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REVIEW ARTICLE

Combating Aminoglycoside Resistance: From Structural and Functional Characterisation to Therapeutic Challenges with RKAAT

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Abstract: A comprehensive knowledge of aminoglycoside-modifying enzymes (AMEs) and their role in bacterial resistance mechanisms is urgently required due to the rising incidence of antibiotic resistance, particularly in *Klebsiella pneumoniae* infections. This study explores the essential features of AMEs, including their structural and functional properties, the processes by which they contribute to antibiotic resistance, and the therapeutic importance of aminoglycosides. The study primarily examines the Recombinant *Klebsiella pneumoniae* Aminoglycoside Adenylyl Transferase (RKAAT), particularly emphasizing its biophysical characteristics and the sorts of resistance it imparts. Furthermore, this study examines the challenges presented by RKAAT-mediated resistance, an evaluation of treatment methods and constraints, and options for controlling infection. The analysis provides a prospective outlook on strategies to address and reduce antibiotic resistance. This extensive investigation seeks to provide vital insights into the continuing fight against bacterial resistance, directing future research efforts and medicinal approaches.

Keywords: Adenylyl transferase, aminoglycoside antibiotics, antibiotic resistance, healthcare-associated infections, *Klebsiella pneumoniae*, recombinant, *Klebsiella pneumoniae Aminoglycoside*, (3") (9) adenylyl transferase (RKAAT).

1. INTRODUCTION

Klebsiella pneumoniae, a bacterium characterised by its Gram-negative cell wall structure, has become a prominent concern in public health due to its increasing resistance to many categories of antibiotics [1]. Among the various mechanisms contributing to its resistance, aminoglycoside-modifying enzymes play a pivotal role in inactivating aminoglycoside antibiotics, rendering them ineffective against infections caused by this pathogen [2]. One such enzyme, the recombinant *Klebsiella pneumoniae Aminoglycoside* (3") (9) Adenylyl Transferase, has attracted significant attention in recent years.

The recombinant *Klebsiella pneumoniae Aminoglycoside* (3") (9) Adenylyl Transferase, commonly abbreviated as RKAAT, is a member of the adenylyl transferase family of enzymes that mediates resistance to aminoglycoside antibiotics. This enzyme confers resistance by catalysing the covalent transfer of an adenylyl group to the 3"-hydroxyl moiety of aminoglycosides, thereby reducing their binding affinity to bacterial ribosomes, and hampering their bactericidal action [3]. Consequently, the presence of RKAAT-expressing strains of *Klebsiella pneumoniae* poses significant challenges in treating infections, resulting in more extended hospitalisation, escalated healthcare expenditures, and elevated death rates. Therefore, understanding the function and mechanisms of RKAAT is paramount in the battle against antibiotic-resistant *Klebsiella pneumonia*.

Despite its significance, comprehensive reviews focusing solely on RKAAT's role in aminoglycoside resistance are still being determined in the scientific literature. This review aims to bridge this knowledge gap by providing a thorough and up-to-date examination of RKAAT, encompassing its structural features, biochemical properties, substrate specificity, and the molecular mechanisms underlying its adenylyl transferase activity. This review delves into the existing literature to synthesise the current understanding of RKAAT and its implications for public health. Furthermore, we explore the challenges associated with combatting RKAAT--mediated resistance and highlight potential strategies to counteract its effects, from developing novel therapeutics to implementing effective infection control measures.

Overall, this comprehensive review aims to unravel the intricate function and mechanisms of RKAAT, offering valuable insights for researchers, clinicians, and policymakers involved in the fight against antibiotic-resistant *Klebsiella pneumoniae* and other pathogens bearing similar resistance determinants. By better understanding RKAAT, we can take significant strides toward preserving the efficacy of aminoglycoside antibiotics and safeguarding public health from the escalating threat of antibiotic resistance.

2. *KLEBSIELLA PNEUMONIAE* AND ANTIBIOTIC RESISTANCE

Klebsiella pneumoniae, a gram-negative bacterium, has emerged as a formidable global health threat, particularly in

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healthcare settings. The many illnesses caused by this opportunistic bacterium include pneumonia, UTIs, bloodstream infections, and infections at surgical sites [4]. Its increasing resistance to multiple antibiotic classes compounds the severity of *K. pneumoniae* infections, making it a prominent member of the notorious group of multidrug-resistant bacteria [5].

Healthcare-associated infections caused by antibiotic-resistant *K. pneumoniae* strains have become a significant challenge, resulting in prolonged hospital stays, increased treatment costs, and higher mortality rates. The nosocomial infections that were once readily treatable with common antibiotics are now becoming insurmountable obstacles due to antibiotic resistance to the limited therapeutic options available. This problem is prevalent among vulnerable populations, such as elderly individuals, immunocompromised patients, and those with underlying health conditions, at higher risk of infection and its adverse outcomes [4, 5].

Unfortunately, the dissemination of antibiotic-resistance genes among *K. pneumoniae* strains has been facilitated by various factors, including the widespread use of antibiotics, inadequate infection control practices, and the horizontal transfer of resistance genes between bacterial populations [6]. In addition, the ability of *K. pneumoniae* to form biofilms on medical devices and within hospital environments further enhances its capacity to persist and propagate [7].

Beyond healthcare settings, the threat of *K. pneumoniae* is not confined to hospitals alone. Increasingly, community-acquired infections caused by drug-resistant strains are being reported, posing an additional challenge for clinicians and public health authorities [8]. Therefore, efforts to combat antibiotic-resistant *K. pneumoniae* require a multifaceted approach encompassing infection prevention and control measures, reasonable antibiotic usage, rapid and accurate diagnostics, and the development of new therapeutic strategies [9]. In this review, understanding the underlying resistance mechanisms, such as those mediated by enzymes like RKAAT, becomes crucial. By elucidating the factors driving the spread of antibiotic resistance in *K. pneumoniae*, we can develop targeted interventions and strengthen our defences against this formidable pathogen.

2.1. Mechanisms of Antibiotic Resistance in *Klebsiella pneumoniae*

The development and spread of antibiotic resistance in *K. pneumoniae* have reached alarming levels, posing a severe threat to public health worldwide [10]. Understanding how this pathogen acquires antibiotic resistance is crucial for devising effective strategies to combat the growing crisis. *K. pneumoniae* uses many basic processes to become resistant to many drugs [11], contributing to its status as a significant multidrug-resistant pathogen.

K. pneumoniae employs diverse strategies for antibiotic resistance. Notably, it produces beta-lactamases like ESBLs and carbapenems that degrade vital antibiotics [11]. Furthermore, Efflux pumps expel drugs, contributing to multidrug resistance [12]. According to Albarri *et al.* [13], they performed a disc diffusion test on *K. pneumoniae* isolates, which revealed elevated resistance rates to several drugs. It was seen that ciprofloxacin-resistant isolates had higher levels of efflux pump genes (acrA, acrB, oqxA, and oqxB) and regulators (marA, soxS, and rarA). The research reveals a correlation between the minimum inhibitory concentration (MIC) of ciprofloxacin and the expression of efflux pump genes. Similarly, the second study by Albarri *et al.* [14] backs up these findings by showing that the efflux pump inhibitor CCCP effectively lowered the ciprofloxacin MIC values in many isolates of *K. pneumoniae* that are multidrug-resistant. There were more efflux pump genes (acrAB and oqxAB) and transcriptional regulators (marA, soxS, and rarA) in isolates that did not respond to ciprofloxacin. Both results show that clinical isolates of *K. pneumoniae* are not sensitive to ciprofloxacin because they have too many efflux pump genes and the regulators that control them. Understanding the molecular processes behind bacterial resistance, particularly efflux pumps, is crucial to devise efficient solutions for antibiotic resistance. Biofilm formation also shields the bacterium from antibiotics and immune attacks [15]. Lastly, modifying aminoglycosides through AMEs reduces their efficacy [16]. These mechanisms collectively enable *K. pneumoniae* to withstand treatment, posing a challenge to combating infections effectively [17].

