JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)

ARTICLE
Vol.29; No.3. October-December | 2021

Employing of CRISPR-Cas System as an Antimicrobial Agent

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Received: 17/10/2021 **Accepted:** 26/12/2021 **Published:** 31/12/2021

Abstract

Background:

Improper use of antibiotics and an incomplete administration of the drug may assist to the emergence of antibiotic-resistant bacteria. Using the CRISPR-Cas9 system acts as a precision biological tool to remove resistance genes from the bacterial genome, thereby reducing the appearance of resistant bacterial strains. CRISPR and Cas9 genes work as an adaptive immune systems in widespread bacteria and Achaea. The fragments of invading DNA were integrated into the host genome at the CRISPR locus, where they serve as transcription templates for the synthesis of RNA that aim Cas nucleases to cut foreign nucleic acids. The Cas enzyme identifies target sites in chromosomal sequences, leading to destroy foreign DNA. In our review, we discuss the mechanism of DNA destruction achieved by CRISPR-Cas systems applied as a powerful technology to eliminate plasmids or destroy bacterial cell.

Key words:

CRISPR-Cas systems, Cas, Antibiotic resistance, curing agent, PFREE.

Citation:

Dalal M.Ridha [1*], Hawraa M.AL-|Rafyai [2] and Zainb Fadhil Jawad [3]. Employing of CRISPR-Cas System as an Antimicrobial Agent. Journal of University of Babylon for Pure and applied science (**JUBPAS**). October-December , 2021. Vol.29; No.3: 306-319.

INTRODUCTION

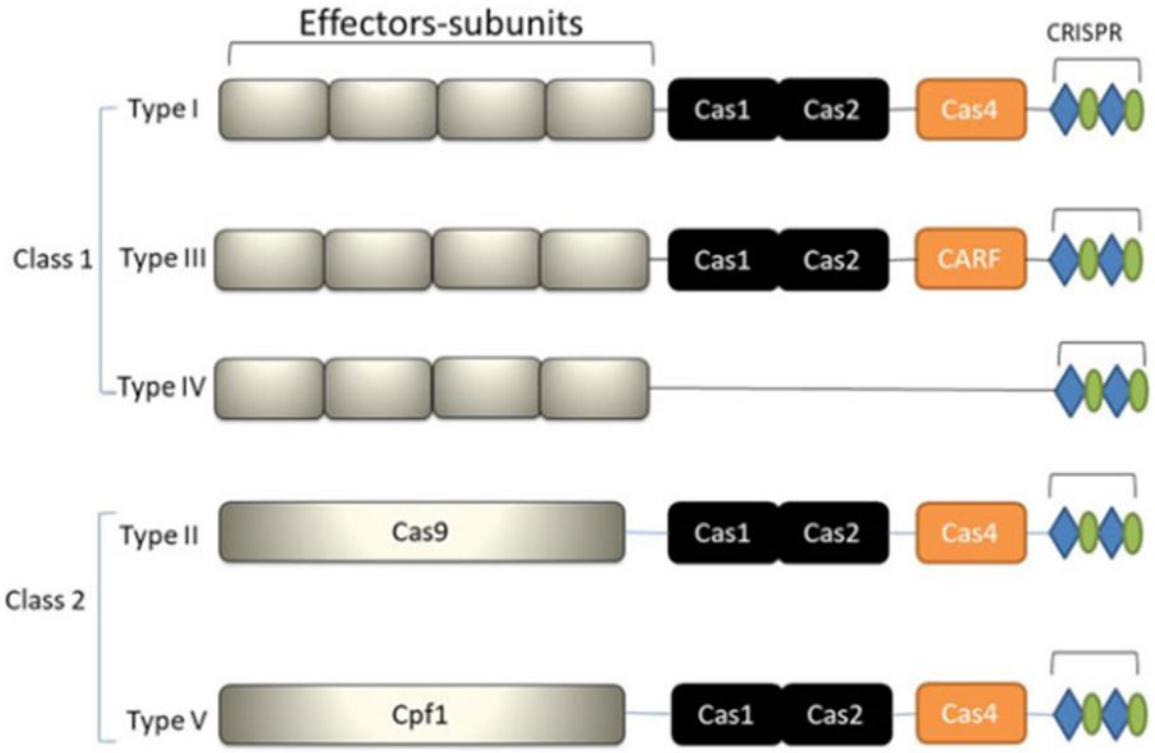
Over the past years, the production of antibiotics has been saved countless lives, while the widespread usage of antimicrobials drives to the development of antibiotic-resistant bacteria. Several studies revealed that CRISPR Cas system restricted the replication of drug-resistant genes by hindering different horizontal gene transfer pathways. The reprogrammed Cas nuclease can be applied to prevent the expansion of the transferred multidrug resistance plasmid by targeting antibiotic resistance genes and curing bacterial resistance plasmids [1]. DNA segments are consist of specific sequences separated by repeating repeats called CRISPR sequences, which are short, clustered, and regularly spaced symmetric repeats. An adaptive immune system in prokaryotes employs a CRISPR array along with Cas binding protein to target the foreign genetic material [2]. The most widespread systems found in almost Archaea and about 50% of bacteria CRISPR Cas system, which exploits the specific sequence RNA-guided endonucleases to split mobile genetic elements and attacker phages. The CRISPR-Cas immune mechanism has based on distinct CRISPR RNA (crRNA) and Cas proteins. It has divided into three phases: spacer acquisition, crRNA biogenesis, and target interference [3]. The CRISPR-Cas also plays a crucial role in the tendency of microbes to resist invasion phage and accommodate to its environment [4]. Recent findings have been shown that CRISPR-Cas systems include six preferable types that fall into two categories. Class I involves types I, III, and IV, which use a single protein set. In contrast, class II uses a particular protein effector nuclease, which includes II, V, and VI [5].The CRISPR-Cas Class I systems use a set of protein-guided RNAs consisting of three to five proteins that adapt rRNA to a target and signal to use the transience nuclease known as Cas3 to destruct DNA. Class II systems; on the other hand, contain a single active protein, Cas9, which has implicated in spacer acquisition, crRNA processing, target identification, and degradation. Because of the dependence of the class II system on a unique protein for function, Cas9 homologs have acquired from different subtypes and species that had applied in gene editing programs over the years [6]. For editing the genome, two CRISPR-Cas effectors, called Cas9 (type II) and Cas12a (Cpf1) had utilized to induce cleavage in double-stranded DNA (dsDNA) targets. While Cas13a (C2c2, type VI), which employs an active detection device for diagnosis pathogens, has been able to induce a break in RNA targets [3]. There are two nuclease domains in Cas9 that are used to create end gaps in two strands. The target DNA strand, which corresponded to the crRNA guide, was cut by the HNH domain as well as the RuvC domain [7].

