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UNLOCKING CRISPR/CAS POTENTIAL TO TACKLE MULTI-DRUG RESISTANT PATHOGENS

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ABSTRACT

Rapid emergence of multi drug resistant super bugs is a major concern for the health of individual world wide. In this scientific era antibiotic resistance is still one of the most leading cause of death among patients. In order to combat this deadly problem latest strategies have been suggested that deal with notorious infections causing pathogenic bacteria. The most common and widely used treatment is prescription of antibiotic drugs. Antibiotics are losing their effectiveness day by day as most of the bacteria has developed resistance against them. It has been proved that these pathogenic organisms are using both phenotypes and genetic modifications in order to enable a natural defence against antibiotics and this lead to elevated resistance to the class of antibiotic that has been used. The clustered regularly interspaced palindromic repeats CRISPR Cas system is promising tool in terms of eliminating resistance. It is a part of bacterial immune system and bacteria uses this system to protect it self from various viruses and bacteriophages. CRISPR Cas is an effective tool for diagnosis and treatment of infections caused by Multi Drug resistant bacteria. CRISPR Cas is a programmable tool

that can act as an efficient antibiotic tool in terms of dealing with MDR pathogens. This tool can be exploited against pathogens and it can eliminate pathogen on basis of sequence specific manner. CRISPR-Cas9 technology is a genome-editing tool that has various applications in cell lines, plants, animals, and even in human clinical trials, and it is seriously being considered as a safe and effective tool for eliminating MDR.

Multidrug resistant pathogens are causing mortality and morbidity in patients and lead to prolong treatment. Bacteria has developed resistance against antibiotics causing them to be ineffective. To deal with rising issue of antibiotic resistance CRISPR Cas system is a life saving tool that can potentially fight against the superbugs and remove them.

Key words: Multi Drug resistance, CRISPR Cas, Cas 9, ESKAPE pathogens

INTRODUCTION

Antibiotic resistance is a grave risk to the health of individuals world wide. Intrinsic resistant mutations and the horizontal transfer of antibiotics resistance genes are the factors that uphold bacteria to be Multi drug resistant, pandrug resistant or extensively resistant to drugs (Wang, *et al.,* 2023).In human beings AMR is arises out by the rough treatment and ill use of antibiotics (Costanzo, V., & Roviello, G. N. 2023).

The evolution of pathogens has led to the accumulation of antibiotic resistance mechanism to the previously discovered antibiotics. Globally the bacteria effect 2 million people every year out of which 700 thousand infected people ends up at losing their lives due to the antibiotic resistance (Jubair, *et al.,* 2021)(Adrizain, *et al.,* 2018). In 1960 the mechanism of multi drug resistance was first perceived in the bacteria that are *shigella* , *salmonella* and is *Escherichia coli* (Zohra, *et al.,* 2021).

Table 1. Three major categories of drug-resistant pathogenic bacteria as per the CDC records (Kundar, R., & Gokarn, K. 2022)

Urgent threats	Serious threats	concerning threats
Carbapenem-resistant AcinetobacterClostridioides difficile (C. difficile)Carbapenem-resistantEnterobacteriaceae (CRE)Drug-resistant Neisseriagonorrhoeae(N. gonorrhoeae	Drug-resistant Campylobacter Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae Vancomycin-resistant Enterococci (VRE) Multidrug-resistant Pseudomonas aeruginosa (P. aeruginosa) Drug-resistant non-typhoidal Salmonella Drug-resistant Salmonella serotype Typhi Drug-resistant ShigellaMethicillin-resistant Staphylococcus aureus(MRSA)Drug-resistant Streptococcus pneumoniae (S. pneumoniae)Drug-resistant Tuberculosis (TB)	Erythromycin-resistant group A Streptococcus Clindamycin-resistant group B Streptococcus

Classification of Resistance

The mechanism of resistance is classified as the following :

- Intrinsic resistance
- Acquired resistance

Intrinsic resistance:

The type of resistance which is due to the functional or structural characteristics of a bacteria is known as intrinsic resistance. In this type of resistance the bacteria lacks target site where a specific antibiotic acts on it.

Acquired resistance

This type of resistance involve drug resistant phenotypes caused by mechanism like genetic mutation or jeans acquisition from various bacterias which is initiated by horizontal gene transfer (Kundar, R., & Gokarn, K. 2022).

Multi-drug resistance organisms: the super bugs

To escape the toxicity of various antimicrobials and antibiotics bacteria has evolved them selves (Jorge, *et al.*,2019). *Staphylococcus aureus, Enterococcus feacium, Enterococcus faecalis, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* are some of multi drug resistance bacteria. Enterobacteria and carbapenem resistance of bacteria are the factors that causes nosocomial infections (Bharti, *et al.,* 2023).

Multi drug resistance phenomenon and infections are caused by gram negative bacteria or gram positive bacteria and sometimes in fungi(Jorge, *et al.,* 2019). Globally *Staphylococcal aureus* and *Enterococcus* species are most noxious super bugs causing infections (Jorge, *et al.,* 2019).

Emergence of Antimicrobial Resistance

To deal with AMR with aid of One health approach and to heighten the world wide efficiency WHO has hurl a call to action on AMR (Tiseo, *et al.*,2022). The main drivers of AMR are the excess use of antibiotics and inappropriate use of them. The unnecessary prescription of antibiotics by physicians which is further supported by wrong drug dosage and incorrect drug choice are other factors that causes emergence of AMR and lead to the ineffectiveness of treatment (Costanzo, V., & Roviello, G. N. 2023).Multidrug resistance organisms causing infections and mortallity are gram positive bacteria (methicillin resistant *Staphylococcus aureus*),gram negative bacilli (carbapenem resistant *Pseudomonas aeruginosa),* carbapenem resistant Enterobacterias and carbapenem resistant Acinetobacter baumannii (Tiseo, *et al.*,2022).

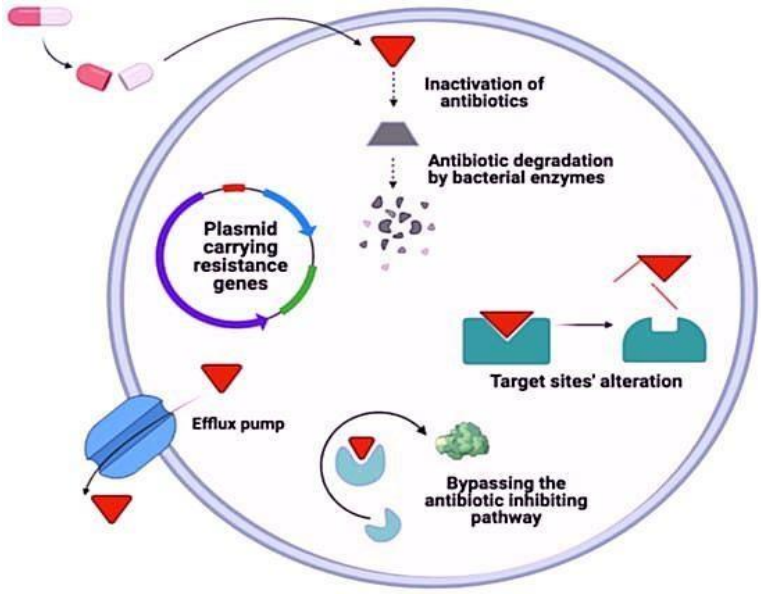

Figure 1. Illustration of the general mechanism of antimicrobial resistance in bacteria (Zohra, *et al.*, 2021)

ESKAPE Pathogens

ESKAPE Pathogens comprises of the six pathogenic bacteria that are multidrug resistant and capable of escaping the action of antimicrobials. The ESKAPE Pathogens are *Enterococcus feacium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* speicies (Mulani, *et al.*, 2019).