3. AMINOGLYCOSIDE-MODIFYING ENZYMES (AMEs): AN OVERVIEW

Aminoglycoside antibiotics are vital therapeutic agents for treating severe bacterial infections [18]. Unfortunately, the clinical efficacy of these antibiotics is increasingly threatened by the emergence and dissemination of AMEs among bacterial pathogens, including *K. pneumoniae* [19]. AMEs are a diverse group of enzymes that chemically modify aminoglycosides, reducing antibiotic activity and rendering these drugs ineffective against the targeted pathogens.

AMEs exhibit distinct activities affecting antibiotic efficacy. For instance, Aminoglycoside Acetyltransferases (AACs) diminish positive charges and disrupt ribosomal interactions [20]. At the same time, Aminoglycoside Adenylyl transferases (AADs) add adenosine monophosphate, weakening ribosomal binding [21], and Aminoglycoside Phosphotransferases (APHs) phosphorylate hydroxyl groups, impairing ribosomal attachment and exerting significant clinical resistance impact [22]. These enzymatic activities are responsible for some of the most clinically substantial aminoglycoside resistance phenotypes.

3.1. Structural Characterisation of Aminoglycoside-Modifying Enzymes (AMEs)

Aminoglycosides have been a crucial element in the arsenal for treating life-threatening illnesses. Regrettably, the resistance levels were so high that they sometimes became ineffective. However, enzymatic modification is the most common mechanism of resistance to aminoglycosides in clinical settings. Aminoglycoside modifying enzymes alter specific -

OH or -NH_2 groups within the 2-deoxystreptamine nucleus or the sugar components. These enzymes may function as nucleotidyltransferases, phosphotransferases, or acetyltransferases (Fig. **1**). The number of aminoglycoside-modifying enzymes discovered so far and the genetic surroundings in which the coding genes are situated are remarkable. Virtually every bacterium can facilitate enzymatic resistance to aminoglycosides.

3.2. Functional Characteristics of Aminoglycoside-Modifying Enzymes (AMEs)

AMEs are expressed by mobile genetic elements, such as plasmids and transposons. The abovementioned factors are paramount in supporting the effective dissemination of AMEs among bacterial populations (Fig. **2**).

Thus, acquiring AME genes through horizontal gene transfer allows bacteria to gain resistance to aminoglycosides, even without prior exposure to these antibiotics [25]. Furthermore, aminoglycoside enzymes often display substrate specificity, meaning different enzymes may modify specific subsets of aminoglycoside antibiotics. Therefore, comprehending the functional characteristics of these enzymes (discussed below) is crucial for unravelling the complex processes by which they provide resistance.

3.2.1. Substrate Specificity

This refers to aminoglycoside enzymes' capacity to alter aminoglycoside types selectively. These enzymes are designed to identify specific functional groups or areas in the antibiotic molecules [26]. This allows them to facilitate alterations that reduce the antibiotics' binding ability to bacterial ribosomes.

3.2.2. Catalytic Processes

The catalytic processes of aminoglycoside enzymes exhibit variability across various enzyme types. Aminoglycoside acetyltransferases (AAC) facilitate the transfer of acetyl groups, aminoglycoside phosphotransferases (APH) facilitate the transfer of phosphate groups, and aminoglycoside nucleotidyltransferases (ANT) facilitate the transfer of nucleotides [27]. These alterations modify the physicochemical characteristics of aminoglycosides, making them less efficient in suppressing bacterial protein production.

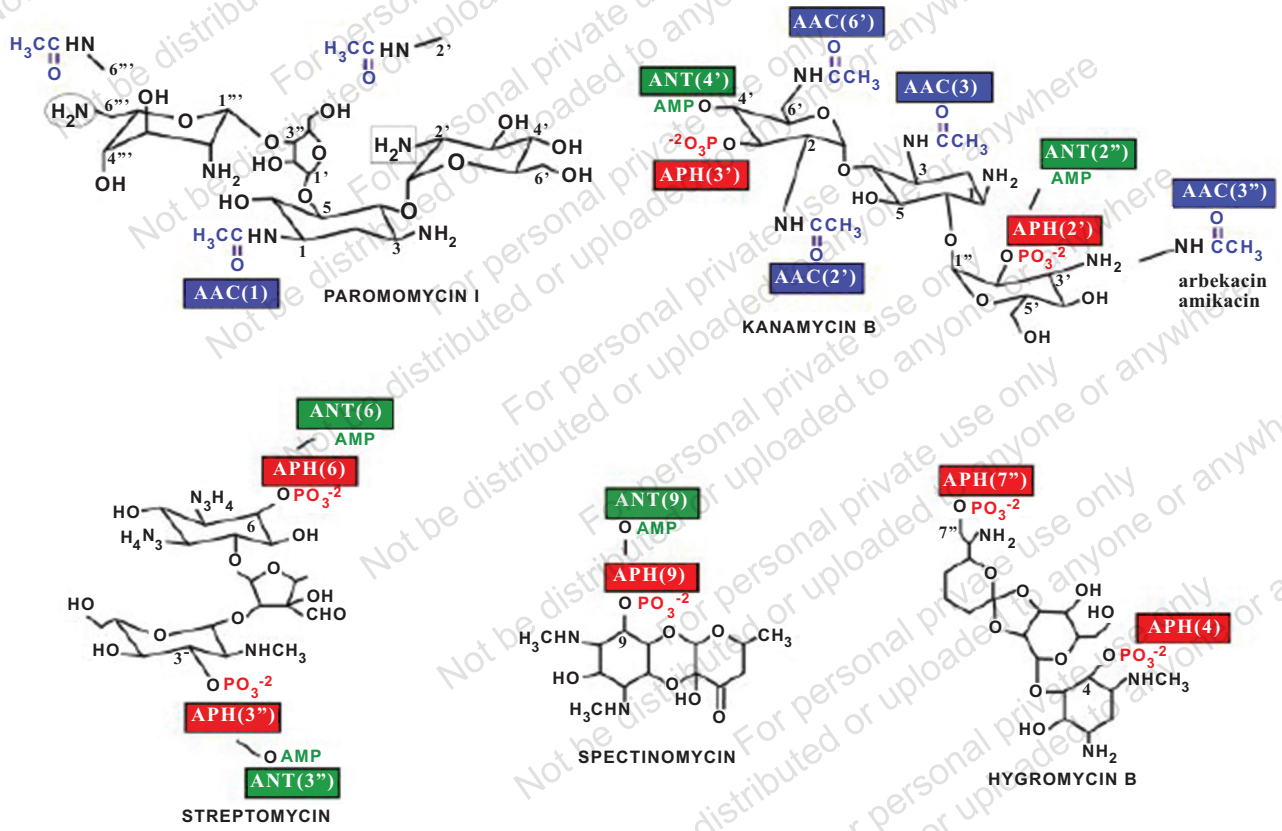

Fig. (1). Characterisation of aminoglycosides Enzymes. The identification of modification sites by AAC, ANT, and APH enzymes. Each kind of alteration is exemplified on one of the substrates. The presence of a square and an oval at positions 2' and 6", respectively, in paromomycin I suggest that while this molecule is primarily acetylated at position 1, the enzymatic reaction also produces 1,2'-di-N-acetylparomomycin and 1,6"-di-N-acetyl paromomycin as additional products [23]. The enzyme AAC (3)-X can facilitate acetylation, specifically at the 3″-amino group in arbekacin and amikacin, as shown by Hotta *et al.* [24]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