Origins of CRISPR-Cas systems

In 1978, five highly-similarities sequences consisting of 29 nucleotides observed as direct repeats interspaced by 32 nucleotides as space in the upstream region of the Iap gene in *Escherichia coli* [8]. Short regular repetitions had distributed among distant phylogenetic groups. Including strains of proteinaceous bacteria , some members of the cyanobacteria, Hyper thermophilic bacteria, and archaea. In addition to strains of gram-positive bacteria[9]. They explained the similarities in identical sequences of spacers in bacteriophages and conjugated plasmids. The existence of similarity belongs to the acquisition of extrachromosomal genetic elements during lateral gene transfer [10]. Examination of the sequence of the CRISPR1 site in diverse *Streptococcus thermophilus* strains indicates the presence of differences between industrial species and phage-resistant strains in the description of spacers. Findings showed the alteration in CRISPR loci of the strains relates to the appearance of a CRISPR spacer similar to a unique sequence in phage. It supplies the protection against the phage that has an identical sequence in their genome [11]. According to CRISPR repeat distributions and Cas gene architecture, in Lactic acid bacteria genomes, eight different families of CRISPR sites were determined. From a phylogenetic and genome evolutionary point of view, the diversity in the CRISPR sequence of the Lactic acid bacteria genome had been interpreted by exchanging bacterial genome between different strains of bacteria through a mechanism known as horizontal gene transfer [4].