ESKAPE pathogens uses acquisition of genetic elements to cause mutations and develop multidrug resistance against oxazoldiones, macrolides, lipopeptides, tetracyclines, fluroquinolones, B lactam B lactamase inhibitors and even against the last line of defense antibiotics including carbapenems, polymyxins and glycopeptides (Mulani, *et al.*, 2019).

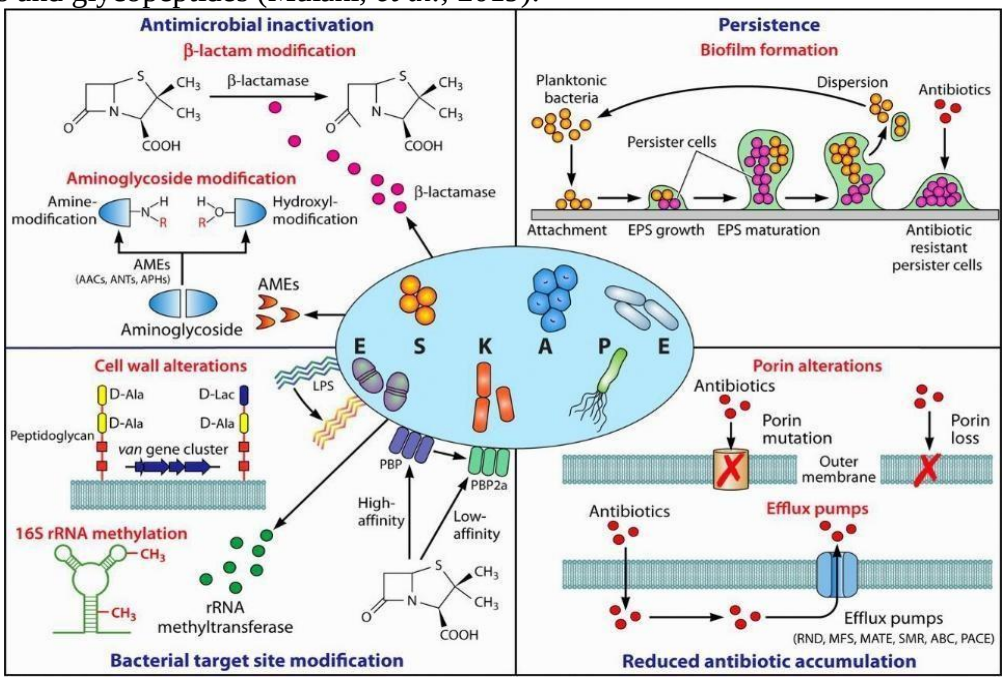

Figure 2- Here we explore the most important AMR determinants that have contributed to the success of ESKAPE pathogens in the modern-day clinical setting (De Oliveira, *et al.*, 2020).

Nosocomial infections are majorly cause by these antibiotic resistance ESKAPE pathogens (Gholizadeh, *et al.*,2020). For the production of new antibiotics and for the research purposes the

World health organization has enlisted ESKAPE pathogens among the top priority (Odoyo, *et al.,* 2023).

Causes of reduced antibiotics penetration and Accumulation

Porins

The poor regulation, imbalance or destruction of outer membrane channels porins caused by mutations are important factors of AMR in gram negative ESKAPE pathogens (De Oliveira, *et al.,* 2020).

Efflux pumps

The efflux pump thrust drugs out of the cells and assits the AMR(De Oliveira, *et al.,* 2020). This phenomena occurs in both gram positive and gram negative bacteria (Singh, *et al.*,2021).

Bio-films

On the biotic and abiotic surface of microorganisms there are well orderly structures that are enclosed and protected (Jorge, *et al.,* 2019). *B*iofilms can grow in sewage caunnels, hospitals, bathrooms, Laboratories, on biomedical equipment, hot springs, rocks and deep sea vents (Singh, *et al.*,2021) (Sharma, D., Misba, L., & Khan, A. U. 2019). Bacterial biofilms are responsible for almost 80 % of infections in human beings (Sharma, D., Misba, L., & Khan, A. U. 2019).

Table 2. Types of antimicrobial resistance at the cellular level (Zohra, *et al.*, 2021).

Resistance	Proposed Mechanism	Examples
Inactivation of Drug	Use of hydrolysis or modification	b-lactamase for b-lactam resistance, acetyltransferases for aminoglycoside resistance
Alteration of Target	Reduction of bindingaffinity to the drug bypassing the drug target.	DNA gyrase mutation for fluoroquinolone resistance
Drug influx Reduction	decreasing permeability	Gram-negative outermembrane Accessory membranefusion proteins
Extrusion of Drug	Efflux pumps	
Horizontal gene transfer	resistance determinant from microorganisms	

Use of phage therapy to oppose Multi-drug resistance of Bacteria

Various types of methods and genomic engineering tools are suggested to combat antibiotics resistance which includes the following methods:

- Use of peptide nucleic acid as an ultra narrow spectrum - PNA

Zinc finger nucleases -ZFNs

- Clustered regularly interspaced palindromic repeats -CRISPR (Odoyo, *et al.,* 2023)

Clustered regularly interspaced palindromic repeats (CRISPR)

Clustered regularly interspaced palindromic repeats shortly known as CRISPR is the part of adaptive immune system of the archae and bacteria (Akbar,*et al.,* 2020).CRISPR belongs to the family of palindromic repeats (Kundar, R., & Gokarn, K. 2022).Researchers discovered CRISPR Cas system while working on nucleotide sequence of the iap gene in bacteria *Escherichia coli* (Wu, *et al.,*

2021).Its a genome editing revolution that to target the sequence of intrest CRISPR cas systems can be engineered (Palacios Araya, *et al.,* 2021).CRISPR Cas system is an efficient antimicrobial by deleting the unnecessary traits within a genome in bacteria (Palacios Araya, *et al.,* 2021).
CRISPR Cas system identifies its targeted DNA by altering the RNA and with the assistance of Cas enzymes which demolishes the nucleic acids (Odoyo, *et al .,* 2023) .This contemporary technology of genome editing can be used by scientists to modify the Genetic code of cells in living organism (Baiko, O., & Klochko, V. 2023).This tool can potentially revulse and bring a radical change in the scope of Biotechnology, pharmaceuticals, agriculture as it helps to expand treatment methods for genetic diseases, the betterment of crops production, pest and disease resistant crops, revolution in the development of bioactive compounds etc(Baiko, O., & Klochko, V. 2023).
CRISPR Cas is an anticipation by attributing the quality of RNA guided destruction to develop the next generation Antimicrobials to tackle with infections caused by AMR pathogens (Duan, *et al.,* 2021).CRISPR antimicrobials has a crRNA which particulate target sequence on genome of bacteria and cas affector that produces double strand break at target site(Wang, *et al.,* 2023)(Selle, *et al.,* 2020).