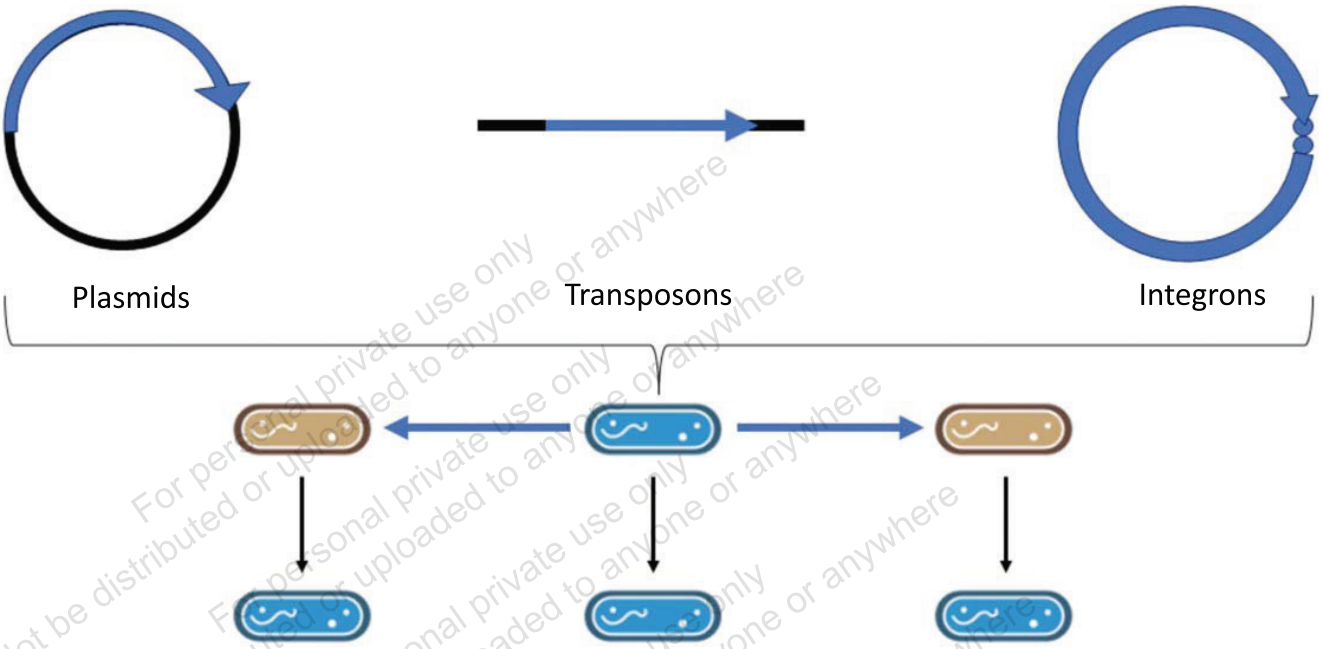

Fig. (2). Genetic elements responsible for the transmission of AMEs. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.2.3. Enzyme Kinetics

Acetyltransferase modifying enzymes (AMEs) often exhibit distinct enzyme kinetics, directly impacting the speed at which they alter aminoglycosides [28]. Gaining a comprehension of these dynamics offers a valuable understanding of the effectiveness of the remodelling process and the possible influence on the effectiveness of antibiotics.

3.2.4. Resistance Spectra:

Various acquired mechanisms of aminoglycoside enzymes resist certain types of aminoglycosides, contributing to the varied range of resistance found in bacterial strains. Some AMEs may provide resistance against a wide variety of aminoglycosides, while others have a more restricted spectrum of effectiveness [29].

3.2.5. Evolutionary Adaptations

These have influenced the functional features of aminoglycoside enzymes due to selection pressure exerted by aminoglycoside antibiotics. The different enzymes found in various types of bacteria result from constantly improving antibiotics and bacteria developing ways to resist them [30].

3.2.6. Antibiotic Efficacy Impact

Aminoglycoside enzymes considerably affect the effectiveness of aminoglycosides by reducing their ability to inhibit bacterial protein synthesis [31]. AME-mediated resistance makes aminoglycoside antibiotics less effective at killing bacteria, which is a problem for therapy.

Ultimately, the functional attributes of aminoglycoside enzymes play a crucial role in the molecular tactics that bacteria use to avoid the effects of aminoglycoside antibiotics. Comprehending these features in-depth is essential for creating specific therapies to combat AME-mediated resistance and maintain the efficacy of aminoglycosides in clinical environments.

3.3. The Mechanism of Action of Aminoglycoside-Modifying Enzymes (AMEs) in Antibiotic Resistance

AMEs are pivotal players in developing resistance to aminoglycoside antibiotics [32]. These enzymes catalyse chemical modifications of aminoglycoside molecules, leading to reduced antibiotic activity and rendering them ineffective against bacterial pathogens [32, 33]. Unfortunately, the acquisition and expression of AME genes by bacteria confer high resistance to aminoglycosides, posing significant challenges for clinical treatment and patient outcomes. Hence, understanding the mechanism of action of AME would help fight against antibiotic resistance. Some of their reported mechanisms of action are highlighted below.

3.3.1. Enzymatic Inactivation of Aminoglycosides

AMEs exert their resistance-conferring effects through various enzymatic activities, including acetylation, adenyla-

tion, and phosphorylation [34]. Each type of AME targets specific chemical groups on the aminoglycoside structure, altering the antibiotic's physicochemical properties and interfering with its binding to the bacterial ribosome, the primary site of action [35]. By modifying critical functional groups on aminoglycosides, AMEs disrupt the antibiotics' ability to interact with ribosomal RNA and proteins (Fig. **3**), thereby impairing protein synthesis and rendering the bactericidal effects of aminoglycosides ineffective [17].

3.3.2. Substrate Specificity

Different AMEs exhibit varying substrate specificities, leading to a diverse spectrum of resistance phenotypes among bacterial strains [36]. Some AMEs may act on a specific subset of aminoglycosides, while others can modify a broader range of antibiotics [37]. This substrate specificity influences the overall resistance profile of the bacterial population and plays a crucial role in shaping the epidemiology of aminoglycoside resistance in clinical settings [38].