Classification of CRISPR-Cas systems

The mechanism of the CRISPR-Cas system involves transcribing and processing the CRISPR array to produce mature CRISPR RNA (crRNA), which often contains repeated sequence regions in 5 and 3 that separated by spacer sequences [12]. Relying on the variety of CRISPR-Cas system, crRNA and Cas protein create a complex to recognize the complementary sequences in target DNA and attack it [13]. There are six types of CRISPR-Cas systems, which had assorted into two groups. Class 1 observed in bacteria and archaea, includes types I, III, and IV, besides 16 subgroups, as shown in Figure (1). It comprises multiple Cas proteins that act as operative ingredients that generate crRNA-binding complexes arbitrated in pre-crRNA processing and interference. While class 2 is identified only in bacteria, including types II, V, and VI, besides 17 subtypes. It contains Cas9, Cas12, and Cas13, which are a large, single, and multidomain crRNA-binding protein existing in type II, type V and type III, respectively. These proteins have needed in all activities involved in interference and pre-crRNA processing [14].



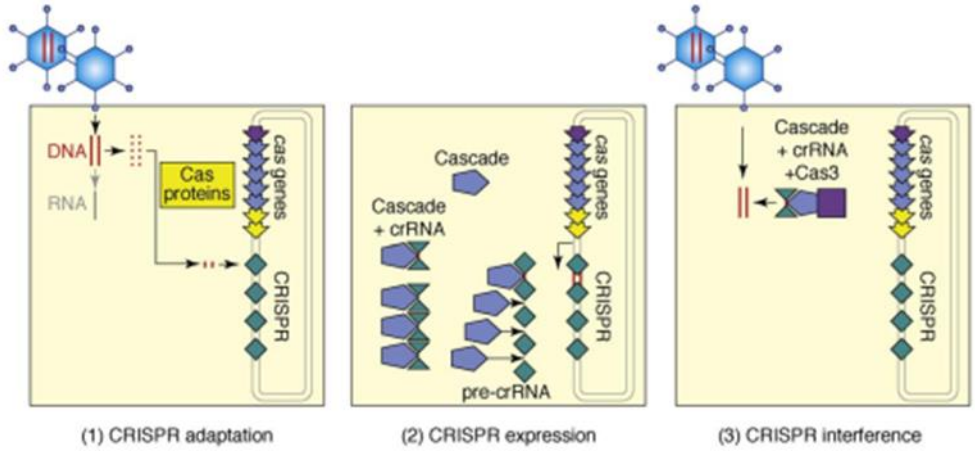
Figure(1): Classification of CRISPR-Cas systems [15].

Each type I CRISPR-Cas site share the ownership of the Cas3 gene, which encodes the single strand of DNA that is responsible for forming enzyme helicase, which is accountable for unwarping the double strand of DNA and the DNA-RNA duplexes [16]. Each subtype contains a specific set of genes and its characteristic features of the Operon Organization. Single operon related to cas1, cas2, and cas3 genes, besides the Cascade subunit genes, encode different subtypes of CRISPR-Cas I systems such as I-C, I-D, I-E, and I-F. On the other hand, various regulators in the Cas genes cluster, including two or more operon, are responsible for expression I-A and I-B subtypes [17]. Cas9 gene found in Type II CRISPR-Cas systems is involved in the formation of a multidomain protein that participates in target DNA cleavage. Based on the attendance of csm2 genes and cmr5, that are encoding small subunits, Type III systems have classified within two subtypes: III-A (Csm module) and III-B (Cmr module) respectively [13]. The backbone effector complexes of Type III CRISPR-Cas consists of one Cas5 subunit and several Cas7 subunits, involving the RRM (RNA Recognition Motif) fold as well as a large subunit and a small subunit. The Cas5 subunit attaches the crRNA and associates with the Cas10 (large subunit). According to the subtypes of Type III, The Csm module, and the Cmr module,

small subunits interact with the crRNA backbone fastened to Cas7. Type III system relies on the repeat sequences of the crRNA precursor that is cleaved by the Cas6 family of endoribonucleases to produce small crRNAs that have combined into a Csm/Cas10 or Cmr/Cas10 complex [18]. Earlier type IV had described as an unexplained type due to its limited existence and loss of the adaptation module. After identifying several genetic arrangements of Type U Cas genes in *Acidithiobacillus ferrooxidans*, it's named Type IV (putative). Integrated elements, plasmids, or phages are responsible for codding all Type IV CRISPR-Cas systems [19]. In all Type IV systems, some genes are accountable for expressing a set of subunits consisting of csf1, csf2, and csf3 called the head, backbone, and tail subunits, respectively. Type IV is the single type containing csf4 (dinG) at CRISPR-Cas sites and is known as a labeled protein for type IV systems [20]. The Type V of CRISPR–Cas system includes Cpf1, which is being in genomes of several bacterial strains, as well as in single archaeal genome nearby Cas1, Cas2, and a CRISPR sequence. Cpf1 is a large protein that comprises a RuvC-like nuclease domain corresponds to the domain Cas9 and the TnpB protein of IS605 family transposons, while the HNH nuclease domain exists in all Cas9 proteins except Cpf1 protein. Cas12a (Cpf1) has expressed outside the CRISPR–Cas setting in several genomes, and its similar to TnpB. It indicates that cpf1 is related to transposable elements [21]. The effector proteins are bilobed, which is comparable to 'jaw-like' architecture holding the guide RNA and the target DNA between the flaps [22].

