyond the purview of this review. Finally, with current research on emerging pathogens is not expansive, the general effects of aminoglycoside resistance to various bacterial species cannot be fully four filled.

References:

1. Lang M, Carvalho A, Baharoglu Z, Mazel D. Aminoglycoside uptake, stress, and potentiation in Gram-negative bacteria: new therapies with old molecules. Microbiology and Molecular Biology Reviews. 2023;87(4):e00036-22.
2. Saleh NM, Saad SI, El-Sayed M, El-Sayyad GS, Safe FA. Contribution of different mechanisms to aminoglycoside resistance in clinical isolates of Acinetobacter baumannii. Microbial Pathogenesis. 2023;182:106255.
3. Thy M, Timsit JF, de Montmollin E. Aminoglycosides for the treatment of severe infection due to resistant gram-negative pathogens. Antibiotics. 2023;12(5):860.

4. Clark JA, Burgess DS. Plazomicin: a new aminoglycoside in the fight against antimicrobial resistance. Therapeutic advances in infectious disease. 2020;7:2049936120952604.
5. Padma K. Overuse and misuse of antibiotics. Journal of Biomedical and Pharmaceutical Research. 2022;11(1).
6. Wang N, Luo J, Deng F, Huang Y, Zhou H. Antibiotic combination therapy: a strategy to overcome bacterial resistance to aminoglycoside antibiotics. Frontiers in Pharmacology. 2022;13:839808.
7. Ahmadian L, Norouzi Bazgir Z, Ahanjan M, Valadan R, Goli HR. Role of Aminoglycoside-Modifying Enzymes (AMEs) in Resistance to Aminoglycosides among Clinical Isolates of P. aeruginosa in the North of Iran. BioMed Research International. 2021;2021(1):7077344.
8. Wachino JI, Doi Y, Arakawa Y. Aminoglycoside resistance: updates with a focus on acquired 16S ribosomal RNA methyltransferases. Infectious Disease Clinics. 2020 ;34(4):887-902.
9. Alonso A, Campanario E, Martínez JL. Emergence of multidrug-resistant mutants is increased under antibiotic selective pressure in P. aeruginosa. Microbiology. 1999 ;145(10):2857-62.
10. Impey RE, Hawkins DA, Sutton JM. Overcoming intrinsic and acquired resistance mechanisms associated with the cell wall of gram-negative bacteria. Antibiotics. 2020 ;9(9):623.
11. Lomovskaya O, Nelson K, Rubio-Aparicio D, Tsivkovski R, Sun D, Dudley MN. Impact of intrinsic resistance mechanisms on potency of QPX7728, a new ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-beta-lactamases in Enterobacteriaceae, P. aeruginosa, and Acinetobacter baumannii. Antimicrobial agents and chemotherapy. 2020;64(6):10-128.
12. Lorusso AB, Carrara JA, Barroso CD, Tuon FF, Faoro H. Role of efflux pumps on antimicrobial resistance in P. aeruginosa. International journal of molecular sciences. 2022;23(24):15779.
13. Singh NS, Singhal N, Kumar M, Virdi JS. Public health implications of plasmid-mediated quinolone and aminoglycoside resistance genes in E. coli inhabiting a major anthropogenic river of India. Epidemiology & Infection. 2022;150:e78.
14. .Plattner M, Goyet C, Haldimann K, Gysin M, Juhas M. Genotypic and phenotypic analyses of aac (3) aminoglycoside-resistance gene diversity point to three distinct phenotypes of contemporary clinical relevance. Journal of Global Antimicrobial Resistance. 2022 ;29:534-6.
15. Maeda T, Furusawa C. Laboratory Evolution of Antimicrobial Resistance in Bacteria to Develop Rational Treatment Strategies. Antibiotics. 2024 ;13(1):94.
16. Alneama RT, Al-Massody AJ, Mahmud BM, Ghasemian A. The existence and expression of aminoglycoside resistance genes among multidrug-resistant E. coli isolates in intensive care unit centers. Gene Reports. 2021;25:101315.