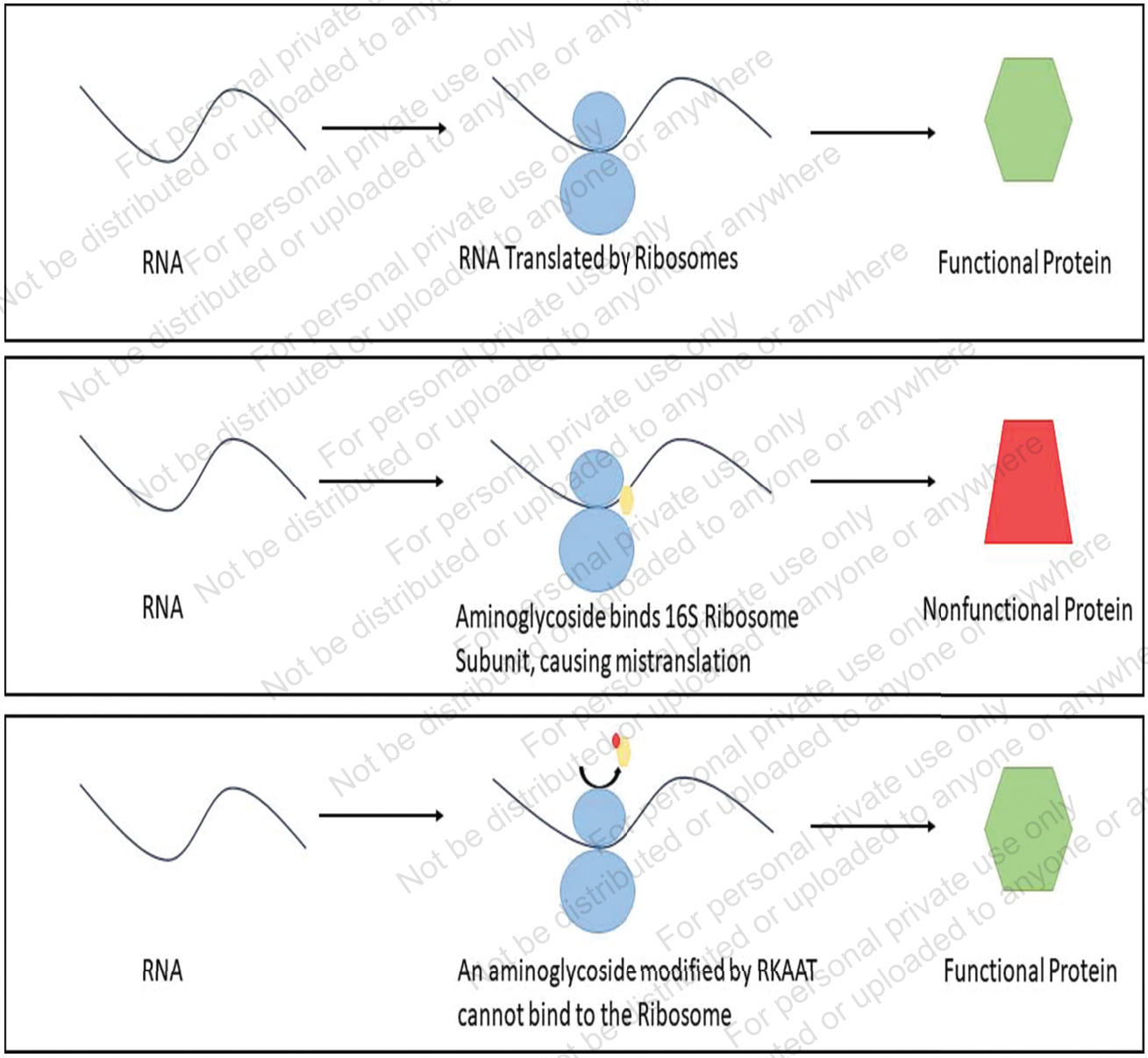

Fig. (3). The Enzymatic inactivation of aminoglycosides. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.3.3. Genetic Context and Dissemination

The AME genes often reside on mobile genetic elements, including plasmids and transposons [39]. This mobility allows for the rapid dissemination of AMEs among bacterial populations through horizontal gene transfer. The transfer of AME genes between different bacterial species and strains contributes to the widespread emergence of aminoglycoside resistance, even in environments where aminoglycosides may not have been used extensively [40].

3.3.4. Combination Resistance

In multidrug-resistant bacterial strains, AMEs frequently coexist with other antibiotic resistance mechanisms, such as beta-lactamases and efflux pumps [41]. This combination of resistance mechanisms presents a significant clinical challenge as it limits the available treatment options and necessitates using more potent and potentially toxic antibiotics to combat infections caused by these resistant pathogens.

Given the clinical significance of aminoglycosides in treating severe infections, understanding the role of AMEs in resistance is vital for guiding antibiotic therapy and implementing effective infection control measures. Strategies to combat AME-mediated resistance include the development of new generations of aminoglycosides that evade AME modifications [42], using combination therapy to prevent or delay resistance, and infection control practices aimed at limiting the spread of resistant strains [41]. Comprehensive surveillance and continued research into the molecular mechanisms of AMEs are essential to preserve the efficacy of aminoglycosides and ensure their continued clinical utility in the face of the growing threat of antibiotic resistance [39, 40].

3.4. Aminoglycosides and their Clinical Significance

Aminoglycosides are a class of broad-spectrum antibiotics with potent bactericidal activity against various Gram-negative and some Gram-positive bacteria [43]. They are crucial in treating severe and life-threatening infections, especially those caused by multidrug-resistant pathogens. Aminoglycosides are characterised by a unique structure composed of amino sugars linked together to form a cyclical ring [44], and they are typically administered parenterally, intravenously or intramuscularly due to their limited oral absorption [32].

The clinical significance of aminoglycosides lies in their efficacy against serious infections caused by bacteria resistant to other antibiotics. They are often used in combination therapy with beta-lactam antibiotics (*e.g.,* penicillins or cephalosporins) to achieve synergistic effects and broaden the spectrum of activity, especially in cases of severe sepsis, endocarditis, and complicated urinary tract infections [45] Commonly used aminoglycosides are listed in Table **1**.

The pharmacokinetics of aminoglycosides are characterised by concentration-dependent killing [46], which means that higher peak serum concentrations relative to the target pathogen's minimum inhibitory concentration (MIC) are associated with increased bactericidal activity. However, these antibiotics also exhibit concentration-dependent toxicity, particularly concerning their potential to cause nephrotoxicity (kidney damage) and ototoxicity (hearing and balance disorders) [47]. Therefore, monitoring serum drug levels is essential to ensure optimal therapeutic efficacy while minimizing adverse effects.

Nevertheless, aminoglycosides have emerged as vital therapeutic choices for managing serious infections, primarily because of increased antibiotic resistance, particularly among Gram-negative bacteria [46]. However, their use should be reasonable and guided by antimicrobial susceptibility testing to prevent the emergence and spread of resistance. In recent years, the emergence of aminoglycoside--modifying enzymes, such as Recombinant *Klebsiella pneumoniae Aminoglycoside* (3") (9) Adenylyl Transferase (RKAAT), has raised concerns about the effectiveness of aminoglycosides, emphasising the need for comprehensive research and surveillance to preserve the clinical significance of these vital antibiotics.