Molecular mechanisms of CRISPR-Cas system

In recent years, many studies had performed to investigate the mechanism of CRISPR-Cas, which represents an adaptive immune system for an extensive range of bacteria and Archaea. For combating the attack of invasive genetic elements, CRISPR systems intercede immunity through three steps: Adaptation, Expression, and Interference, as exhibited in Figure(2) [19].



Figure(2): Molecular mechanisms of CRISPR-Cas [19].

(a) CRISPR adaptation

The first stage involves integrating protospacer (invasive genetic materials) into the CRISPR sequence that creating distinct spacer, which assists the hostess organism to recognize and preserve foreign genetic materials for a stimulating adaptive immune system at a later time [23]. However, in all CRISPR-Cas types, the Cas1 and Cas2 proteins are related to the spacer acquisition process, but in CRISPR-Cas Type III-C, III-D, and IV systems, there are specific proteins that manage the process. Also, C2C3 andCas1 homolog proteins that make up the CRISPR-Cas Type V-C system participate in the acquisition of spacer [13]. Recent studies have explained Cas1 and Cas2 of the type I-E system in *E. coli*. It forms a complex which assists in the insertion of different spacers in a mode that is similar to transposases and viral integrases [24]. The active site of Cas2 is unable to acquiring spacer, despite Cas1 and Cas2 work as a nuclease. A different spacer is usually integrated at the leader-repeat edge of the CRISPR sequence, while the first repeat of the arrangement is duplicated [25]. The requirements and targets of the adaptation machinery are responsible for the existence of several differences in mechanisms of the various CRISPR-Cas Types. Cas4 in Type I-B CRISPR Cas systems had required for spacer acquisition; despite both Cas1 and Cas 2 can activate the taking of spacer in most investigated Type I CRISPR Cas systems [26]. Pseudomonas aeruginosa has the I-F CRISPR-Cas system, which needs the interference machinery to enhance the acquisition of different spacers [27]. The integration target sequence in Type I, II, and V CRISPR-Cas systems demands the presence of a proto-spacer adjacent motif termed (PAM), which is essential for cleavage by Cas nuclease. For protospacer selection, the domain of Cas9 in the Type II-A CRISPR-Cas system recognizes PAM array (a short specific sequence located downstream of the

target DNA sequence) [28]. It is assumed the incorporation of the different spacers into the CRISPR sequence, Cas9 directs Cas1, Cas2, and perhaps Csn2. Findings have demonstrated in type I-E, the existence of the interference machinery, as well as the Cas1–Cas2 complex, enhances the incidence of combined spacers contiguous to a suitable PAM [29]. Furthermore, during the priming process, the interference machinery of several types I CRISPR-Cas systems can activate the acquisition of different spacers according to the crRNA-guided adhesive to a protospacer that has detected at an initial infection [30].

