A review on RNA targeting antibiotics and how to overcome antibiotic resistance

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Abstract: Antibiotics target ribosomes at distinct locations within functionally relevant sites. They exert their inhibitory action by diverse modes, including competing with substrate binding, interfering with ribosomal dynamics, minimizing ribosomal mobility, facilitating miscoding, and blocking the nascent protein exit tunnel. Although the ribosome is a highly conserved organelle, they possess a subtle sequence this enables drug selectivity they facilitating clinical usage. The increased understanding of RNA structure and function is likely to be to a broad variety of potential therapeutic targets for small molecules. The continued development of technologies for drug ability assessment and inhibitor discovery against new RNA targets will be invaluable. We should also focus on other newer drugs which also target RNA in next future.

Keywords: Antibiotic, RNA, Multidrug resistance.

I. INTRODUCTION

RNAs have diverse structures, including bulges and internal loops, which can form tertiary contacts or serve as ligand-binding sites. The recent increase in structural and functional information related to RNAs has put them in the limelight as a drug target for small molecule therapy. In addition, the recognition of the marked difference between prokaryotic and eukaryotic rRNA has led to the development of antibiotics that specifically target bacterial rRNA, reduce protein translation and thereby inhibit bacterial growth. To facilitate the development of new antibiotics targeting RNA, we here review the literature concerning such antibiotics, mRNA, riboswitch, and tRNA and the key methodologies used for their screening. Many antibiotics are known to target ribosomal RNA (rRNA) in prokaryotes to inhibit the growth of bacteria. To facilitate the discovery of improved antibiotics targeting RNA, we describe the secondary structures of partial rRNA and indicate the binding sites for tetracycline, puromycin, lincomycin, and other antibiotics. With the development of new drug discovery technologies, targeting RNA for better antibiotics is emerging as a new frontier in drug discovery. Deoxyribonucleic acid (DNA) is the major genetic material in eukaryotes and generally exists in the form of double-stranded helices. In contrast, ribonucleic acid (RNA) can fold into numberless tertiary structures that reflect its diverse functions. Thus, it serves as the genetic material in some viruses, as the mediator of genetic information from DNA to protein, as the structural component in many ribonucleoproteins (RNPs), and, in some cases, as a catalyst. RNA is usually associated with RNA-binding proteins (RBPs), which protect, stabilize or transport it and regulate its interaction with other molecules. RNA plays many crucial roles in protein synthesis, transcriptional regulation, and retroviral replication making it a prime target for drug action. The recent publication of high-resolution crystal structures of prokaryotic rRNA subunits has transformed our understanding of RNA. The structures of RNA alone, and of RNA–protein complexes reveal a variety of tertiary structures and patterns of RNA–protein interaction. RNA can fold into complex three-dimensional structures comprising loops, pseudoknots, bulges, and turns which afford specific binding sites for small molecules. Compared to DNA, RNA is not only more flexible but lacks repair mechanisms that enhance its susceptibility to the action of therapeutics. These include both natural and synthetic compounds that can influence the biological activity of RNA by changing its configuration or inhibiting its catalytic function. [**1.2.3.4.5]**