4. RECOMBINANT *KLEBSIELLA PNEUMONIAE* AMINOGLYCOSIDE ADENYLYL TRANSFERASE (RKAAT)

Inactivation of antimicrobials is employed by Gram-negative bacteria like antibiotic-resistant *Klebsiella pneumoniae (K. pneumoniae*) strains. *K. pneumoniae* is the leading cause of hospital-acquired infections, including pneumonia [48]. Previously, *K. pneumoniae* infections could be treated with aminoglycoside or combinations of beta-lactam and aminoglycoside antibiotics. However, it has been shown that the isolates that cause various hospital-acquired infections are resistant to these classes of antibiotics (Zhang *et al.,* 2020) [49]. Studies have revealed that *K. pneumoniae* employs multiple mechanisms that allow the bacterium to become resistant to once-effective antibiotics. The most common mechanism is the synthesis of beta-lactamases and AMEs. *K. pneumonia*'s increased resistance to these antibiotics creates an urgent need to develop a new generation of more robust and effective antibiotics that can fight off the pathogen. The most effective approach to combat antimicrobial drug resistance is to conduct rational drug discovery using proteins such as AMEs as drug targets [38, 48].

Furthermore, an enzyme has been identified in *K. pneumoniae*, and it is a potential drug target. The enzyme is called aminoglycoside (3") (9) adenylyl-transferase (UniProtKBP08881 (S3AD_KLEPN), and it is a member of the ANT class of AMEs. The enzyme is abbreviated Kp3"ANT to show that it was identified in *K. pneumoniae*. The enzyme has been shown to mediate resistance to two aminoglycoside antibiotics: streptomycin and spectinomycin [50]. Kp3"ANT falls within the ANT (3") and ANT (9) subclasses because it catalyses adenylation at position 3" of streptomycin and adenylation at position 9 of spectinomycin, and ATP is used as a substrate in the enzymatic process (Fig. **4**). The amino acid sequence of the Kp3"ANT was compared to that of an aminoglycoside (3") (9) adenylyl-transferase enzyme from a

Salmonella enterica bacterial species using the EMBL-EBI EMBOSS Needle pairwise sequence alignment tool. Moreover, the results revealed that the two enzymes share a sequence identity of only 46.96%. This suggests that although the enzyme carries out similar enzymatic reactions, they have significant structural differences.

Table 1. Commonly used aminoglycosides and their clinical significance

Aminoglycoside Antibiotic	Clinical Significance	Bacterial Targets
Gentamicin	Broad-spectrum antibiotics effective against Gram-negative and some Gram-positive bacteria	*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*
Tobramycin	Effective against various Gram-negative pathogens, including Pseudomonas aeruginosa	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii*
Amikacin	Broad-spectrum aminoglycoside with increased resistance to modifying enzymes	Multidrug-resistant Gram-negative pathogens

Note: Source [32, 45].

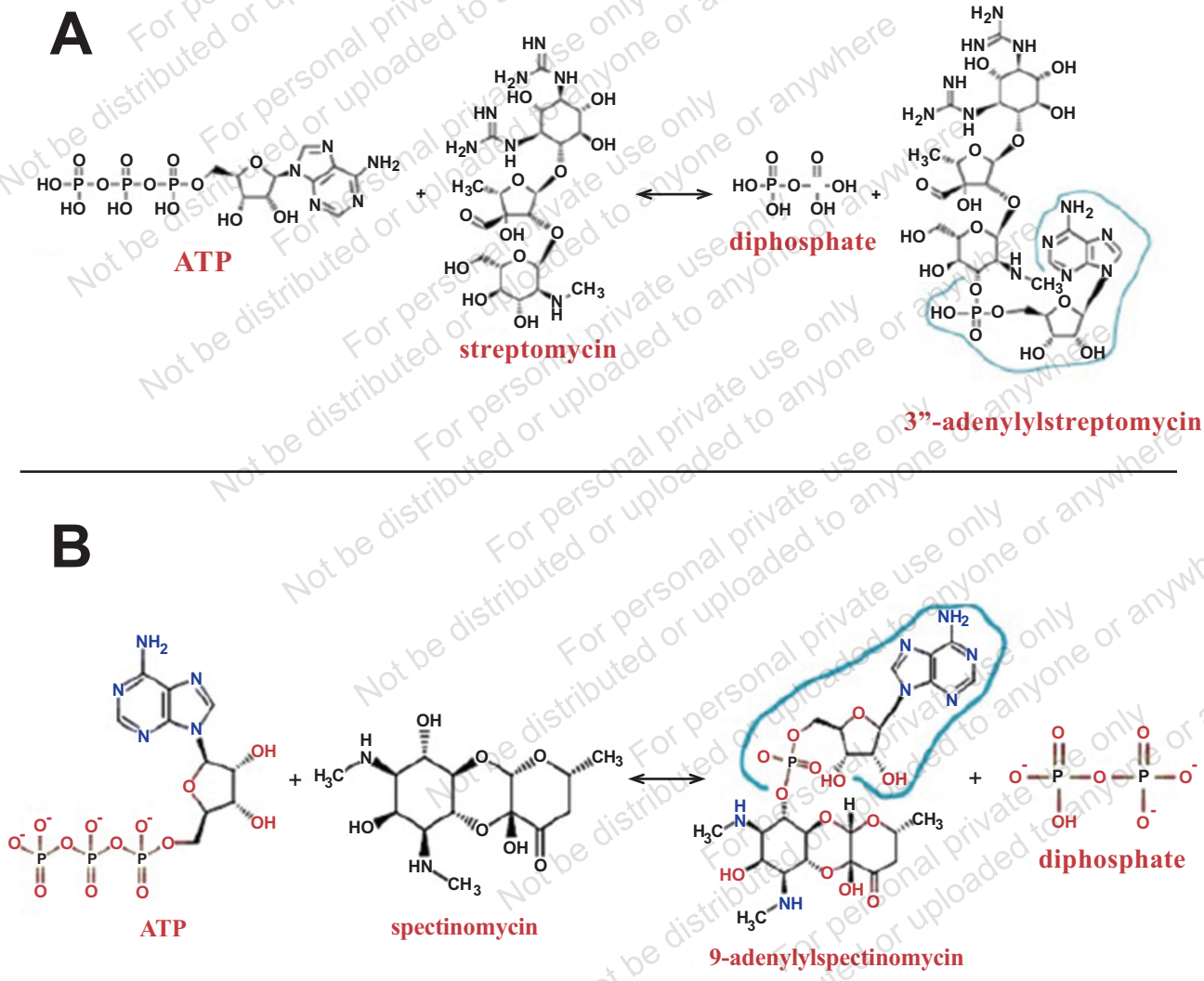

Fig. (4). The enzymatic reactions catalysed by Kp3”ANT. (**A**) The enzymatic reaction involves streptomycin as a substrate. (**B**) the enzymatic reaction involving spectinomycin as a substrate. The names of the molecular structures of the compounds are indicated below the structures in red. The adenosine monophosphate (AMP) groups transferred from ATP onto streptomycin or spectinomycin are enclosed in light blue regions. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

4.1. Biophysical Characterisation

Biomolecules such as proteins develop and progress various diseases [51]. Hence, it is essential to comprehend the mechanisms behind the functionality of these proteins.

Determining their structures in the case of enzymes is essential because this can help understand how these proteins interact with their natural ligands. This concept of determining the structure of a protein and gaining knowledge about the protein functions is called biophysical characterisation [52]. Moreover, this concept is essential in drug discovery, structural biology, and diagnostic research. The emergence of the ability to express and purify recombinant proteins rapidly and cost-effectively has led to an urgent need for developing and optimising techniques that can be used for the biophysical characterisation of recombinant proteins [53]. Various high-resolution techniques are used for the biophysical characterisation of small molecules, including mass spectrometry and crystallography [54]. Readily available low-resolution methods are usually used for the biophysical characterisation of large complex macromolecules such as proteins. These low-resolution methods include far-UV circular dichroism (CD) and fluorescence spectroscopy [55].