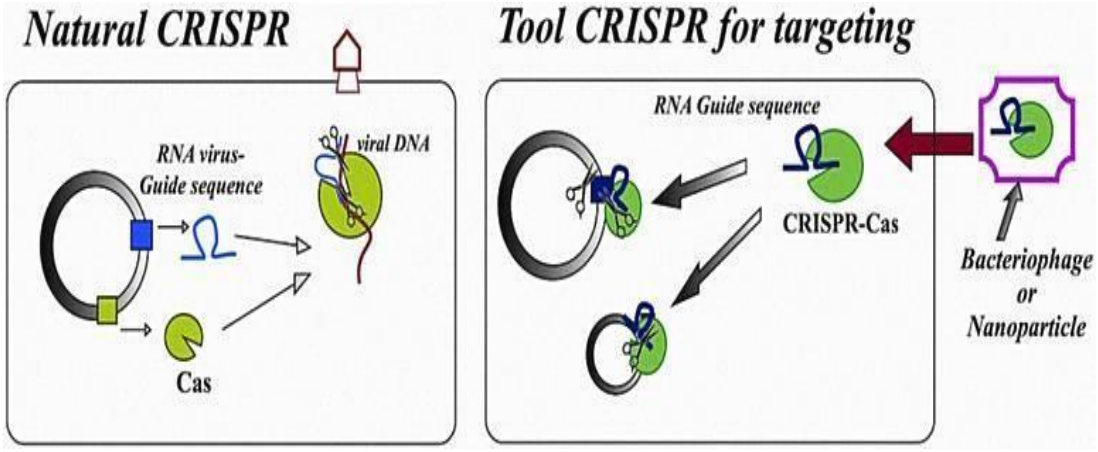

Figure 3- Schematic drawing showing the natural CRISPR–Cas complex found in bacteria, which functions as an "immune system" against viruses, and the CRISPR–Cas tool used as an agent, based on the complex naturally present in bacteria (Lima, *et al* ., 2019) .

Applications of CRISPR Cas

CRISPR based immunity

The Immunity mechanism of CRISPR Cas systems can be categorised in three stages i.e adaptation, expression and interference (Gholizadeh, *et al.,*2020)(Wu, *et al.,* 2021).

Adaptation

Affector protiens assit the cas 1 and cas 2 protiens to conduct this step.The DNA is cleaved by the proto spacer adjacent motif which has type specific short sequence for identification of spacer sequence i.e 2 -3 nucleotide (Kundar, R., & Gokarn, K. 2022).

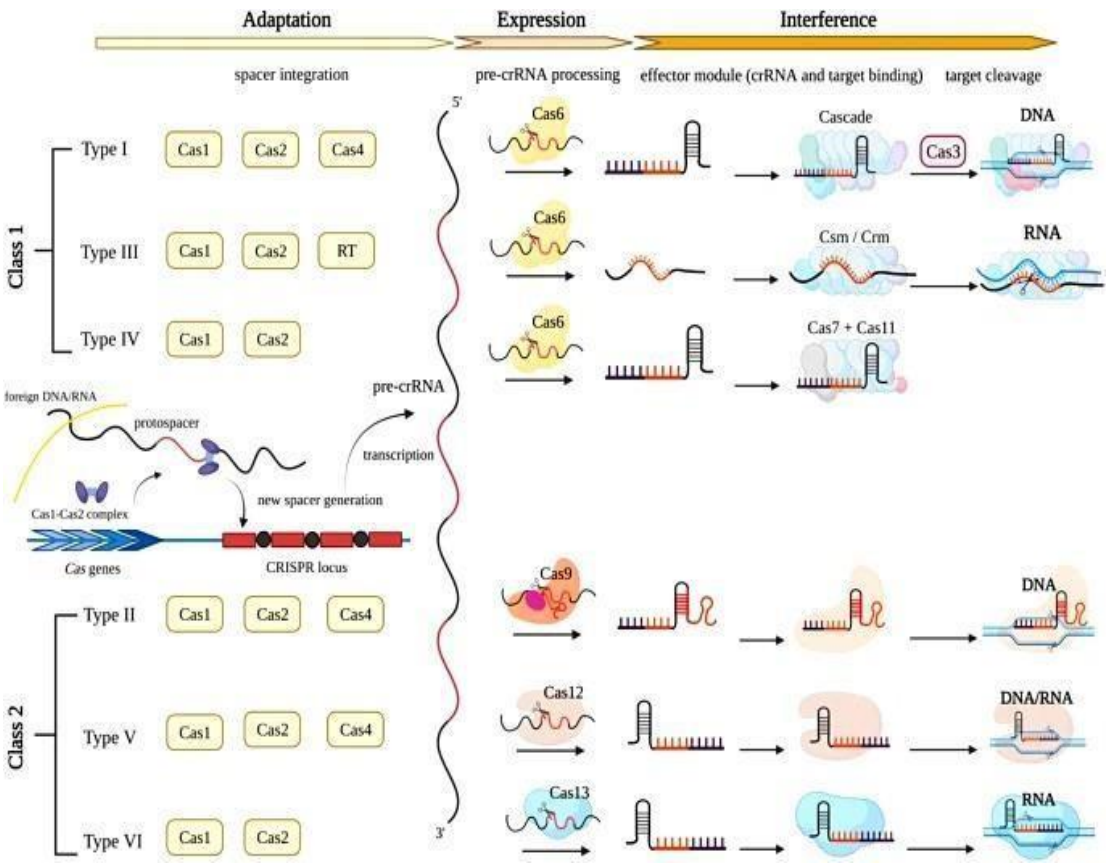

Figure 5- Classification and function of the CRISPR-Cas system in bacteria. Cas effectors are classified based on generic organization, and the functional modules of CRISPR-Cas systems are shown(Wu, *et al.*, 2021).

Expression

It is followed by biogenesis of CRISPR RNA with aid of transcription of CRISPR locus at spacer sequen*ce* (Kundar, R., & Gokarn, K. 2022).

Interference

This step involves the mature CRISPR RNA forms a complex with Cas nucleases guides the Cas machinery to complementary sequence on invading nucleic acid (Kundar, R., & Gokarn, K. 2022).

Classification of CRISPR Cas system

To comprehend the functions of CRISPR Cas system in bacteria and to develop editing tools for genome classification of these systems is compulsory(Wu, *et al.*, 2021).This system is classified on the basis of Cas protiens and module sequence into two classes , six types and 33 subtypes (Wu, *et al.*, 2021) .

Type I system

Type I CRISPR-CAS systems are chief systems present in bacteria that value for about 60 % of all CRISPR systems discovered (Baiko, O., & Klochko, V. 2023). Cas 8 is used by type I systems for hybridization of crRNA and consist of Cas 3 helicase nuclease protein that lead to digestion of invader chromosome and identification of protospacer adjacent motif (Padilha,*et al.*, 2020).

Type II system

Type II system requires only a single effector protien with a convenient system but type 1 system requires a cascade complex and Cas 3 nuclease to attain interference on target sequence (Duan, *et al.*, 2021).The artlessness of this system makes it easier to incorporate into phage genome delivery as compared to type I Broadly used type II CRISPR Cas system consists of three components:

- Cas 9 an endonuclease
- CrRNA CRISPR RNA
- TracrRNA transactivating CrRNA (Chaudhary, *et al.,* 2021)

Type III system

Type IIIsystem cleaves invader messenger RNA by using the Cas 7 backbone but the Cas 10 HD nucleases transcribed the DNA(Padilha,*et al.,* 2020).Type III is classified into 4 subtypes i.e 111 A, B, C and D. All the sub types encode for the homologous proteins that have the same functions. The diversion of these proteins and their sub types are recognized by multiple sequence alignment tools for example BLAST(Padilha,*et al.,* 2020).