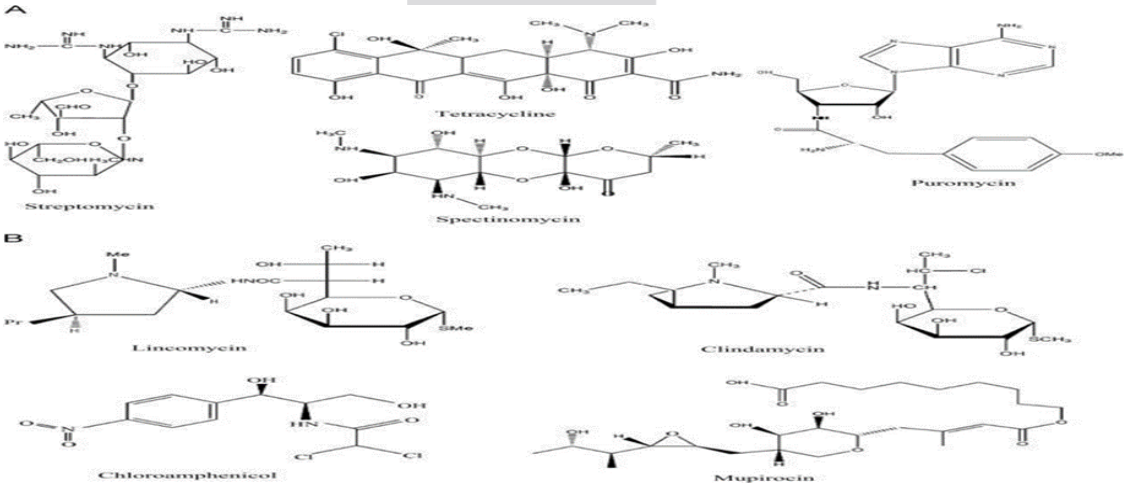


Fig -1: Different types of antibiotics

Many antibiotics are known to target rRNA in prokaryotes and thereby alter protein translation. Their diverse structures witness the importance of this class of drugs and the long period of their development since their discovery in the 1930s. Concomitant with the marketing of powerful antibiotics has emerged the phenomenon of antibiotic resistance which poses a serious threat to human health. The recent slowdown in the pace of novel antibiotic development has further complicated the global health issue. However, antibiotics remain an attractive area for investment and represent the third largest therapeutic area with global financing. To assist in the discovery of new antibiotics, here summarize the most commonly used drugs targeting bacterial rRNA in clinical development rRNA is the most commonly exploited RNA target for small molecules. The bacterial ribosome comprises the 30S and 50S ribonucleoprotein subunits contain several binding sites for known antibiotics and is an attractive target for novel antibacterial agents. The large difference between prokaryotic and eukaryotic rRNA enables rRNA-targeting against a broad spectrum of pathogenic bacteria. Bacterial ribosomes have two ribonucleoprotein subunits of which approximately two-thirds are RNA. The bacterial rRNA includes 5S, 16S, and 23S rRNA, the smallest (5S rRNA) being a ~120 nt RNA. The smaller 30S subunit contains a single ~1500 nt RNA (16S rRNA) and about 20 different proteins while the larger 50S subunit contains ~2900 nt RNA (23S rRNA) and about 30 different proteins21. Recently, the application of X-ray crystallography has elucidated many antibiotic-binding sites ribosomal subunits facilitating the design of novel antibiotics. **[6.7.8.9.10.11]**. Now we talk about mi RNA. MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression at the post-transcriptional level. It is now well-established that the overexpression of some miRNAs (oncogenic miRNAs) is responsible for the initiation and progression of human cancers and the discovery of new molecules able to interfere with their production and/or function represents one of the most important challenges of the current medicinal chemistry of RNA ligands. In this, the study of the ability of 18 different antibiotics, known as prokaryotic ribosomal RNA, to bind to oncogenic miRNA precursors (stem-loop structured pre-miRNAs) to inhibit miRNA production. In vitro inhibition, binding constants, thermodynamic parameters, and binding sites were investigated, and highlighted that aminoglycoside and tetracyclines represent interesting pre-miRNA ligands with the ability to inhibit Dicer processing. **[12,13,14]**

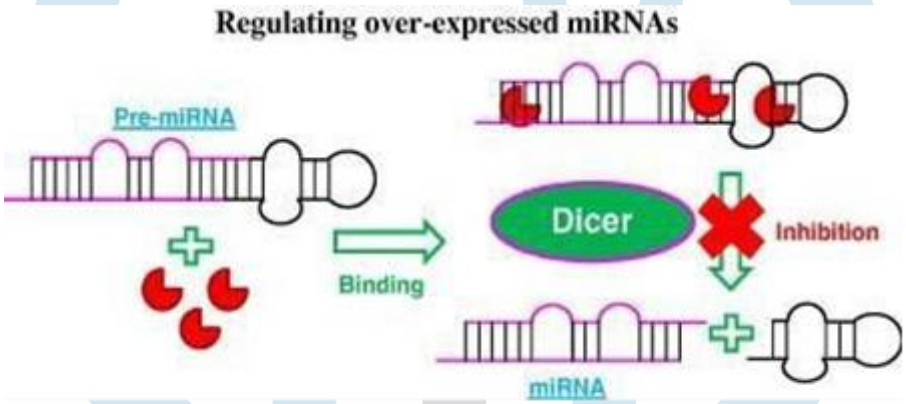


Fig – 2 Regulation overexpressed miRNAs

II. AMINOGLYCOSIDE ANTIBIOTICS

An aminoglycoside is a group of well-known antibiotics that have been used successfully for over half a century. The drugs are binding to specific sites in prokaryotic rRNA and affect the fidelity of protein synthesis. The rRNA aminoacyl tRNA is a major target for aminoglycoside which because of the difference between prokaryotic 16s and human 18s rRNA, selectively kills bacterial cells. Binding of the drug to the 16s subunit near the A site of the 30s subunit leads to a decrease in translational accuracy and inhibition of the translocation of the ribosome Examples- Streptomycin, Gentamycin**. [15,16,17,18,19].**