(b) CRISPR expression

The crRNA biogenesis stage includes the transcription of the CRISPR sequences followed by the building and shaping process for generating crRNA. Activation adapts the immune system in bacteria requires transcription of the CRISPR sequence into a long CRRNA precursor (pre-crRNA) that had converted into an adult crRNA guide containing the foreign genetic information from previous infection [12]. Throughout the processing stage, the Cas6 protein in type I and III systems generate intermediate species of crRNAs that are surrounded by a short 50 tag. While in type I-C systems, the protein Cas5d is accountable for processing pre-crRNA to create intermediate crRNAs containing an 11 nt 50 tags[31]. In most type I system, splicing of the 30 ends of the intermediate crRNA is performed by an undiscovered nuclease, generating adult crRNA species consisting of a complete spacer-part (50 termini) and a repeat-part (30 termini), that formed a hairpin construction [32].In Type II systems, tracrRNA is involved in the processing of pre-crRNA. Cas9 had required for stabilizing crRNA: tracrRNA duplex then is identified and processed by the RNase III host, which provides the intermediate form of crRNA, which matures through a still unknown mechanism that generates adult small guide RNA [33]. The current studies have demonstrated the attendance of promoter sequences in the Type II-C CRISPR-Cas system of *Neisseria meningitidis*, customize each repeat, which is involved in generating intermediate crRNA species [34]. Cpf1 in the type V-A CRISPR-Cas system processes premature crRNA. Then processed crRNA have employed to cut detected DNA [35].

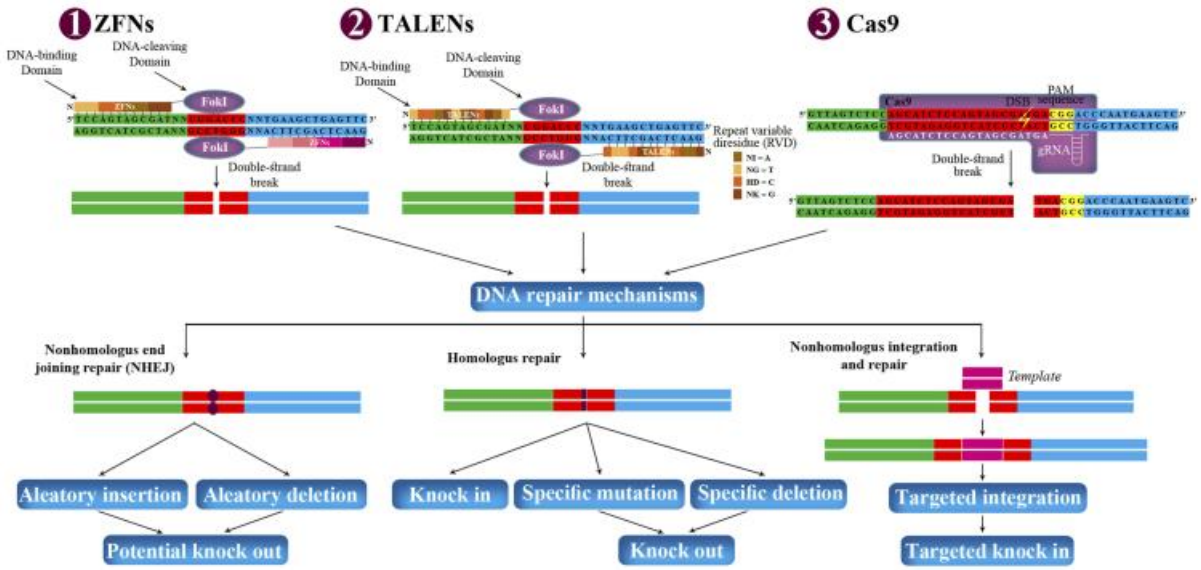
(c) CRISPR interference

During the interference step, crRNA directs the Cas protein to identify target nucleic acids for destruction. In class 1 system CRISPR Cascade participate in target deterioration, while a unique effector protein in class 2 systems is required in interference [36]. The PAM sequences,that occurred in different sites of bacterial genome are accurate target for Type I, II, and V systems to prevent self-targeting [37]. Diagnosis between individual and non-individual in type III systems have achieved by detecting the 50 tags of the adult crRNAs, which should not match with the

target to permit destruction by the complex [38]. Cascade of Type I systems works to localize the invasive DNA in a crRNA-dependent mechanism and employs the Cas3 enzyme to induce a cleavage on the strange DNA and afterward destroys the target DNA. The duplex TracrRNA: crRNA in CRISPR-Cas Type II systems directs the effector Cas9 protein to cause a double-strand split in the DNA. The interference mechanism for Type III systems includes Cas10-Cmr or Cas10-Csm complexes, which had recruited to detect both DNA and RNA. Interestingly, the overlap of the Type III-A and Type III-B systems rely on the transcription of detected DNA.