Type VI system

CRISPR C2c2 now known as CRISPR Cas 13a is latest system which is related to the Class 2 type VI system. This system has a single protein Cas 13a Rnase which works in the CrRNA guided manner and is responsible for cleaving single stranded RNA molecule (Duan, *et al.,* 2021).

Cas protiens of CRISPR System

Cas protiens have a role in fields of biotechnology, field of agriculture and field of medical research and they are widely known for thier genome engineering role in these fields(Hillary, V. E., & Ceasar, S. A. 2023). CRISPR-CAS mediated genome editing has now much improved after the discovery of Cas proteins such as Cas 12, Cas 13 and Cas 14 (Hillary, V. E., & Ceasar, S. A. 2023).

Cas 1 and Cas 2 proteins

In the spacers of E.coli type II CRISPR system there are two proteins i.e cas 1 and cas 2 (Padilha,*et al.,* 2020). The array of CRISPR cas 1 and cas 2 constructs a heterohexameric complex whose function is to cataylezes spacer integration with two transesterification reactions and these reactions are supported by nucleophilic attack of 3 hydroxyl on double stranded prespacer on each strand at phosphodiester backbone (Lee, *et al.,* 2019).

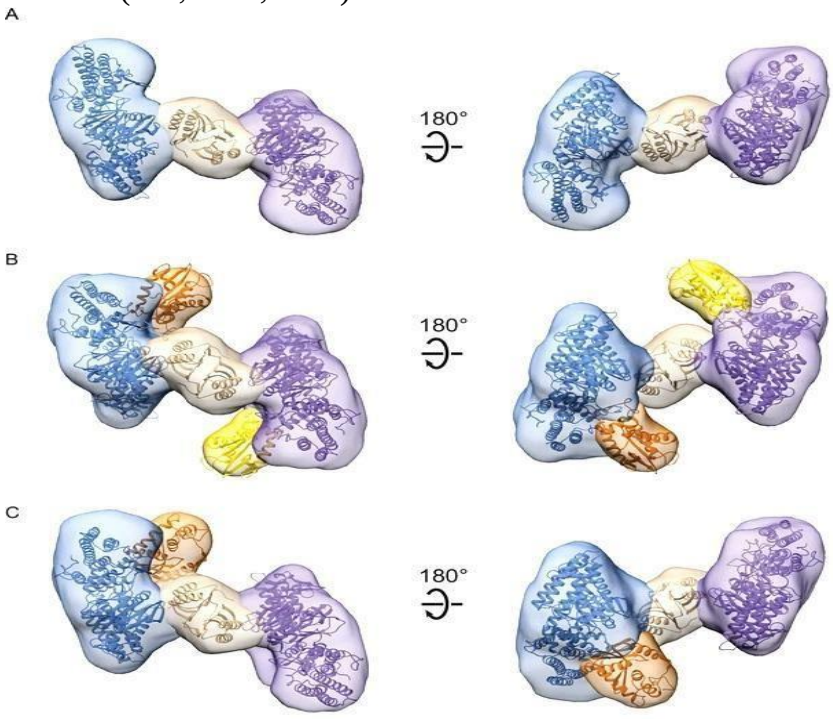

Figure 6- Architecture of Cas1-Cas2 and Cas4-Cas1-Cas2 complexes formed in the presence of CRISPR hairpin target DNA. (A) Segmented density forCas1-Cas2 reconstruction. Two copies of a structural model of BhCas1 dimer (see Materials and methods) were fit in the two assigned Cas1 densities (blue, purple). The crystal structure of BhCas2 (PDB 4ES3) was used

for fitting to density assigned to Cas2 (tan). (B–C) Segmented density for (B) symmetrical and (C) asymmetrical reconstructions of Cas4-Cas1-Cas2. BhCas1 and BhCas2 structural models are fit to segments and colored as in (A). Two copies of a structural model of BhCas4 are fit into assigned Cas4 densities in (B) (orange, gold). One copy of BhCas4 structural model is fit into assigned Cas4 density in (C) (orange) (Lee, et al., 2019).

Cas 4 proteins

Cas 4 proteins is found in abundance in type I, II, and V systems.Deletion of cas 4 lessen adaptation performance and cause acquisition of non functional spacers from areas that are PAM insufficient (Lee, *et al.*, 2019)(Almendros , *et al.*, 2019).

Cas 9 protein

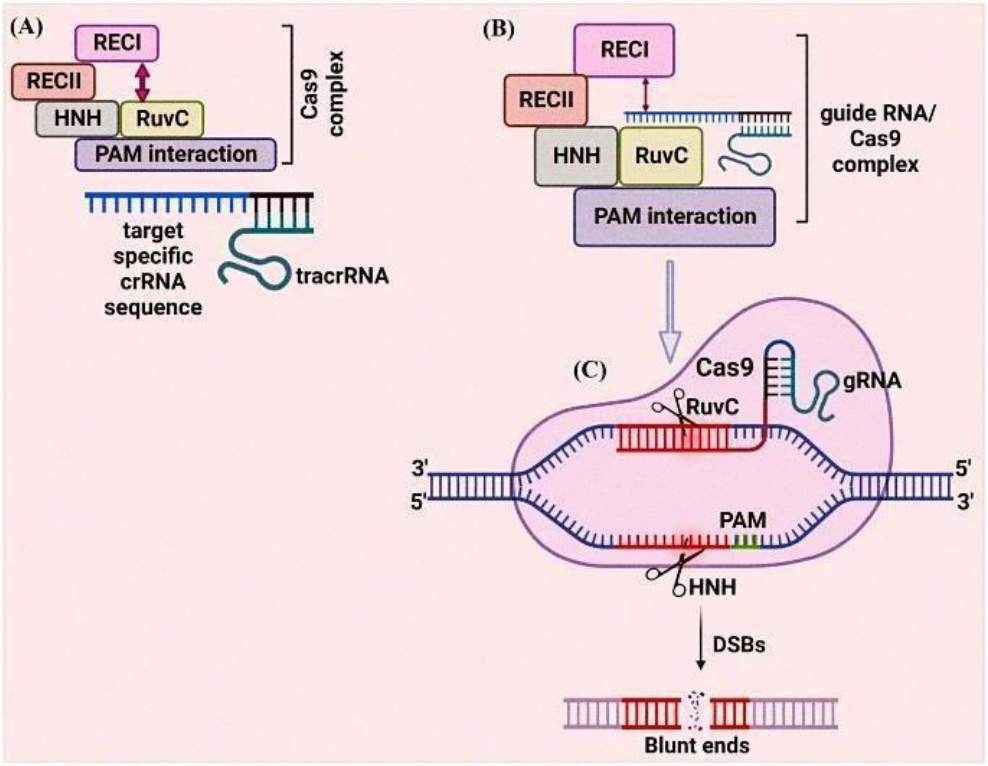

Figure 7- The mechanism of Cas9 protein has been studied extensively. Cas9 protein has six domains (1) Recognition lobe (REC I), (2) REC II, (3) Arginine-rich bridge helix, (4) PAM Interacting, (5) HNH, and (6) RuvC. REC I is the major domain responsible for binding with the gRNA; the REC II function is not studied . The arginine-rich bridge helix initiates cleavage activity upon binding to targeted sequences (Hillary, V. E., & Ceasar, S. A. 2023).