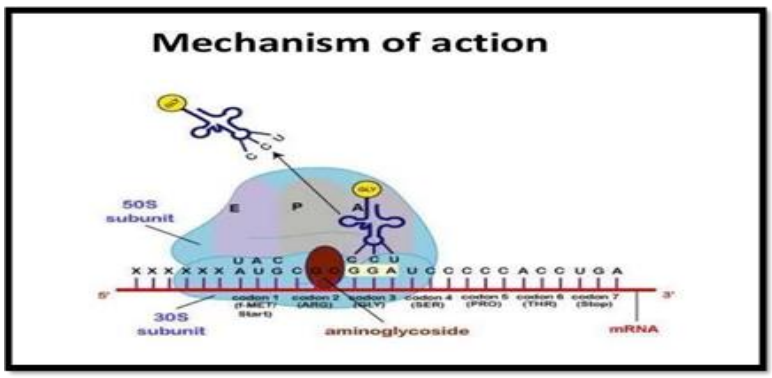
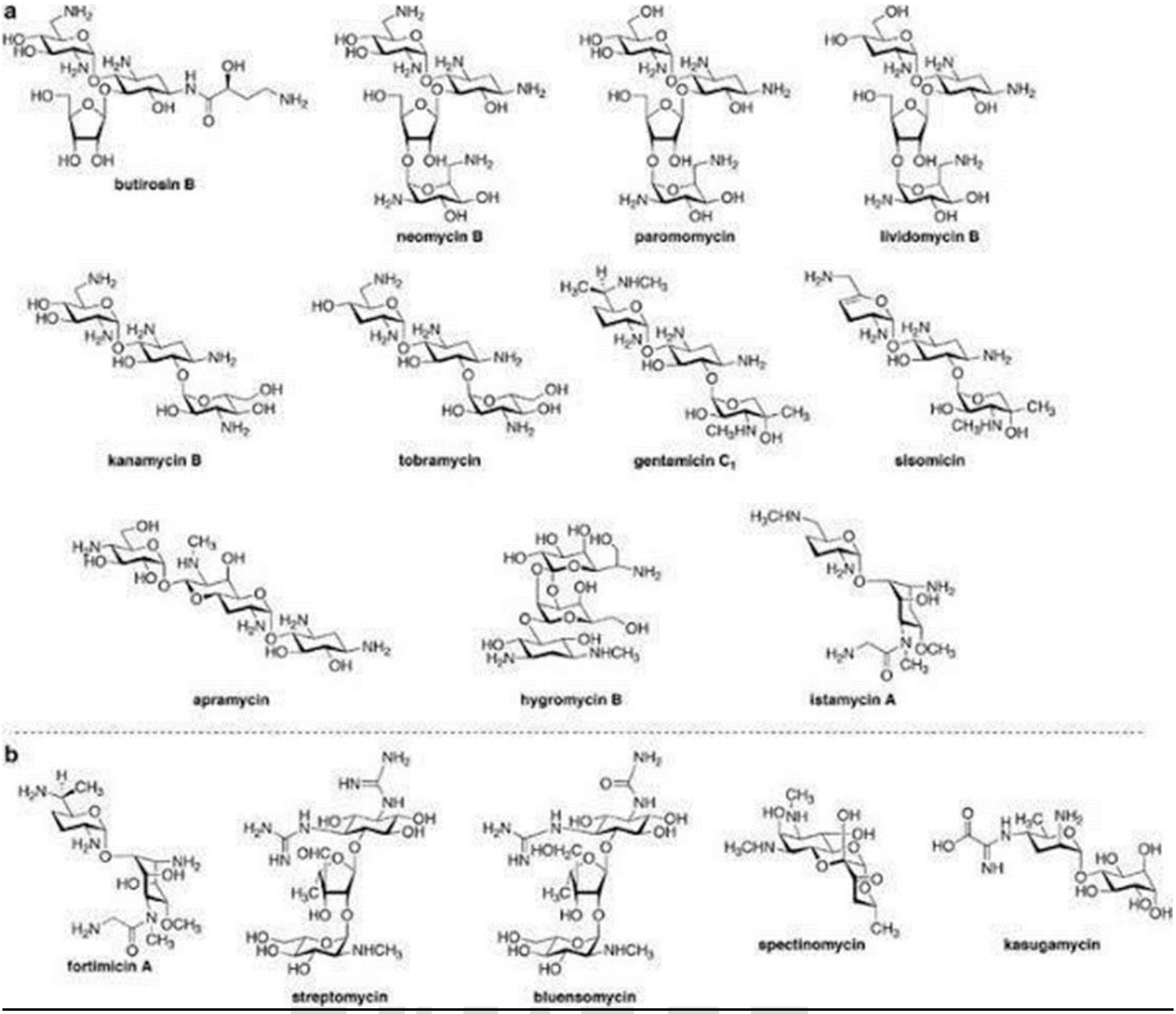


Fig -3: Mechanism of Aminoglycoside antibiotics



- **Molecular recognition of aminoglycoside antibiotics by ribosomal RNA and resistance enzymes:**

The potential of RNA molecules to be used as therapeutic targets by small inhibitors is now well established. In this fascinating wide-open field, aminoglycoside antibiotics constitute the most studied family of RNA-binding drugs. Within the last three years, several x-ray crystal structures were solved for aminoglycosides complexed to one of their main natural targets in the bacterial cell, the decoding aminoacyl–tRNA site (A site). Other crystallographic structures have revealed the binding modes of aminoglycosides to the three existing types of resistance-associated enzymes. The present review summarizes the various aspects of the molecular recognition of aminoglycosides by these natural RNA or protein receptors. The analysis and the comparisons of the detailed interactions offer insights that help design new generations of antibiotics. The progress of transcription is synthesized by complex molecules, among which DNA-dependent RNA polymerase (RNAP) is composed of core subunits (α2, β, and β') and a factor that is required for specific recognition of the promoter site and the initiation of transcription. Despite their ubiquity and structural and functional similarities, bacterial RNAPs do not share extensive sequence homology with eukaryotic RNAPs. Bacterial RNAP is an attractive target for the development of anti-bacterial drugs as its inactivation would lead to bacterial cell death. This will present the state of knowledge on the assembly and function of RNAP subunits in bacteria with a special focus on insights provided by structural analysis of a key component factor. Thorough retrospection has been provided for a better understanding of progress and problems in targeting RNAP by traditional chemical compounds. Recent progress using innovative strategies including structural biology and phage-based screening, especially antisense technology, has shed light on developing the first set of macro-molecule RNAP inhibitors. In particular, the exploration of targeting RNAP 70 for the realization of broad-spectrum antisense bactericidal effect gram-negative time bacteria presents the first successful example of RNA-peptide conjugate showing attractive potential as conventional broad-spectrum antibiotics, in which possible way the antisense antibiotics might develop into meet the range and type of usage in future health care.

1. Aminoglycoside antibiotics are known to target ribosomal, retroviral, and catalytic RNAs with high

affinity and specificity. Recently, in vitro selection experiments have identified RNA aptamers that bind to aminoglycoside antibiotics with nanomolar affinity and stringent specificity, allowing discrimination between closely related family members. There has, to date, been limited structural information on the molecular basis of such saccharide-RNA recognition.

2. A solution-structure determination of the tobramycin-RNA aptamer complex, obtained using NMR and molecular dynamics. The structure gives insight into the molecular features associated with saccharide-RNA recognition. Tobramycin adopts a defined alignment and binds to the RNA major groove centered about a stem-loop junction site. A portion of the bound tobramycin is encapsulated between the floor of the major groove and a looped-out cytosine residue that forms a flap over the binding site in the complex.