Far-UV CD is used quantitatively to characterise proteins regarding their secondary structural elements [56]. Optically active substances can absorb different amounts of right-handed and lefthanded circular polarised light. Furthermore, this difference in the absorption of the two forms of circularly polarised light can be quantified. Proteins contain peptide bonds that are chiral and, therefore, optically active. A protein's secondary structural elements can be quantified by observing the difference in the absorption of the two forms of circularly polarised light, which correspond to the different conformations of peptide bonds in each element.

The technique is well-established and widely used [57]. Similarly, Fluorescence spectroscopy is a technique that uses a range of fluorescent dyes to evaluate the capacity of recombinant enzymes to adopt tertiary structures and investigate their interactions with endogenous ligand molecules [58]. The fluorescent dyes are susceptible to their environment, and their fluorescence is quenched in hydrophilic environments (external protein environment). However, when the dyes bind to predominantly hydrophobic regions of a protein (active site), their fluorescence is no longer quenched, and they fluoresce with high intensity. The fluorescence spectra of these dyes also change in the presence of competitive ligand molecules that naturally bind to the active site of an enzyme [59]. Therefore, the fluorescence spectra of the dyes can be observed in different conditions, and changes can be used to assess enzymes in terms of 3D structure and ligand binding [60].

To enhance the antibacterial efficacy of aminoglycosides, it is imperative to comprehend the structural and molecular intricacies associated with these modifying enzymes. Considerable advancements have been achieved in comprehending the composition, functioning, and inhibitory characteristics of many APH enzymes [61, 28]. The complete characterisation of the AAC family has been furthered by investigations into the structure and function of AAC(3) and AAC(6′) enzymes. The crystal structures of ANT(4′)-Ia (PDB ID: 1KNY) (21), ANT(4′)-IIb (PDB ID: 4EBJ and 4EBK), and ANT(6)-Ia (PDB ID: 2PBE) have been successfully determined [62-64]. However, it is essential to note that the overall understanding of the ANT enzyme family remains limited.

The differentiation of the five primary types of ANT enzymes is based on their regioselectivity regarding the chemical modification of substrate aminoglycosides at positions 2, 3, 4, 6, and 9. The aminoglycoside 2″-O-nucleotidyltransferase [ANT(2″)-Ia] is a highly prevalent enzyme among Gram-negative bacteria in North America. Its discovery dates to 1971 [65], when it was initially identified as a clinical strain of *K. pneumoniae*. The ESKAPE diseases, namely Pseudomonas, Acinetobacter, and Enterobacter, have been recognised for their significant clinical importance, and this genetic variant has disseminated to numerous other bacterial species [66].

4.2. Resistance Phenotypes Conferred by Aminoglycoside AdenylylTransferase (AGAT)

Recombinant *Klebsiella pneumoniae Aminoglycoside* Adenylyl Transferase (AGAT) is a critical determinant of aminoglycoside resistance in bacterial pathogens. AGAT's enzymatic modification of aminoglycosides confers specific resistance phenotypes, impacting the susceptibility of bacterial strains to these antibiotics [67]. The resistance phenotypes observed in AGAT-expressing strains can vary depending on the modified aminoglycosides and the bacterial species involved. Some of the critical resistance phenotypes conferred by AGAT (Table **2**) are as follows:

Table 2. Key resistance mechanisms and associated phenotypes in RKAAT-expressing strains.

Resistance Mechanism	Impact on Aminoglycosides	Phenotypic Effect	Resistance Mechanism
RKAAT-mediated adenylylation	Adenylylation of aminoglycosides, reducing ribosomal binding	High-level aminoglycoside resistance	RKAAT-mediated adenylylation
Efflux Pumps	Active expulsion of aminoglycosides from bacterial cells	Reduced intracellular drug accumulation, decreased susceptibility	Efflux Pumps
Beta-lactamases	Hydrolysis of beta-lactam antibiotics, preventing bacterial cell wall damage	Multidrug resistance, reduced effectiveness of beta-lactam antibiotics	Beta-lactamases

4.2.1. High-Level Aminoglycoside Resistance

The most prominent phenotype observed in bacterial strains expressing AGAT is high-level resistance to aminoglycoside antibiotics. AGAT-mediated adenylation of aminoglycosides, such as gentamicin and tobramycin, significantly reduces their binding affinity to bacterial ribosomes, leading to impaired bactericidal activity. This high-level resistance phenotype can render conventional aminoglycoside treatment ineffective against AGAT-expressing pathogens [68].

4.2.2. Multi-Aminoglycoside Resistance

RKAAT's ability to modify specific aminoglycoside substrates, including gentamicin, tobramycin, and amikacin, contributes to multi-aminoglycoside resistance in bacterial strains. By targeting a diverse range of aminoglycosides, AGAT confers resistance against multiple members of this antibiotic class, further limiting treatment options [69].

4.2.3. Preservation of Sensitivity to Other Antibiotics

While AGAT-mediated resistance primarily affects aminoglycosides, it does not alter bacterial strains' susceptibility to other antibiotics. As a result, AGAT-expressing strains may remain sensitive to non-aminoglycoside antibiotics, allowing for alternative treatment options in cases where aminoglycosides are not viable [70].

4.2.4. Enhanced Multidrug Resistance

AGAT often coexists with other antibiotic resistance mechanisms in multidrug-resistant bacterial strains, such as beta-lactamases and efflux pumps. The combination of AGAT-mediated aminoglycoside resistance and other resistance mechanisms contributes to an enhanced multidrug-resistant phenotype, limiting the efficacy of multiple classes of antibiotics against these strains [71].

4.2.5. Cross-Species Transfer of Resistance

AGAT, encoded on mobile genetic elements, can facilitate the transfer of aminoglycoside resistance between different bacterial species. Horizontal gene transfer allows AGAT and other resistance genes to spread within bacterial populations, promoting the dissemination of multidrug-resistant strains (Fig. **5**) [71].

The resistance phenotypes conferred by AGAT can have significant clinical implications. Infections caused by AGAT-expressing strains of *K. pneumoniae* and related pathogens may be challenging to treat, especially when aminoglycosides are essential therapeutic options [72]. The significance of antimicrobial management and infection control strategies is underscored by AGAT and its link with multidrug resistance.

Moreover, there is a need for the advancement of innovative therapeutic approaches to address the issue of RKAAT-induced aminoglycoside resistance effectively.

Hence, understanding the resistance phenotypes conferred by RKAAT is vital for guiding antibiotic therapy and developing strategies to combat multidrug-resistant bacterial infections effectively.

5. CHALLENGES IN COMBATING RKAAT-MEDIATED RESISTANCE

The emergence and propagation of RKAAT-mediated aminoglycoside resistance among bacterial pathogens carry profound clinical implications, engendering multifaceted challenges for patient care and public health. RKAAT often intertwines with other resistance mechanisms, yielding multidrug-resistant strains that substantially curtail viable treatment options [73]. This need for more effective antibiotics places clinicians in a precarious position, grappling with limited choices to combat infections effectively.