Using the CRISPR-Cas system as struggle antibiotic resistance

The scarcity of efficient tools to create double-strand cleave in specific site DNA made the alteration DNA within cells restricted [39]. Previous approaches had based on Zinc finger nuclease (ZFNs) and transcription activator-like effector nuclease (TALENs), which are restrictive enzymes directed to break down specific sequences of DNA as explained in Figure(3). The gene-editing tools had linked to a non-specific restriction enzyme (FokI), which gives nuclease activity to ZFNs and TALENs. TALENs and ZFNs are comparable to creating knockdown or knockout genes and cause double-strand splits at the wanted location in the DNA. Nevertheless, the large size of TALENs contrasted with ZFNs makes it troublesome to introduce TALENs into cells [40]. The ability of these manipulated restriction enzymes to identify a target site is limited.in the other hand; CRISPR-Cas systems depend on the sequence of RNA to recognize specific sequences [39]. The CRISPR-Cas system discriminates between commensal and pathogenic bacterial strains by targeting detected sequences. So it has been adopted to target antibiotic-resistant pathogens in heterogeneous bacterial residents, allowing the delivery of antibacterial agents to the pathogenic bacteria, and in some examples administering treatments to Host cells infected with pathogenic bacteria [41]. Extended-spectrum beta-lactamase (ESBL)-*Eschrechia coli* have plasmid-mediated antibiotic resistance genes that can be easily transmitted to other individuals of the bacterial inhabitants by horizontal gene transfer. For returning the antibiotic susceptibility of these bacteria, the CRISPR / Cas9 technique had employed to remove plasmids responsible for drug resistance[42]. A recent study revealed that genetically modified CRISPR-cas9 phages could target virulent *Staphylococcus aureus*. These results confirmed that Cas9 re-programmed utilized to recognize pathogenic bacteria and restrict the spread of plasmid-transmitting antibiotic resistance genes[43]. There is a set of studies that have embraced the search for the CRISPR Cas delivery mechanism that has involved the use of transformable plasmids or phages to identify target cells.



Figure(3):latent genome alteration employing ZFNs, TALENs, and Cas9[40].

PRPs are vectors for the expanse of antibiotic resistance genes in *Enterococcus faecalis* Communities. The recent finding approved that the PRP pPD1, a cognate CRISPR antimicrobial plasmid which modified by inserting the cas9 gene and a CRISPR guide RNA under the direction of the bac A activater can diminish the existence of antibiotic resistance in *Enterococcus faecalis* Communities[44]. In another study, Lorstein and colleagues designed a plasmid removal system called (PFREE), which has constructed by amplifying the backbone of a Pmazsk plasmid. It includes CRISPR-Case 9 and target sequences of four different types of (ColE1) plasmids to eliminate different plasmids within one step, as displayed in figure(4).