3. The emergence of antibiotic-resistant pathogens and their impact on human health continues to be a major concern in the medical community. Rational modification of existing antibiotics aimed at improving their efficacy requires a molecular view of their receptor-binding sites. It provided such a molecular view for a member of the aminoglycoside antibiotic family that targets RNA. **[20,21,22]**

III.MACROLIDE ANTIBIOTICS

The Macrolides are inhibition of bacterial protein biosynthesis, and they are thought to do this by preventing peptidyl transferase from adding the growing peptide attached to tRNA to the next amino acid as well as inhibiting ribosomal translation. Examples - Erythromycin, Clarithromycin, and Azithromycin. The acute problems associated with macrolide resistance; several new compounds have been designed. These include macrolide derivatives in which the core macro lactone ring has higher flexibility by increasing the number of atoms, similar to the 15-member ring Azithromycin well as 16-member ring derivatives such as Tylosin, Carbomycin-A, Spiramycin, and Josamycin.

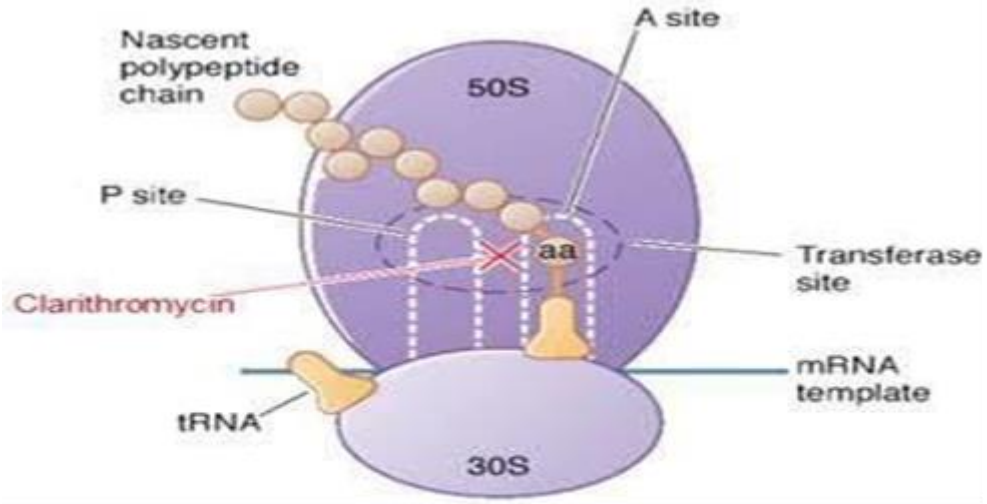


Fig - 5: Mechanism of action of macrolide Antibiotics

- **Mechanism of action:**

The macrolide antibiotic erythromycin interacts with bacterial 23S ribosomal RNA (rRNA) making contacts that are limited to hairpin 35 in domain II of the rRNA and the peptidyl transferase loop in domain V. These two regions are probably folded close together in the 23S rRNA tertiary structure and form a binding pocket for macrolides and other drug types. Erythromycin has been derivatized by replacing the L-cladinose moiety at position 3 with a keto group (forming the ketolide antibiotics) and by an alkyl-aryl extension at positions 11/12 of the lactone ring. All drugs footprint identically within the peptidyl transferase loop, giving protection against chemical modification at A2058, A2059, and G2505, and enhancing the accessibility of A2062. However, the ketolide derivatives bind to ribosomes with widely varying affinities compared with erythromycin. This variation correlates with differences in the hairpin 35 footprints. Erythromycin enhances the modification at position A752. Removal of cladinose lowers drug binding 70-fold, with concomitant loss of the A752 footprint. However, the 11/12 extension strengthens binding 10-fold, and position A752 becomes protected. **[23,24,25,26,27,28,29]**

- **The role of ribosomal RNAs in macrolide resistance**

Macrolides are bacteriostatic antibiotics that interfere with the peptidyl transfer function of the ribosome. Investigation of the molecular mechanisms underlying macrolide resistance in Mycobacterium smegmatis, a eubacterium carrying two rRNA

operons. Surprisingly, drug resistance was associated not with alterations in ribosomal proteins, but with a single point mutation in the peptidyl transferase region of one of the two 23S RNA genes, i.e. A2058→G or A2059→G. This mutation resulted in a heterozygous organism with a mutated and a wild-type rRNA operon respectively. Reverse transcriptase sequencing indicated the expression of both wild-type and mutated rRNAs. The mutated operon was introduced into genetically engineered rrn− strains of M. smegmatis carrying a single functional rRNA operon and into parental M. smegmatis with two chromosomal rRNA operons, using gene transfer as well as gene replacement techniques. The results obtained demonstrate the dominant nature of resistance. As exemplified in results on macrolide resistance, a complete set of genetic tools is now available, which allows questions of dominance vs. recessively and gene dosage effects in eubacterial ribosomal nucleic acids to be addressed experimentally in vivo. **[30,31,32]**

IV. LINCOSAMIDE ANTIBIOTICS

Lincosamide antibiotics (clindamycin, lincomycin) inhibit bacterial protein synthesis via a mechanism Similar to that of the macrolides, although it is not chemically Related. Mechanisms of resistance include methylation of the Binding site on the 50S ribosomal subunit and enzymatic inactivation. Gram-negative aerobes are intrinsically resistant because of Poor penetration of clindamycin through the outer membrane. Cross-resistance between clindamycin and macrolides is common. Good tissue penetration occurs after oral absorption. Clindamycin undergoes hepatic metabolism, and both intact drugs and metabolites are eliminated by biliary and renal excretion. **[33,34,35,36]**