The ramifications extend to treatment outcomes, where infections instigated by RKAAT-producing strains face heightened prospects of therapeutic inadequacy. The scarcity of suitable antibiotics magnifies the likelihood of treatment failure, fostering a fertile ground for complications, prolonged ed hospitalisations, and elevated mortality rates in afflicted patients [66]. Moreover, the repercussions encompass the broader epidemiological landscape, characterised by the horizontal transmission of RKAAT and sociated resistance genes across diverse bacterial species. This ease of cross-species transmission accentuates the menace of resistance dissemination, fostering challenging outbreaks and precipitating the swift diffusion of resistance within healthcare facilities [74].

The prevalence of RKAAT-mediated resistance orchestrates a complex interplay of clinical and public health difficulties. As treatment options dwindle and infections become refractory, the urgency to devise innovative strategies, reinforce infection control measures, and cultivate antibiotic stewardship initiatives intensifies. Only through a comprehensive and collaborative effort can we confront these challenges and safeguard the efficacy of our antibiotic arsenal [66, 72].

5.1. Therapeutic Approaches and Limitations

Combatting RKAAT-mediated resistance presents several challenges, but various therapeutic approaches (Table **3**) are being explored:

5.1.1 Combination Therapy

Combining amino-glycosides with other antibiotics, such as beta-lactams or fluoroquinolones, may help overcome resistance and improve treatment outcomes. However, the potential for increased side effects and drug interactions needs careful consideration [75].

5.1.2 Development of Novel Aminoglycosides

Researchers are investigating the development of novel aminoglycoside antibiotics that are less susceptible to RKAAT-mediated modification. These new agents may provide alternative treatment options for resistant infections [76]. Plazomicin, also known as ACHN-490, is an exam le.

Apramycin analogues and Plazomicin are novel aminoglycosides specifically engineered to resist modification by aminoglycoside-modifying enzymes. They can effectively combat many multidrug-resistant bacteria, including those that produce AMEs.

5.1.3 Novel Antibiotics

Teixobactin is a widely found antibiotic that explicitly attacks the process of bacterial cell wall formation. This treatment's unique method reduces the likelihood of developing resistance, offering a promising solution for fighting against antibiotic-resistant bacteria.

5.1.4 Phage Therapy

Eliava Phage Therapy Centre in Georgia has been at the forefront of utilising bacteriophages to treat bacterial diseases. Phage treatment uses phages to selectively target and eradicate bacterial strains resistant to antibiotics [77].

5.1.5 Antibiotic Adjuvants

Clavulanic acid is often used with beta-lactam antibiotics as a beta-lactamase inhibitor. It improves the effectiveness of beta-lactam antibiotics by inhibiting their breakdown by bacterial beta-lactamases [78].

5.1.6 Combining Antibiotics

For example, the Trimethoprim/Sulfamethoxazole combination, these antibiotic combination targets several stages in bacterial folate production, showcasing synergistic effects and diminishing the probability of resistance formation [79].

5.1.7 Host-Targeted Therapies

An example is the Quorum-sensing inhibitors. These inhibitors impair bacterial communication mechanisms and are currently being investigated [79]. These medicines specifically target the host to disrupt the virulence and pathogenicity of bacteria. Table **3** summarises the therapeutic approaches for addressing RKAAT-Mediated Resistance.

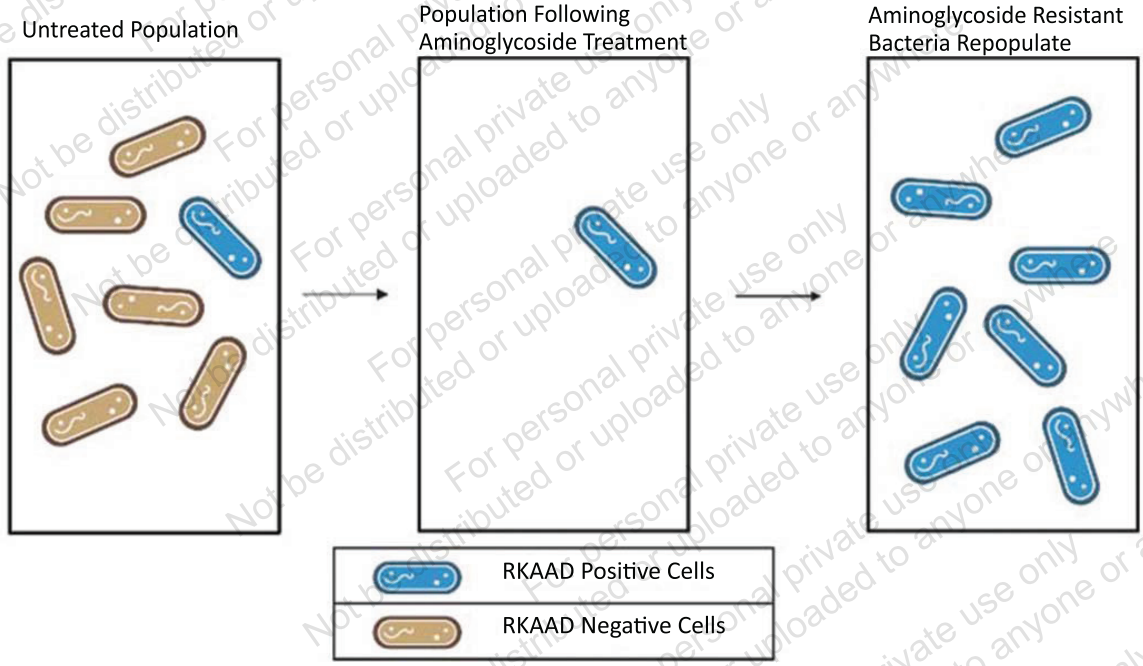

Fig. (5). Evolutionary Aspects of RKAAT Dissemination. This figure presents the evolutionary aspects of RKAAT dissemination, highlighting the selective pressure imposed by antibiotic use, the fitness cost associated with resistance, and the role of compensatory mutations in promoting the survival of RKAAT-expressing strains. It illustrates how horizontal gene transfer contributes to the rapid spread of RKAAT and other resistance genes among bacterial populations. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 3. Therapeutic approaches for addressing RKAAT-mediated resistance.

Therapeutic Approach	Description	Potential Benefits
RKAAT Inhibitors	Small molecules that block RKAAT activity	Restoration of aminoglycoside efficacy
Novel Aminoglycosides	Development of modified aminoglycosides resistant to RKAAT	Alternative treatment options
Combination Therapy	Combinations of antibiotics to enhance the effectiveness	Synergistic effects, overcoming resistance

Table 4. Infection control strategies for limiting RKAAT-mediated resistance spread

Infection Control Strategy	Description	Implementation
Enhanced Surveillance	Regular monitoring of antibiotic resistance patterns	Hospital and community surveillance programs
Isolation Precautions	Isolation of patients infected with multidrug-resistant strains	In healthcare settings
Hand Hygiene	Strict adherence to hand hygiene protocols	Hospitals, clinics, and public places
Antibiotic Stewardship	Rational prescribing and responsible antibiotic use	Healthcare facilities and community health programs

5.2. Infection Control Strategies

Robust infection control measures (Table **4**) are pivotal to curbing the transmission and outbreaks of RKAAT-producing strains. Enhanced surveillance must track antibiotic resistance trends, particularly RKAAT-mediated resistance, pinpointing outbreaks for swift intervention [80]. Isolation precautions, effectively isolating patients with multidrug-resistant strains, thwart cross-transmission among patients and healthcare staff. Rigorous hand hygiene practices must be advocated to minimise the disseminating of resistant bacteria within healthcare settings [81].