Components of CRISPR Cas

CRISPR Cas is widely known as a genome editing tool has a single guide RNA associated with two components and an endonuclease(Chourasia, *et al.*, 2022).

SgRNA

TracRNA and CrRNA combines to form a single guide RNA. crRNA is also called a spacer sequence that assists CRISPR Cas 9 system to target site and joins to tracr RNA. A functional guide RNA is formed when tracr RNA binds to crRNA for cas 9 to identify and arrange direction(Chourasia, *et al.*, 2022).

CAS 9

Cas 9 functions as an endonuclease and pair molecular scissors for targeted DNA sequence. It consists of six domains: Rec I, Rec II, bridge helix, Ruv C, HNH and PAM(Chourasia, *et al.*, 2022).

CRISPR -Cas as a tool to kill mutli drug resistant pathogens

In modern era CRISPR Cas is the most widely studied genome editing tool.This tool is fast ,cheap and an effective method for gene editing to treat genetic defects, remove pathogenic bacteria and to deal with viral infections(Kim, T. H., & Lee, S. W. 2022).It has been proven that CRISPR Cas system is effective against controlling the escalation of antibiotics resistance (Kundar, R., & Gokarn, K. 2022)(Wu, et al., 2021)(Kim, T. H., & Lee, S. W. 2022).

Antibiotic resistance can be controlled by following startgies like development of new antibiotics, targeting the functional protiens or genome by using bacteriophage, peptides or enzymes that may be natural or synthetic (Janik, *et al.,* 2020). These startgies are however giving unsatisfactory results and the mortallity rate will reach 10 million annually by 2050 due to the drug resistance(Janik, *et al.*, 2020)(Gholizadeh, *et al.,* 2020) (Shabbir, *et al., 2019).*According to researches that CRISPR Cas system can be efficient against antibiotic resistance of bacteria(Janik, *et al.,* 2020). CRISPR-Cas is used as following:

- To diagnose drug resistant pathogens
- To eliminate AMR of gram negative Bacteria

CRISPR-Cas role in Diagnostic

For the diagnosis purposes CRISPR Cas system is widely used as a detector. Alterations in CRISPR cas has improved the specificity and cost effectiveness of formerly used diagnostic tools for example Polymerase chain reaction PCR, isothermal based amplification sequencing and assay (Kundar, R., & Gokarn, K. 2022).To overcome AMR , for diagnosis of mutations for betterment of drug therapies and to detect variety of pathogens next generation tools that are single nucleotide specific are of great importance (Kundar, R., & Gokarn, K. 2022)(Li, *et al.*,2022).

The nucleic acid present in sample is detected by detection tools that are altered using CRISPR based system (Kundar, R., & Gokarn, K. 2022)(Li, *et al.*,2022).For detection of various types of pathogens amplification method are developed that are combined with CRISPR Cas system(Li, *et al.*,2022)(Wang, X., Shang, X., & Huang, X. 2020).

Neutralizing antibiotic resistant gene

Partially repeating RNA based spacers guide cas proteins to target DNA and to cleave it for encoding matching protospacers.To specifically target and cleave the DNA on basis of CRISPR array information CRISPR-Cas system can be used and it targets the specific gene for antibiotic resistance in a bacterial population(Gholizadeh, *et al.*,2020). Neutralizing the antimicrobial resistance genes bacterial sensitivity can be retrieved with CRISPR Cas system as it acts as an antimicrobial agent (Wu, *et al.,* 2021).

CRISPR Cas systems are engineered in such a way that they have implicit to remove resistance plasmid (Wu, *et al.,* 2021).This system can efficiently differentiate between commensal and Pathogenic bacteria as it has sequence specific targeting which is its key feature(Aslam, *et al.,* 2020). The programming of Cas protiens enables the direct targeting and cleavage of DNA and lead to flanking of RNA based spacers with aid of partial repeats to encode conforming protospacers (Javed, *et al.,* 2023) .In vivo conditions the DNA is cleaved and targeted by the assistance of CRISPR array system (Javed, *et al.,* 2023).Priorly CRIPR array is used to target the particular genes that possess antibiotics resistance in a bacteria (Javed, *et al.,* 2023). Explicit targeting of bacteria and directional transformation like abundant benefits can be obtained by CRISPR Cas system while dealing with Pathogens at genetic stratum. The targeting takes place in following manner:

- To inactivate specific strains that include drug resistance strains and drug sensitive strains CRISPR cas system targets them.
- CRISPR Cas targets drug resistance strains and genes to inert thier cells .
- Aim to reinstate the sensitivity of drug resistant genes and others(Zhang, F., & Cheng, W. 2022).

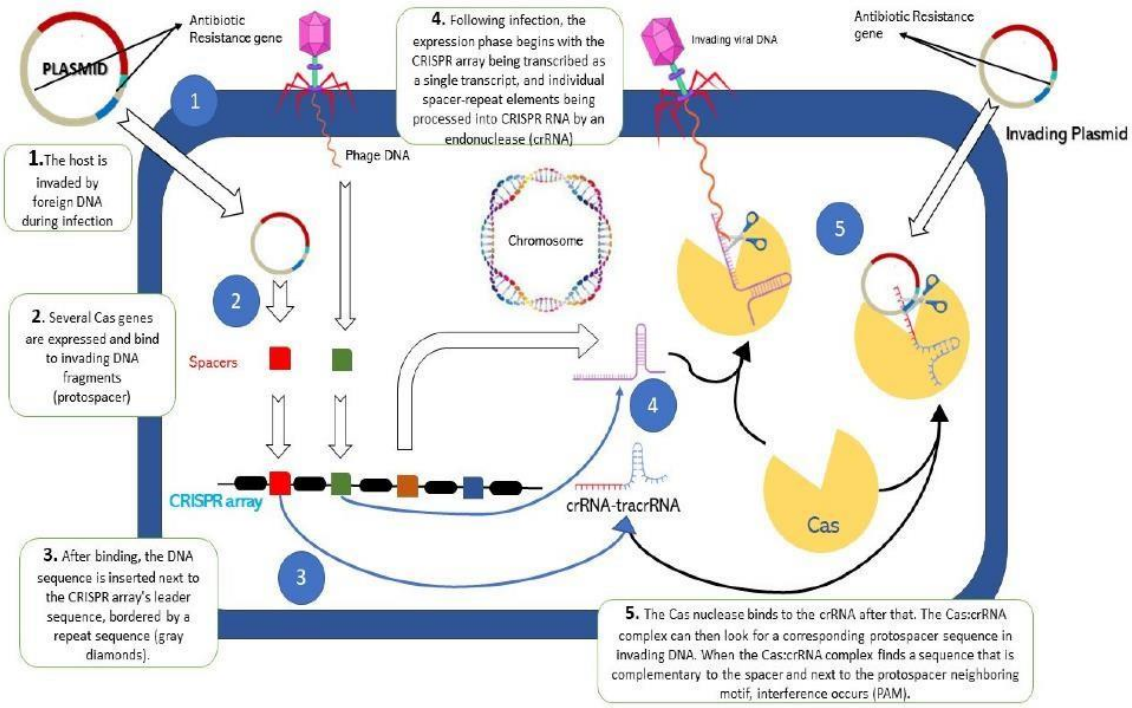

Figure 8. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Associated (CRISPR-Cas) and bacterial antibiotic virulence. 1. The host is invaded by foreign DNA during infection. 2. Spacer acquisition. 3. Incorporation of spacers in CRISPR array. 4. Formation of crRNA and transRNA complex containing specific spacers. 5. CrRNA guide cas genes to target invading DNA (Javed, *et al.*, 2023).