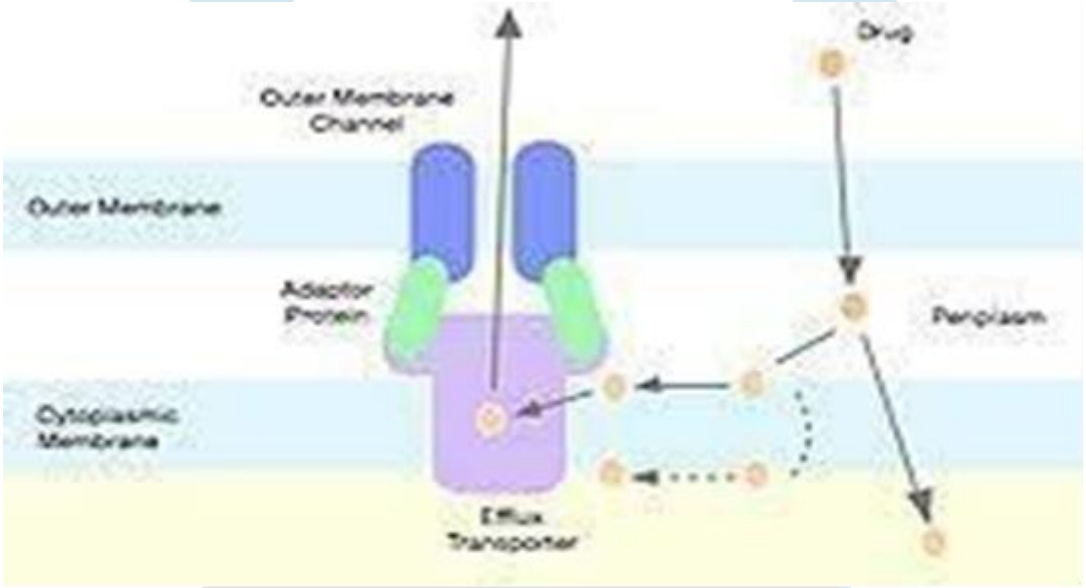


Fig – 6: Lincosamide antibiotics mechanism of action

V.STREPTOGRAMINS

Quinupristin-dalfopristin, a combination of 2 streptogramins, is bactericidal and has a duration of antibacterial activity longer than the half-lives of the two compounds (post-antibiotic effects).

Antibacterial activity Includes penicillin-resistant pneumococci, methicillin-resistant (MRSA) and vancomycin-resistant staphylococci (VRSA), and Resistant E faecium; E faecalis is intrinsically resistant via an efflux Transport system. Administered intravenously, the combination Product may cause pain and an arthralgia-myalgia syndrome. Streptogramins are potent inhibitors of CYP3A4 and increase Plasma levels of many drugs, including astemizole, cisapride, Cyclosporine, diazepam, nonnucleoside reverse transcriptase Inhibitors, and Warfarin. [**37,38,39,40]**

VI.OXAZOLIDINONES

The first of a novel class of antibiotics (oxazolidinones), Linezolid is active against drug-resistant gram-positive cocci, Including strains resistant to penicillin (ex-MRSA, PRSP) And vancomycin (eg, VRE). The drug is also active against L monocytogenes and corynebacterial. Linezolid binds to a unique Site located on the 23S ribosomal RNA of the 50S ribosomal Subunit, and there is currently no cross-resistance with other Protein synthesis inhibitors. Resistance (rare to date) involves A decreased affinity of linezolid for its binding site. Linezolid is Available in both oral and parenteral formulations and should Be reserved for the treatment of infections caused by multidrug-resistant gram-positive bacteria. The drug is metabolized by the liver and has an elimination half-life of 4–6 h. Thrombocytopenia and neutropenia occur, most commonly in immunosuppressed patients. Linezolid has been implicated in Serotonin syndrome when used in patients taking selective Serotonin reuptake inhibitors (SSRIs).

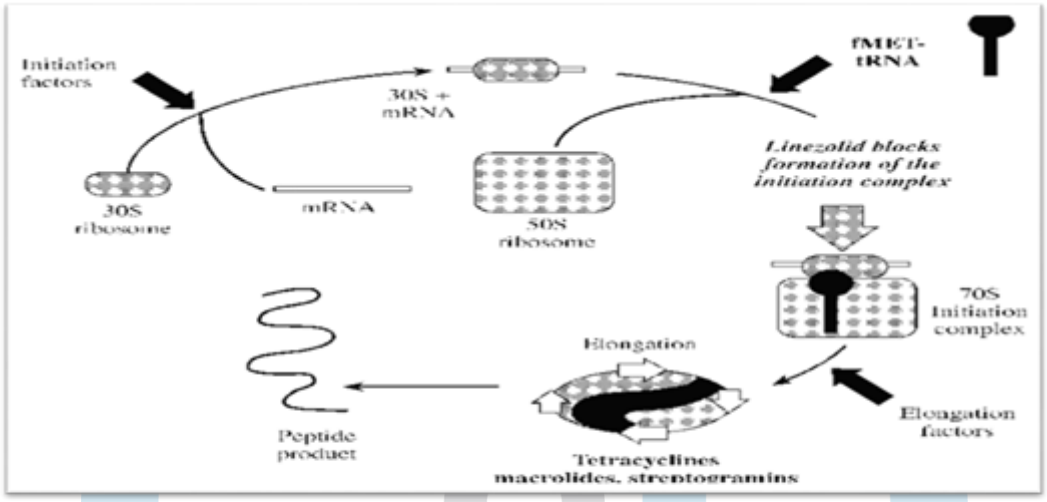


Fig -7: Mechanism of action of oxazolidinones

VII. ANTIBIOTIC RESISTANCE

Antibiotic resistance refers to the responsiveness of a microorganism to an AMA and is akin to the phenomenon of tolerance seen in the higher organism. It has two types, they are –

- **Natural resistance –** Some microbes have always been resistant to certain AMA. They lack the metabolic process or the target site which is affected by the particular drug. This is generally a group or species characteristic. Gram-negative bacilli are normally unaffected by penicillin G, aerobic organisms are not affected by metronidazole, anaerobic bacteria are not inhibited by aminoglycoside antibiotics, or M. Tuberculosis is insensitive to Tetracyclines. This type of resistance does not pose a significant clinical problem.