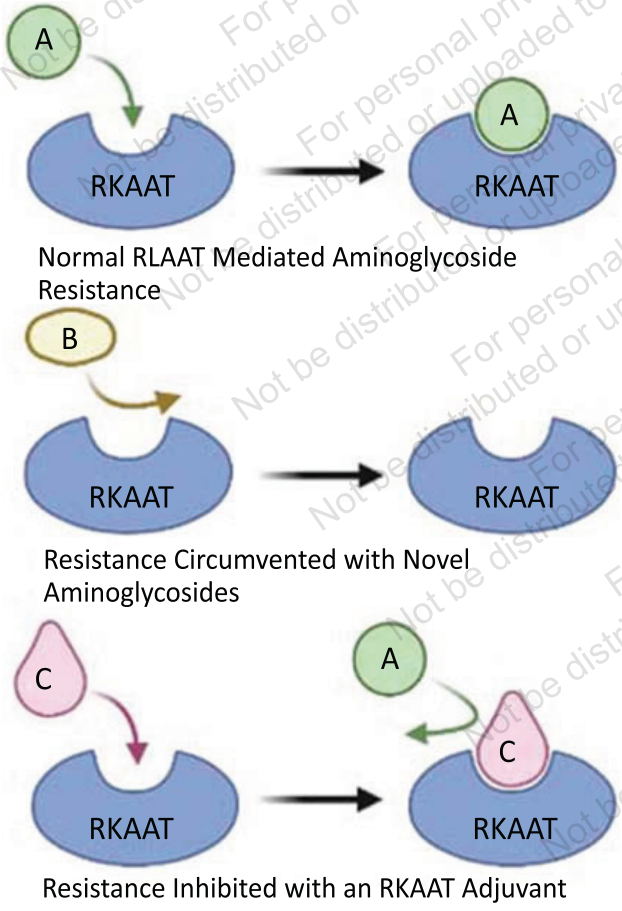

Fig. (6). Strategies for Addressing RKAAT-Mediated Resistance. Fig. (**5**) outlines the different strategies for combating RKAAT-mediated resistance. It can include illustrations of RKAAT inhibitors, novel aminoglycosides, and combination therapies that overcome resistance. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Comprehensive environmental cleaning is imperative, eradicating potential reservoirs of resistant organisms. Antimicrobial stewardship programs promoting judicious antibiotic use are crucial in curbing the emergence and spread of RKAAT-mediated resistance. Tackling these challenges necessitates a holistic approach, uniting healthcare providers, researchers, policymakers, and the public. By implementing robust infection control strategies and fostering innovative therapies, we can alleviate the impact of RKAAT-mediated resistance and safeguard the effectiveness of aminoglycoside antibiotics in combatting bacterial infections. In doing so, we fortify healthcare systems against multidrug opposition, ensuring effective treatment and preserving public health Table **5**.

In summary, Fig. (**6**) depicts an overview of the various approaches employed to address resistance mediated by RKAAT. The displayed figure also illustrates the significance of antibiotic stewardship and infection control measures in effectively mitigating the dissemination of resistance.

6. FUTURE DIRECTIONS

The increasing antibiotic resistance presents a significant problem regarding RKAAT-mediated aminoglycoside resistance, requiring the development of new solutions. Using state-of-the-art technology, scientists decipher the complex interactions of molecules, revealing the crucial significance of RKAAT [86]. Researchers are motivated to create inhibitors and new treatments to enhance the effectiveness of aminoglycosides. RKAAT-resistant infections in clinical settings result in extended hospital stays and exacerbate consequences. Precision medicine, which involves the analysis of pathogen genomes, is emerging as a promising approach that provides customised therapies. The One Health strategy promotes cooperation across medical, veterinary, and ecological fields, disrupting the transfer of resistance. Education is paramount to fostering awareness, promoting judicious antibiotic use, and fostering a shared dedication to combating antibiotic resistance [87]. The continuous story demonstrates the ability to adapt, persevere, and be willing to work together, with opportunities for further investigation and creativity, crafting a future in which antibiotic resistance is managed and successful therapies are dominant.

In the wards, the impact of RKAAT-mediated resistance was palpable. Infections caused by RKAAT-producing strains proved unyielding to conventional treatments, leading to [88] prolonged hospital stays, complications, and even loss of life. The urgency to find innovative solutions became undeniable, prompting a paradigm shift in treatment approaches.

Table 5. Overview of strategies to overcome RKAAT-mediated resistance in *Klebsiella pneumoniae*.

Control Strategy	Mode of Action	Key Findings	References
Next-Generation Aminoglycosides	Modification resistance, enhanced efficacy	Plazomicin, designed to resist AME modification, shows broad-spectrum activity against multidrug-resistant bacteria.	[82]
AME Inhibitors	Enzyme inhibition, prevention of modification	Synthetic analogues of apramycin act as potential inhibitors, aiming to disrupt enzymatic modification and restore aminoglycoside efficacy.	[83]
Novel Antibiotic Classes	Unique modes of action reduced resistance risk.	Teixobactin, a recently discovered antibiotic, exhibits a novel mechanism targeting bacterial cell wall synthesis, reducing susceptibility to resistance.	[84]
Phage Therapy	Bacteriophage-mediated bacterial lysis	Phage therapy, exemplified by the Eliava Phage Therapy Center, utilises bacteriophages to infect and lyse antibiotic-resistant bacterial strains.	[77]
Antibiotic Adjuvants	Beta-lactamase inhibition enhanced antibiotic activity	Clavulanic acid, a beta-lactamase inhibitor, enhances the efficacy of beta-lactam antibiotics by preventing degradation.	[78]
Antibiotic Combinations	Synergistic effects reduced resistance risk.	Trimethoprim/Sulfamethoxazole combination targets different steps in bacterial folate synthesis, showing synergistic effects.	[79]
Host-Targeted Therapies	Disruption of bacterial virulence mechanisms	Quorum-sensing inhibitors interfere with bacterial communication systems, disrupting virulence and pathogenicity.	[85]

CONCLUSION

RKAAT-mediated aminoglycoside resistance represents a significant challenge in the treatment of bacterial infections. The enzymatic modification of aminoglycosides by RKAAT reduces their effectiveness and contributes to multidrug-resistant phenotypes in bacterial strains. The clinical implications of RKAAT-mediated resistance underscore the urgency of finding practical solutions to combat antibiotic resistance. Thus, addressing RKAAT-mediated resistance requires a multifaceted approach encompassing research, drug development, infection control measures, and antibiotic management. Therefore, persistent research into the molecular bases of resistance, exploring new therapeutic options and implementing strategies for responsible antibiotic use are essential in the fight against RKAAT-mediated and antibiotic resistance.

As we move forward, a collaborative and proactive approach is necessary to confront the challenges of antibiotic resistance. By embracing innovative technologies, promoting global cooperation, and investing in research and development, we can work towards a future where effective antibiotics remain a cornerstone in treating infectious diseases, ensuring the health and well-being of individuals worldwide.

LIST OF ABBREVIATIONS

AMEs = Aminoglycoside-modifying Enzymes

RKAAT = *Klebsiella pneumoniae* Aminoglycoside Adenylyl Transferase

AAC = Aminoglycoside Acetyltransferases

APH = Aminoglycoside Phosphotransferases

ANT = Aminoglycoside Nucleotidyltransferases

CD = Circular Dichroism

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The author(s) declare no conflict of interest, financial or otherwise.

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