The CRISPR Cas single guide RNA finds its way to target specific gene or plasmids with antibiotic resistance with the assistance of Cas9 endonuclease (Chourasia, *et al.*, 2022). CRISPR nano complex or bacteriophage is used to prosperly deliver the CRISPR Cas 9 to antibiotic resistance Bacteria then CRISPR Cas system moves towards the target site of antibiotic resistance by following CRISPR guide RNA(Chourasia, *et al.*, 2022).

Cas 9 proteins have two domains i.e Ruv C and HNH that helps in cleavage of antibiotic resistance gene and eradication of gene. This whole mechanism lead to backsliding of antibiotics sensitivity of pathogen(Chourasia, *et al.*, 2022).

Treatment of AMR in gram negative bacteria

By aiming the particular genes that behold antibiotic resistance the baffling issue of AMR can be resolved by CRISPR Cas technology (Kundar, R., & Gokarn, K. 2022). This technology can be used to target the against both genes either they are chromosomes encoded genes or plasmid encoded genes.for this purpose the approximations are focused on pathogens or the genes (Kundar, R., & Gokarn, K. 2022)(Getahun, *et al.*,2022).

For the sake of editing genes in contrast to class I CRISPR-CAS class 2 is considered as a more tempting choice *(Pereira, et al., 2021)* .Crispr RNA can be used to target gene of interest by targeting it specifically and by cutting it with nucleases. The single guide RNA was transformed by combining Cas 9 crRNA and tracRNA and it is considered as an achievement in field of editing genes but some disclaiming effects are still there to put down the implict of CRISPR Cas system (Pereira, *et al.*, 2021).

CRISPR interference system

CRISPR interference system is another system used to aim MDR genes. Repressor domain is combined together with dead Cas9 which is inactive form of Cas9 and forms a kruppel associated box. This lacks the activity of endonuclease which is present in Cas9 (Kundar, R., & Gokarn, K. 2022).

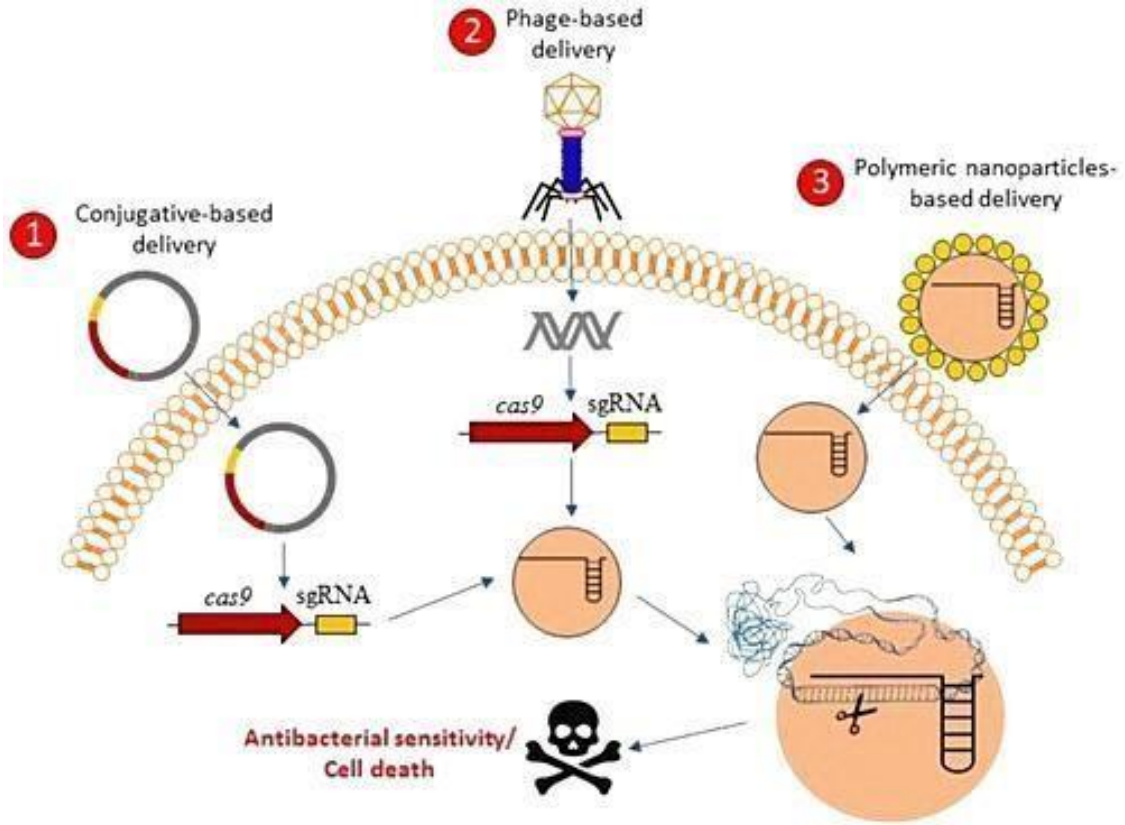

Figure 9- Graphical concepts model of CRISPR-Cas delivery for antibacterial affecting. CRISPR-Cas-based antibacterials could be delivered into the bacterial cells through the three proposed delivery mechanisms including 1) conjugative-based delivery, 2) phage-based delivery, and 3) polymeric-nanoparticles-based delivery. After the delivery of CRISPR-Cas systems in the bacterial cells, the bacterial cells might be resensitized against antibacterial agents or be killed based on the antibacterial resistant genes targets or essential genes targets, respectively(Gholizadeh, et al.,2020).

CRISPR Cas altered phage to target AMR genes

In chromosome thier are antibiotic resistance genes against which the CRISPR Cas 9 is inserted with assistance of bacteriophage based tool(Balcha, F. B., & Neja, S. A. 2023). The nucleic Acids that have homogeneous sequence are invaded to aim or deceased them in bacterial genome at CRISPR loci by introduction of bacteriophages and plasmids(Balcha, F. B., & Neja, S. A. 2023). Bacteriophage fused with CRISPR Cas system is beneficial as it only targets to eliminate antibiotic resistant pathogen but not the antibiotic sensitive bacteria which helps to reduce the egression of bacteria that is resistant(Wei, *et al.,* 2020).

Acquisition of CRISPR Cas system in a war with antibiotic resistance

The base pairing principle is used by type II system CRISPR Cas DNA endonuclease cas 9 by a gRNA that identifies the targeted DNA. The specificity of base pairing minimize the efficacy of CRISPR gRNA that assits Cas9 nucleases to target invading pathogen(Balcha, F. B., & Neja, S. A. 2023). CRISPR Cas system is potent technology that can differentiate between a commensal and Pathogenic bacteria(Balcha, F. B., & Neja, S. A. 2023) . At the end of discussion CRISPR Cas system fused with phage is conducive against Antimicrobial resistance of bacteria(Wei, *et al.,* 2020) .

The notorious antimicrobial resistance is behind 700 000 deaths yearly and in European union 25000 deaths yearly *(Ukuhor, H. O. 2021).*Non serious attitude in terms to attend the AMR lead to millions of deaths globally and by 2050 AMR will emerge as a more frequent reason for mortality than cancer (Ukuhor, H. O. 2021).