- **Acquired resistance –**It is the development of resistance by an organism due use of an AMA over some time. This can happen with any microbes and is a major clinical problem. However, the development of resistance is dependent on the microorganism as well as on the drug. Some bacteria are notorious for the rapid acquisition of resistance, Ex- staphylococci, coliform, and tubercle bacilli.

- **Multidrug resistance –** It is the new problem that happened today. Large amounts of antibiotics used for human therapy, as well as for farm animals and even for fish in aquaculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically in on-resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. This review discusses our current knowledge of the molecular mechanisms involved in both types of resistance. [**41,42,43,44,45]**

TYPES OF ALTERNATIVES TO PREVENT ANTIBIOTIC RESISTANCE (FOCUS ON RNA)

1. Antisense antibiotics: The nightmare of multi-drug resistant bacteria will still haunt us if no panacea is ever found. Efforts on seeking desirable natural products with bactericidal properties and screening chemically modified derivatives of traditional antibiotics have lagged behind the emergence of new multi-drug resistant bacteria. The concept of using antisense antibiotics, now as revolutionary as is on the threshold has experienced ups and downs in the past decade. In the past five years,

however, significant technological advances in the fields of microbial genomics, structural modification of oligonucleotides, and efficient delivery systems have led to fundamental progress in the research and in vivo application of this paradigm. The wealth of information provided in the microbial genomics era has allowed the identification and/or validation of several essential genes that may serve as possible targets for antisense inhibition; antisense oligodeoxynucleotides (ODNs) based on the 3rd generation of modified structures, e.g., peptide nucleic acids (PNAs) and phosphonodiamidite morphine oligomers (PMOs) have shown great potency in gene expression inhibition in a sequence-specific and dose-dependent manner at low micromolar concentrations; and cell-penetrating peptide-mediated delivery system has enabled the effective display of intracellular antisense inhibition of targeted genes both in vitro and in vivo. The new methods show promise in the discovery of novel gene-specific antisense antibiotics that will be useful in the future battle against drug-resistant bacterial infections. This review describes this promising paradigm, the targets that have been identified, and the recent technologies on which it is delivered.

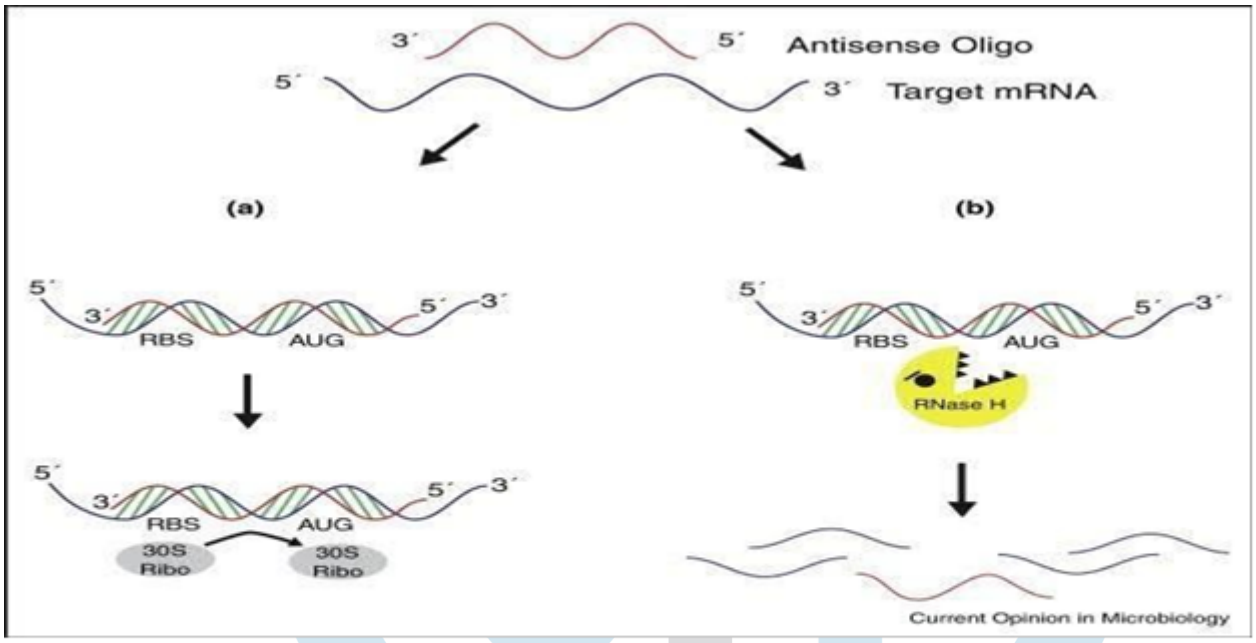


Fig –8: Antisense antibiotics

2. Molecular strategies for preventing antibiotic resistance:

Overuse of antibiotics in humans and livestock has led to the rapid evolution of bacteria that are resistant to multiple drugs such that even Vancomycin, the drug of last resort, is no longer effective against some strains. Apart from the discovery and exploitation of the natural peptide antimicrobial agents that form part of the innate immune systems of plants and animals, few new antibiotics have developed in recent years. Here are strategies designed to exploit recent advances in molecular biology, including recombinant DNA.