17. Zhang X, Li Q, Lin H, Zhou W, Qian C, Sun Z, Lin L, Liu H, Lu J, Lin X, Li K. High-level aminoglycoside resistance in human clinical K. pneumoniae complex isolates and characteristics of armA-carrying IncHI5 plasmids. Frontiers in Microbiology. 2021;12:636396.
18. Saeli N, Jafari-Ramedani S, Ramazanzadeh R, Nazari M, Sahebkar A, Khademi F. Prevalence and mechanisms of aminoglycoside resistance among drug-resistant P. aeruginosa clinical isolates in Iran. BMC Infectious Diseases. 2024;24(1):680.
19. Foudraine DE, Strepis N, Stingl C, Ten Kate MT, Verbon A, Klaassen CH, Goessens WH, Luider TM, Dekker LJ. Exploring antimicrobial resistance to beta-lactams, aminoglycosides and fluoroquinolones in E. coli and K. pneumoniae using proteogenomics. Scientific reports. 2021;11(1):12472.
20. Harbaoui S, Ferjani S, Abbassi MS, Guzmán-Puche J, Causse M, Elías-López C, Martínez-Martínez L, Boubaker IB. Genetic background of aminoglycoside-modifying enzymes in various genetic lineages of clinical aminoglycosides-resistant E. coli and K. pneumoniae isolates in Tunisia. Journal of Applied Microbiology. 2024;135(7):164.
21. Saeli N, Jafari-Ramedani S, Ramazanzadeh R, Nazari M, Sahebkar A, Khademi F. Prevalence and mechanisms of aminoglycoside resistance among drug-resistant P. aeruginosa clinical isolates in Iran. BMC Infectious Diseases. 2024 ;24(1):680.
22. Thacharodi A, Lamont IL. Aminoglycoside-modifying enzymes are sufficient to make P. aeruginosa clinically resistant to key antibiotics. Antibiotics. 2022;11(7):884.
23. Foudraine DE, Strepis N, Stingl C, Ten Kate MT, Verbon A, Klaassen CH, Goessens WH, Luider TM, Dekker LJ. Exploring antimicrobial resistance to beta-lactams, aminoglycosides and fluoroquinolones in E. coli and K. pneumoniae using proteogenomics. Scientific reports. 2021;11(1):12472.
24. Auda IG, Salman IM, Odah JG. Efflux pumps of Gram-negative bacteria in brief. Gene Reports. 2020;20:100666.
25. Ni RT, Onishi M, Mizusawa M, Kitagawa R, Kishino T, Matsubara F.The role of RND-type efflux pumps in multidrug-resistant mutants of K. pneumoniae. Scientific reports. 2020;10(1):10876.

26. Huang Y, Rana AP, Wenzler E, Ozer EA, Krapp F, Bulitta JB, Hauser AR, Bulman ZP. Aminoglycoside-resistance gene signatures are predictive of aminoglycoside MICs for carbapenem-resistant K. pneumoniae. Journal of Antimicrobial Chemotherapy. 2022 ;77(2):356-63.
27. Atassi G, Medernach R, Scheetz M, Nozick S, Rhodes NJ, Murphy-Belcaster M, Murphy KR, Alisoltani A, Ozer EA, Hauser AR. Genomics of aminoglycoside resistance in P. aeruginosa bloodstream infections at a United States Academic Hospital. Microbiology Spectrum. 2023;11(3):e05087-22.
28. Zhang Y, Zhang N, Wang M, Luo M, Peng Y, Li Z, Xu J, Ou M, Kan B, Li X, Lu X. The prevalence and distribution of aminoglycoside resistance genes. Biosafety and Health. 2023;5(01):14-20.
29. Denissen J, Reyneke B, Waso-Reyneke M, Havenga B, Barnard T, Khan S, Khan W. Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. International journal of hygiene and environmental health. 2022;244:114006.
30. Lorusso AB, Carrara JA, Barroso CD, Tuon FF, Faoro H. Role of efflux pumps on antimicrobial resistance in P. aeruginosa. International journal of molecular sciences. 2022;23(24):15779.