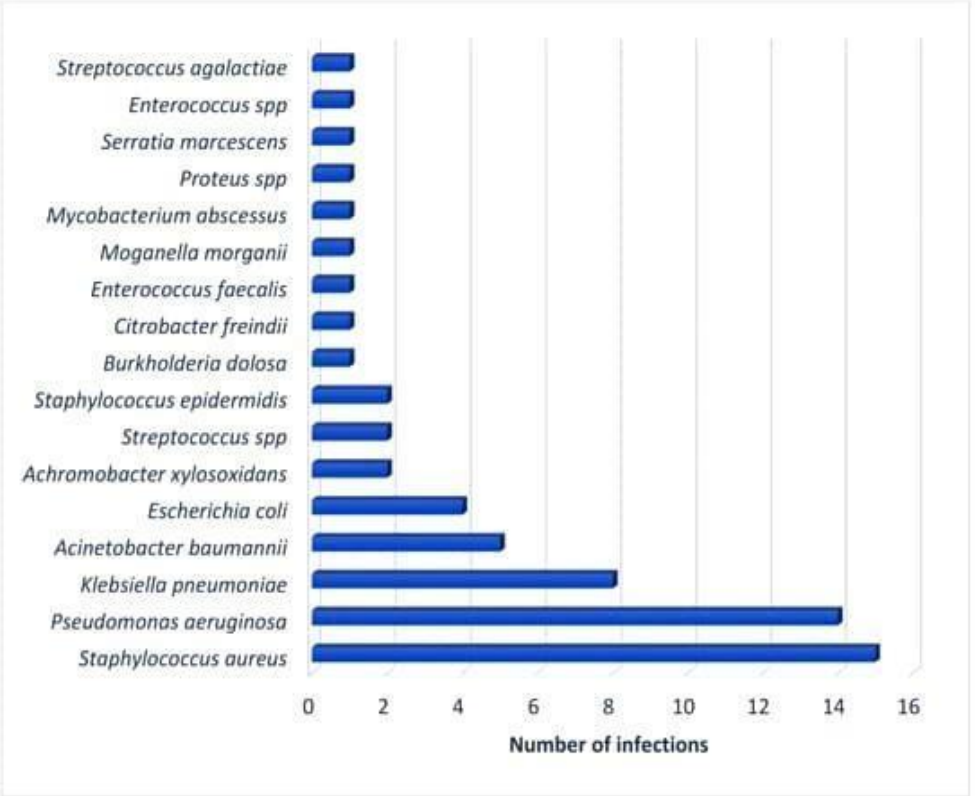

Figure 10. Number of infections caused by each of the multi drug-resistant bacteria mentioned in the articles(Aranaga, *et al.*, 2022)

Table 3. Narrative of pathogenic bacterial strains (ESKAPE) that instigated nosocomial infection(Zohra, *et al.*,2021).

Bacterial Strain	Gram Staining Type	Resistance Type	Antibiotics	Treatment Option	Resistance Level
Acinetobacter	Negative	Multidrug	Ceftazidime aminoglycoside fluoroquinolonesc arbapenems	Carbapenems,b-Lactamase inhibitors, Tigecycline, Aminoglycosids,Poly myxin therapy,Synergy, and combination therapy	High level
E.coli	Negative	Multidrug	Cephalosporins ESBL-producers fluoroquinolonesa minoglycosides	GyrB/ParE programme, EV-035	High level
K.pneumoniae	Negative	Multidrug	Cephalosporin(ES BL-producers) fluoroquinolones aminoglycosides carbapenems	POL7080 and ACHN-975 compounds	High level
Enterococcus	positive	Multidrug	Ampicillin, aminoglycoside glycopeptides	RX-04 lead series 50S ribosomal subunit; inhibit translation by stabilizing a distorted mode of P-tRNA binding	High level
S.aureus	positive	Multidrug	β-lactam antibiotics (except new anti	RX-04 lead series, 50S ribosomal subunit; inhibit translation by stabilizing a distorted	High level

			methicillin resistant S. aureus cephalosporins),macrolides fluoroquinolonesaminoglycosides	mode of P-tRNA binding	

Table 4- Applications of type I and III CRISPR/Cas systems in bacteria, including genome editing and transcriptional regulation (Liu, *et al.*, 2020)

Cas protein	specie	Type of modification
Cas3	*C. tyrobutyricum*	Genome editing, single- and multi-gene deletions (100%)
Cas3	*E. coli*	Genome editing
Cas3	*H. hispanica*	Genome editing, deletion and single nucleotide substitution
Cas3	*H. volcanii*	CRISPRi, the promoter region (down to 8%), the coding strand (down to 88%), the template strand (down to 8%)
dCas3	*E.coli*	CRISPRi (82%)
Cas 10	*S.aureus*	Genome editing, deletions and insertions

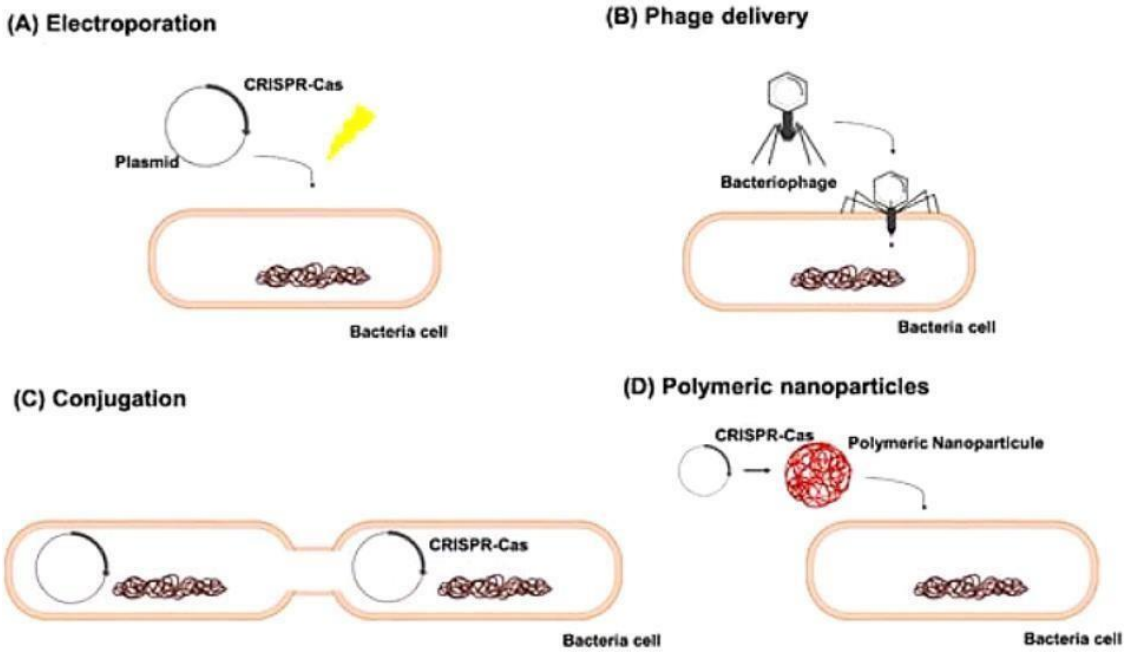

Figure 11. Different means of CRISPR-Cas delivery into the target cells: (A) plasmid electroporation, (B) phage delivery, (C) conjugation and (D) polymeric nanoparticles(González , *et al.*,2021).