3. Roles of Regulatory RNA for Antibiotic Resistance in Bacteria and Their Potential Value for Novel Drug Targets:

The emergence of antibiotic resistance mechanisms among bacterial pathogens increases the demand for novel treatment strategies. Lately, the contribution of non-coding RNAs to antibiotic resistance and their potential value as drug targets became evident. RNA attenuator elements in mRNA leader regions couple the expression of resistance genes to the presence of the cognate antibiotic. Trans-encoded small RNAs (rRNAs) modulate antibiotic tolerance by base pairing with mRNAs encoding functions important for resistance such as metabolic enzymes, drug efflux pumps, or transport proteins. Bacteria respond with extensive changes in their sRNA repertoire to antibiotics. Each antibiotic generates a unique sRNA profile possibly causing downstream effects that may help to overcome the antibiotic challenge. In consequence, regulatory RNAs including rRNAs and their protein interaction partners such as Hfq may prove useful as targets for antimicrobial chemotherapy. Indeed, several compounds have been developed that kill bacteria by mimicking ligands for riboswitches controlling essential genes, demonstrating that regulatory RNA elements are druggable targets. Drugs acting on sRNAs are considered for combined therapies to treat infections. In this review, Regulatory RNAs respond to and establish a resistance to antibiotics in bacteria. Approaches to target RNAs involved in intrinsic antibiotic resistance or virulence for chemotherapy will be discussed.

The emergence and spread of resistance to antibiotics represent a major threat to human health and urgently calls for novel antimicrobial compounds and therapies. Traditionally, efforts to find novel treatment options have focused on bacterial proteins as drug targets, whereas exploiting regulatory RNA elements were only considered of late. In bacteria, regulatory RNAs act at the post-transcriptional level to control bacterial physiology, development, and virulence. Evidence is accumulating that regulatory RNAs are also important players in the bacterial response and resistance to antibiotics, making these molecules promising targets for antimicrobial chemotherapy.

Regulatory RNAs in bacteria comprise a heterogeneous group of molecules that act by various mechanisms to modulate cellular processes in response to cognate stimuli. These RNAs are often called non-coding RNAs (ncRNAs) as they usually operate on their own without needing to be translated. Regulatory RNAs include two major classes, which are RNA attenuates and small RNAs (sRNAs). RNA attenuators are part of the mRNA that they regulate and therefore act in cis. Attenuators are sensory RNAs as they respond directly to environmental signals by toggling between alternative secondary structures either favoring or preventing the expression of downstream genes. Classical attenuators monitor the ability of the ribosome to translate a short leader peptide. Another class of RNA attenuators comprises riboswitches, which respond to cognate small molecule ligands. The ligand binds to the riboswitch aptamer region and thereby alters the structure of an adjacent RNA element, i.e., the expression platform, dictating whether or not gene expression can occur. An additional major class of bacterial regulatory RNAs is sRNAs, which are expressed independently from their targets and distinguished as cis- or trans-encoded cis-encoded sRNAs, also called antisense RNAs, are transcribed in the opposite direction of their target genes and consequently they are fully complementary to their targets. Although there is an ongoing debate about whether the often-pervasive antisense transcription represents a meaningful response or simply reflects transcriptional noise, it became clear that antisense RNAs mediate a plethora of physiological effects through the duplex formation with target transcripts. Finally, trans-encoded sRNAs regulate distantly encoded target RNAs by base-pairing through partial complementarity, but other mechanisms are also known. Trans-encoded sRNAs often rely on proteins, such as Hfq, ProQ, and CsrA for activity and function (In Gram-negative bacteria, Hfq accelerates sRNA/target RNA duplex formation, thereby modulating translation, decay, or transcription of the target RNA As Hfq and CsrA are essential for the activity of numerous cognate sRNAs, their inhibition was shown to down-regulate sRNA networks controlling multiple virulence-relevant processes, which eventually can render bacteria not only non-infective but also more susceptible to antibiotic). In the following chapters, Recent advances in bacterial RNA research demonstrate the impact of various ncRNA classes on the resistance and tolerance to antimicrobials and discuss the suitability of these riboregulatory for antimicrobial chemotherapy. The implication of ncRNAs in Antibiotic Resistance and Tolerance Control of Antibiotic Resistance by RNA Attenuation – A Widespread Phenomenon.

Over the past years, an ever-increasing number of studies reported mechanisms controlling antibiotic resistance genes at the post-transcriptional level. This type of regulation generates an immediate response, which is beneficial when antibiotic concentrations increase rapidly. RNA-based attenuation mechanisms are known to couple the expression of resistance genes to the presence of cognate antibiotics. The classic example is provided by the erm gene of Staphylococcus aureus and its variants, which confer resistance to macrolide antibiotics. They encode enzyme methylation of a residue in 23S rRNA, which interferes with drug binding the leader region of the term's mRNA encodes a short peptide (Figure 9). Efficient translation of this triggers the formation of an attenuator structure that sequesters the term ribosome binding site (RBS) shutting down translation. The binding of erythromycin causes the ribosome to stall, which allows the formation of an alternative RNA structure in which the RBS is exposed, favoring translation (Figure 9)

Chloramphenicol as well as tetracycline resistance genes of Bactericides are controlled by a similar mechanism). Importantly, translation attenuation is not simply the consequence of translation inhibition per se as each of the different attenuators exhibits a high specificity and responds to a different subset of antibiotics. The binding of the antibiotic by the translating ribosome alters the properties of the ribosomal peptidyl transferase center in a drug-specific manner, thereby inhibiting peptide bond formation between specific combinations of amino acids that are present in the leader peptide **[46]**