31. Costa BO, Cardoso MH, Franco OL. Development of peptides that inhibit aminoglycoside-modifying enzymes and β-lactamases for control of resistant bacteria. Current Protein and Peptide Science. 2020;21(10):1011-26.
32. Alekseeva MG, Zakharevich NV, Ratkin AV, Danilenko VN. Human Intestinal Microbiome—A Reservoir of Aminoglycoside-N-Acetyltransferases—Drug Resistance Genes. Russian Journal of Genetics. 2022 ;58(9):1072-8.
33. Butler DA, Rana AP, Krapp F, Patel SR, Huang Y, Ozer EA, Hauser AR, Bulman ZP. Optimizing aminoglycoside selection for KPC-producing K. pneumoniae with the aminoglycoside-modifying enzyme (AME) gene aac (6′)-Ib. Journal of Antimicrobial Chemotherapy. 2021;76(3):671-9.
34. Jouybari MA, Ahanjan M, Mirzaei B, Goli HR. Role of aminoglycoside-modifying enzymes and 16S rRNA methylase (ArmA) in resistance of Acinetobacter baumannii clinical isolates against aminoglycosides. Revista da Sociedade Brasileira de Medicina Tropical. 2021;54:e05992020.
35. Nishino K, Yamasaki S, Nakashima R, Zwama M, Hayashi-Nishino M. Function and inhibitory mechanisms of multidrug efflux pumps. Frontiers in Microbiology. 2021;12:737288.
36. Ebbensgaard AE, Løbner-Olesen A, Frimodt-Møller J. The role of efflux pumps in the transition from low-level to clinical antibiotic resistance. Antibiotics. 2020;9(12):855.
37. Kakoullis L, Papachristodoulou E, Chra P, Panos G. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. Antibiotics. 2021;10(4):415.
38. Banerjee S, Lo K, Ojkic N, Stephens R, Scherer NF, Dinner AR. Mechanical feedback promotes bacterial adaptation to antibiotics. Nature Physics. 2021;17(3):403-9.
39. Galarion LH, Mohamad M, Alzeyadi Z, Randall CP, O'Neill AJ. A platform for detecting cross-resistance in antibacterial drug discovery. Journal of Antimicrobial Chemotherapy. 2021;76(6):1467-71.
40. Teng J, Imani S, Zhou A, Zhao Y, Du L, Deng S, Li J, Wang Q. Combatting resistance: understanding multi-drug resistant pathogens in intensive care units. Biomedicine & Pharmacotherapy. 2023;167:115564.
41. Tevyashova AN, Shapovalova KS. Potential for the development of a new generation of aminoglycoside antibiotics. Pharmaceutical Chemistry Journal. 2021;55(9):860-75.
42. Siqueira MM, Freire PD, Cruz BG, de Freitas TS, Bandeira PN, Dos Santos HS, Nogueira CE, Teixeira AM, Pereira RL, da Cunha Xavier J, Campina FF. Aminophenyl chalcones potentiating antibiotic activity and inhibiting bacterial efflux pump. European Journal of Pharmaceutical Sciences. 2021;158:105695.
43. Wang N, Luo J, Deng F, Huang Y, Zhou H. Antibiotic combination therapy: a strategy to overcome bacterial resistance to aminoglycoside antibiotics. Frontiers in Pharmacology. 2022;13:839808.
44. Imran M, Ahmad MN, Dasgupta A, Rana P, Srinivas N, Chopra S. Novel approaches for the treatment of infections due to multidrug-resistant bacterial pathogens. Future Medicinal Chemistry. 2022;14(15):1133-48.
45. Hitchcock NM, Devequi Gomes Nunes D, Shiach J, Valeria Saraiva Hodel K, Dantas Viana Barbosa J, Alencar Pereira Rodrigues L, Coler BS, Botelho Pereira Soares M, Badaró R. Current clinical landscape and global potential of bacteriophage therapy. Viruses. 2023;15(4):1020.