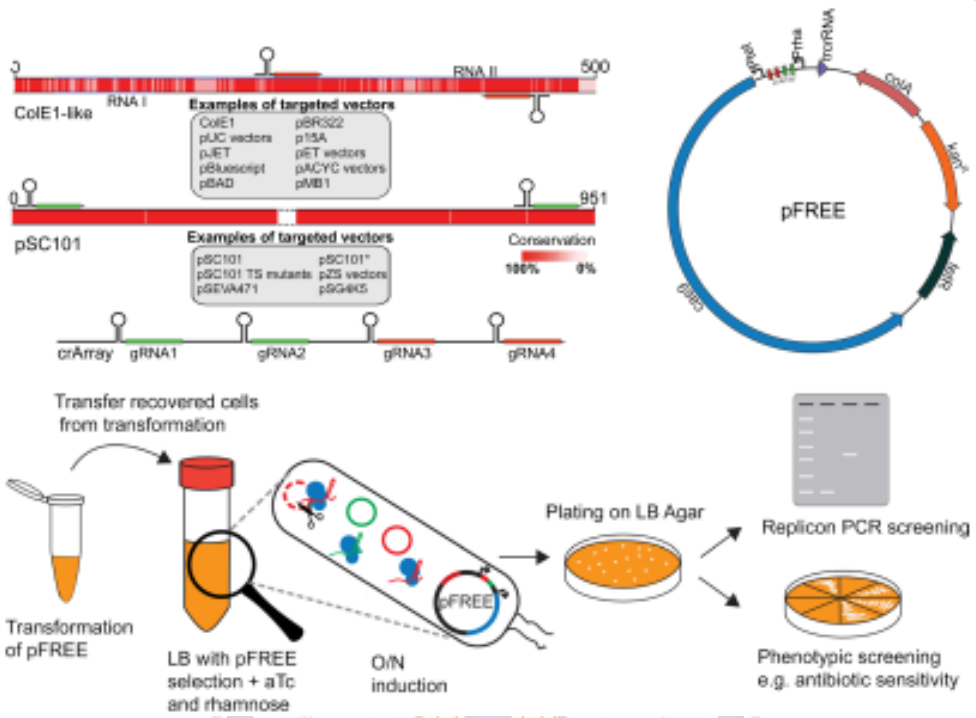


Figure (4): The complete plasmid removal system (PFREE) scheme [45].

The researchers prepared *Escherichia. coli* strains are contained three compatible plasmids and transferred them to a transformation medium containing PFREE plasmids. The mixture incubated for different periods, and then samples were taken from the transformation mixture and grown on Luria broth agar for one night. It found that 80% of the tested cells were entirely devoid of the plasmid, while 10% of the tested cells contained one or more plasmids. As well as, it found that all the tested cells did not contain pFREE plasmids[45].

Conclusion:

CRISPR-Cas systems had utilized to detect antibiotic resistance and virulence genes in bacteria. It is an attractive choice for specific, programmable sequence tools. When ingested by the capsid of phages in vitro, it can effectively execute the target population and can also decrease the settlement of the target population in vivo. Resistance genes carried on a plasmid can be removed by applying the CRISPR-Cas technique, which leads to producing sensitive bacteria against antibiotics. The CRISPR-depend approach has assisted limiting the random extermination of conceivably beneficial bacteria, moreover enabling the non-target inhabitants to flourish and maintain the biological niche. CRISPR-based technologies present a new method to preserve the configuration of microbial populations instead of applying it as a traditional, widespread-spectrum antibiotic.

Acknowledgments:

The authors thank the microbiology laboratory, College of Science, University of Babylon for supplying necessary facilities to achieve this research.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

ان الاستخدام الغير دقيق والمنتظم للمضادات الحيوية ادى الى ظهور سلالات بكتيرية مقاومة لطيف واسع من المضادات الحيوية. يعمل نظام كريسبر كاس 9 كأداة بيولوجية دقيقة لإزالة جينات المقاومة من الجينوم البكتيري ، وبالتالي التقليل من ظهور السلالات البكتيرية المقاومة للمضادات الحيوية. تمتلك العديد من البكتيريا والعتائق سلسلة من التكرارات على كروموسوماتها تتخللها تسلسلات معينة مشتقة من الفيروسات والبلازميدات ,حيث تعمل بمثابة نظام مناعي تكيفي تستخدمه البكتريا لحماية نفسها من الاصابة بالفايروسات ,اكتشف العلماء مقاطع متكررة في الحمض النووي لبكتريا القولون حيت لايتغير ترتيبها ان قرأت طردا او عكسا لانها متناظرة وبعد فترة من الزمن اكتشف الباحثون ان هذه المقاطع من الحمض النووي تماثل المقاطع المكتشفة في فيروسات معينة وان البكتريا تحتفظ بها ضمن المادة الوراثية العائدة لها كاحتياطي تحسبا لعودة اي هجوم فيروسي ,فان حدث هجوم تقوم البكتريا بمقارنته الحمض النووي الفيروسي مع ما محفوظ لديها من الحمض النووي. عندما تكون البكتريا معرضة لهجوم فيروسي فانها تنتج نوعين من الحمض النووي الريبوزي القصير , اللذان يشكلان مع بروتين آخر مركبا يطلق عليه كاس 9. يعتبر بروتين كاس9 من الانزيمات التي لها القدرة على قطع الحمض النووي عندما تتمكن سلسلة تعرف باسم الحمض النووي الريبوزي الموجه من تحديد الهدف ضمن الجينوم الفيروسي, بعد ذلك يقوم كاس9 بقطع تسلسلات الحمض النووي المستهدفة و بالتالي يؤدي الى الحاق ضرر كبير للجينوم الفيروسي. في مراجعتنا، نناقش آلية تدمير الحمض النووي بواسطة المقص الجيني نظام كريسبر كاس 9 المستخدم كتقنية قوية وفعالة للقضاء على البلازميدات أو تدمير الخلايا البكتيرية.