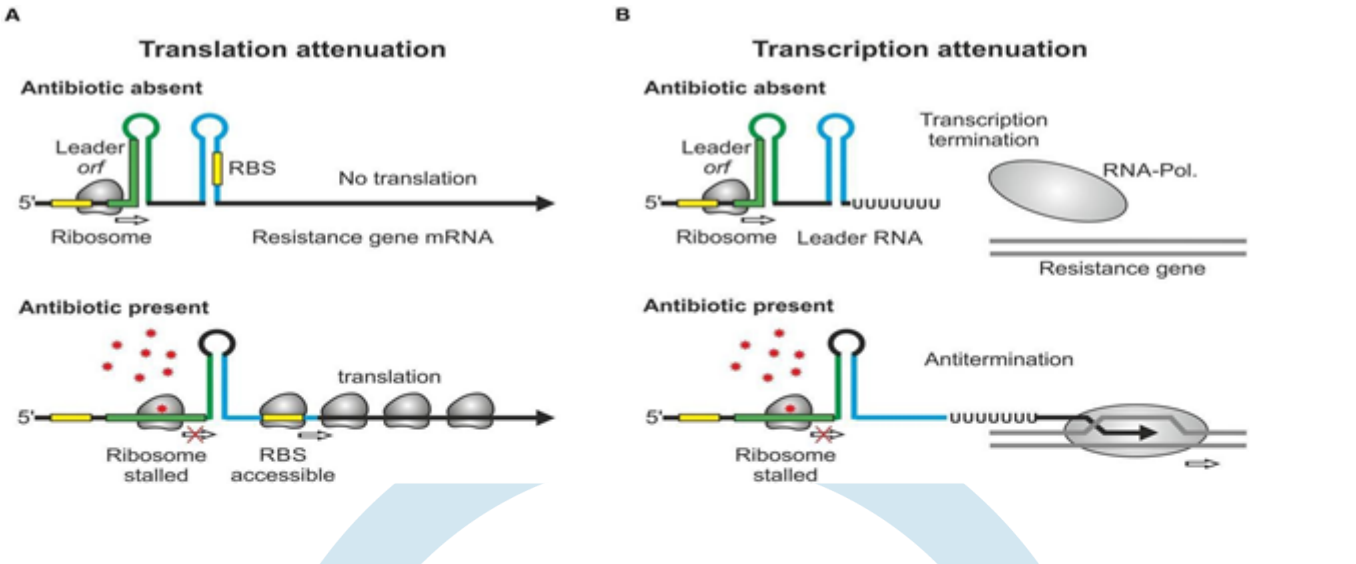


Fig- 9: Regulation of antibiotic resistance genes by RNA attenuation. (A) Regulation by translational attenuation. The resistance gene erm encodes a short orf in the leader region. When the leader is translated, the mRNA folds into a secondary structure, which represses the translation of the resistance gene by sequestration of the RBS (top). Presence of the cognate antibiotic stalls the ribosome in the leader. This triggers the formation of an alternative structure allowing ribosomes to access the RBS and translate the resistance gene (bottom). (B) Regulation by transcriptional attenuation. In the case of ermK and similar attenuators, translation of the leader of causes the RNA polymerase to terminate at an intrinsic terminator. Antibiotic-induced ribosome stalling in this or favors the formation of an ant terminator structure allowing RNA-polymerase to continue transcription beyond the terminator.

Temperate and lytic bacteriophages programmed to sanitize and kill antibiotic-resistant bacteria:
Antibiotic resistance to pathogens is a growing concern to human health, reviving interest in phage therapy. This therapy uses phages (natural bacterial enemies) to kill pathogens. However, it encounters many obstacles such as delivery barriers into the tissues and bacterial resistance to phages. Here, the use of phages for delivering a programmable DNA nuclease, clustered regularly interspaced short palindromic repeats (CRISPR)– CRISPR associated (Cas), to reverse antibiotic resistance and eliminate the transfer of resistance between strains. This approach combines CRISPR-Cas delivery with lytic phage selection of antibiotic-sensitized bacteria. The strategy may reduce the prevalence of antibiotic-resistant bacteria in treated surfaces and the skin of medical personnel, as it uses phages in a unique way that overcomes many of the hurdles phage therapy encounters the threat of pathogen resistance to antibiotics requires the development of novel antimicrobial strategies. Here is a proof of concept for a genetic strategy that aims to sensitize bacteria to antibiotics and selectively kill antibiotic-resistant bacteria. Use of temperate phages to deliver a functional clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated (Cas) system into the genome of antibiotic-resistant bacteria. The delivered CRISPR-Cas system destroys both antibiotic resistance-conferring plasmids and genetically modified lytic phages. This linkage between antibiotic sensitization and protection from lytic phages is a key feature of the strategy. It allows the programming of lytic phages to kill only antibiotic-resistant bacteria while protecting antibiotic-sensitized bacteria. Phages designed according to this strategy may be used on hospital surfaces and hand sanitizers to facilitate ate replacement of antibiotic-resistant pathogens with sensitive ones**. [47,48,49,50]**

VIII.Conclusion

The revolution has revealed many RNAs as potential targets for novel antibiotic drugs. Targeting RNA is challenging and complementary to traditional drug discovery that focuses on proteins and may have some advantages. First, more sites are accessible at the RNA level while targeting proteins is usually restricted to their active sites. Secondly, it is cost-effective to subject RNAs to high-throughput screening. With developments in new drug discovery technologies, targeting RNAs for better antibiotics is emerging as a new frontier in drug discovery along with preventing multi-drug resistance.

IX.Declaration of Competing Interest

The authors declare that they have no known competing financial or personal relationship that could have appeared to influence the work reported in this paper.

X.Acknowledgment

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