Mortal prescription of antibiotics lead to resistance in bacteria also known as multi-drug resistance (Yang, *et al.*,2021). The efficiency of antibiotics to treat infections is declining due to rising threat of multi drug resistance (Yang, e*t al.*,2021).The figures of bacterial infections in past few years due to multi-drug resistant pathogens is a cogent evidence of diversion of bacterial antibiotic resistance (Yang, *et al.*,2021).

In the past few years multidrug resistant pathogens have four-fold globally(Basak, *et al.*, 2016). Currently Antibiotic resistance is effecting recovery of patients by increased fatality,prolonging the hospital stay period and by drastically effecting the economy of a country(Basak, *et al.*, 2016).

Among many notorious mechanisms of developing Resistance in bacteria drug efflux mechanism through multi drug efflux also called Mex system is at top that ejects antibiotics away from the cell and drug cannot percolate the cell(Basak, *et al.,* 2016). The 3 components of Mex system work through extruding out the molecules from cell with active transport or with transporters for example: MexB,MexY, membrane proteins on outer surface that forms channels like OprM and linking protiens like MexA and MexX(Basak, *et al.,* 2016).Reserches shows that attachment of phage with Multidrug resistant *Pseudomonas aeruginosa* the OprM of MexAB and MeXY systems of Pathogen will develop phage Resistance and lead to impairment of liability of efflux pump to eject antibiotic drugs(Shaglouf, K. R. 2018).

To overcome antibiotic resistance numerous tactics have been employed such as introduction of new clasess of antibiotic drugs , attack the pathogenic bacteria with bacteriophages , production of synthetic and natural peptides or enzymes that action upon genes and proteins of Pathogens(Gholizadeh, *et al.,* 2020). Misuse and excess consumption of antibiotics has lead to antibiotic resistance phenomenon in bacteria by manipulating thier genes(Gholizadeh, *et al.,*2020).

The most extensive perspective against dealing with antibiotic resistance is CRISPR CAS system. Antibiotic resistant strains of various bacteria can be controlled by CRISPR Cas system as per according to latest reports(Gholizadeh, *et al.,*2020).

CRISPR Cas system works in three stages i.e adaptation, expression and interference. In the adaptation stage the homologous foreign DNA piece with length of 30basepairs merge into CRISPR Cas locus and lead to selection of host genome protospacers adjacent motifs(Gholizadeh, *et al.,* 2020).In the next step RNA is transcripted into pre-crRNA and then crRNA . The last stage is interference in which double stranded DNA is cleaved by Cas protiens along with aid of crRNA(Gholizadeh, *et al.,*2020).

The drug resistance in bacteria can be guarded with assistance of CRISPR cas system as it attacks and manipulate specific genes that carry resistance or their imprints(Tao, *et al.,* 2022). Ding et al worked on TALEN and CRISPR Cas 9 for gene editing and results predict that efficacies ranges from 0% to 34% and 51% to 79% and CRISPR Cas is more efficient than TALEN(Tao, *et al.,* 2022).Furthermore for the research purposes CRISPR Cas system is much simpler and basic to employ than TALEN as TALEN has to be resynthesized and CRISPR guide is way easier system(Tao, *et al.,* 2022).

This rebellious military tactic can be prosperously to target rather then defend Pathogen for treatment of anew infections caused by multi drug resistance (Aslam, *et al.*, 2020).CRISPR Cas system can produce numerous far off results that can be potentially unfavorable (Aslam, *et al.,* 2020).

Succeeding study can fill the gaps related to expand CRISPR Cas as an antibacterial. To employ CRISPR Cas technology these are the requirements: detection of worthy delivery vector , engineering a suitable host range vector, convulated appeal that has CRISPR Cas to attack resistance carrying sequences so that multidrug resistance can be controlled(Aslam, *et al.,*2020).

CRISPR Cas system is proficient against targeting the sequence of genes in bacteria that carry resistance in clonal population of bacteria CRISPR Cas system can be employed nowadays to reduce the AMR genes(Akbar, *et al.,*2020). In natural surroundings pathogenic bacteria are prototypical organisms that are much complicated after employment of CRISPR Cas technology(Akbar, e*t al.,*2020).

Abeyantly active species are developed as an unwanted outcome of CRISPR Cas based antibiotic technology when a specific strain is eliminated from the plasmids of bacteria and lead to changes in metabolic pathways (Akbar, *et al.,* 2020).

Keeping an eye on merits of CRISPR Cas technology demerits like mutational changes in resistance are negligible(Akbar, *et al.,* 2020). however the mutations in bacteria can be controlled by mixing that means employment of various targeting sequences to decline resistance phenomenon (Akbar, e*t al.,* 2020).

To deal with obstacles like toxicity and to polish the efficacy of CRISPR Cas system in various hosts of pathogenic bacteria different nucleases like Cas9 and Cas12a can be used(Akbar, *et al.,* 2020). Prominently employing domestic CRISPR Cas technology is a auspicious and occasionally technique to manipulate genes in case of intractable pathogens(Xu, *et al.,* 2021).

Other than editing genes in prokaryotes CRISPR Cas system supply massive good time for wide range of applications(Xu, *et al.*, 2021). CRISPR mediated toolkit is an ever growing gene manipulation tool in prokaryotes as it has comprehensibility, pliancy and efficacy to modify genes of native CRISPR Cas (Xu, *et al.*, 2021).

The CRISPR Cas system was employed for the Very first time in year 2012 to develop cleavage double stranded DNA(Liu, M. 2020). Mali et al proposed that CRISPR Cas system can manipulate DNA of mammalian cells (Liu, M. 2020). This highly efficient system is basic and easy to work with and is extensively employed in research purposes to edit genes of plants and animals (Liu, M. 2020). In 2014 few researchs describes the application of this system for editing antibiotic resistant genes and to combat multidrug resistance(Liu, M. 2020).

The emerging escalation of multi drug resistance in bacteria demands an immediate action in terms of developing proxy approaches except antibiotics. Comprehensive studies and researches has shown numerous effective results and CRISPR Cas technology is one of the fruitful outcome for the elimination of pathogenic bacteria from the patient suffering from infection. As time goes by there have been variety of advancements in CRISPR Cas system by imposing and modifying its mechanisms to make it favourable in health care field. CRISPR Cas system is a benign antimicrobial tool as it can particularly attack genes that are multidrug resistant without disturbing other pathogens. CRISPR Cas system is a promising tool to deal with antimicrobial resistance however it can also be effective for precised, simpler and particular identification of pathogenic bacteria. Timely diagnosis in medical field can be ensured by making these diagnostic and identification tools economical. The in vivo aiming of pathognic bacteria can be enhanced in terms of efficiency and harmlessness by various tactics. Excluding in vitro researches variety of systems should be employed in in vivo researches to understand other obstacles and to enhance efficacy of therapy. In vivo templates can be enhanced by this type of research data so that inaccurate outcomes can be declined. In future CRISPR Cas system would be an ideal candidate to deal with notorious resistance mechanism in various strains of bacteria by fixing the genetic module. Latest strategies employing CRISPR Cas system should be pursued to broaden so that current obstacles can be resolved with aid of researchers from medical and genetic engineering field using modern genetic engineering tools.

Suggestions

- This is the need of hour to develop tools and strategies to address the provocation of multidrug resistance in pathogens.
- The delivery of CRISPR Cas system can be enhanced by looking for more conjugative plasmids.
- When Working with CRISPR Cas system the response of pathogenic bacterial population is unpredictable therefore sustainable outcomes should be administered.

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