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REVIEW

Suppressing the CRISPR/Cas adaptive immune system in bacterial infections

P. Gholizadeh[1,2] · M. Aghazadeh[3] · M. Asgharzadeh[4] · H. S. Kafil[5]

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Abstract Clustered regularly interspaced short palindromic repeats (CRISPR) coupled with CRISPR-associated (Cas) proteins (CRISPR/Cas) are the adaptive immune system of eubacteria and archaebacteria. This system provides protection of bacteria against invading foreign DNA, such as transposons, bacteriophages and plasmids. Three-stage processes in this system for immunity against foreign DNAs are defined as adaptation, expression and interference. Recent studies suggested a correlation between the interfering of the CRISPR/Cas locus, acquisition of antibiotic resistance and pathogenicity island. In this review article, we demonstrate and discuss the CRISPR/Cas system's roles in interference with acquisition of antibiotic resistance and pathogenicity island in some eubacteria. Totally, these systems function as the adaptive immune system of bacteria against invading foreign DNA, blocking the acquisition of antibiotic resistance and virulence factor, detecting serotypes, indirect effects of CRISPR self-targeting, associating with physiological functions, associating with infections in humans at the transmission stage, interfering with natural transformation, a tool for genome editing in genome engineering, monitoring foodborne pathogens etc. These results showed that the CRISPR/Cas system might prevent the emergence of virulence both in vitro and in vivo. Moreover, this system was shown to be a strong selective pressure for the acquisition of antibiotic resistance and virulence factor in bacterial pathogens.

✉ H. S. Kafil
Kafilhs@tbzmed.ac.ir

[1] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[4] Infectious and Tropical Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction

CRISPR/Cas systems are the adaptive immune system of eubacteria and archaebacteria known as clustered regularly interspaced short palindromic repeats (CRISPR) coupled with CRISPR-associated (Cas) proteins [1–6]. Cas proteins play a key role in the CRISPR/Cas immune system function and indicate the system activity [7]. The CRISPR/Cas systems protect these organisms against potentially dangerous foreign DNAs, such as transposable elements, phages and plasmids [1, 2]. The mechanism in these systems is similar to that of RNA interference (RNAi) in eukaryotes since they use small RNAs (sRNA) to detect a specific foreign sequence and neutralise the invading DNA genomes [4, 8]. However, the CRISPR/Cas system integrates a small segment of DNA derived from foreign nucleic acid into the CRISPR locus of the host genome that leads to immunity against the invader [8]. Three-stage processes of CRISPR/Cas systems (types I, II and III) have been defined for immunity against invading foreign DNAs: adaptation, expression and interference (Fig. 1) [6, 10–12].

(1) During the adaptation stage, a short segment of homologous to plasmid or phage is integrated into the leader side of the CRISPR locus (approximately 30 bp) [1, 13, 14]. Protospacer adjacent motifs (PAMs) have selected spacer precursors of invading DNAs. PAMs differ between variants of the CRISPR/Cas systems and are usually several nucleotides

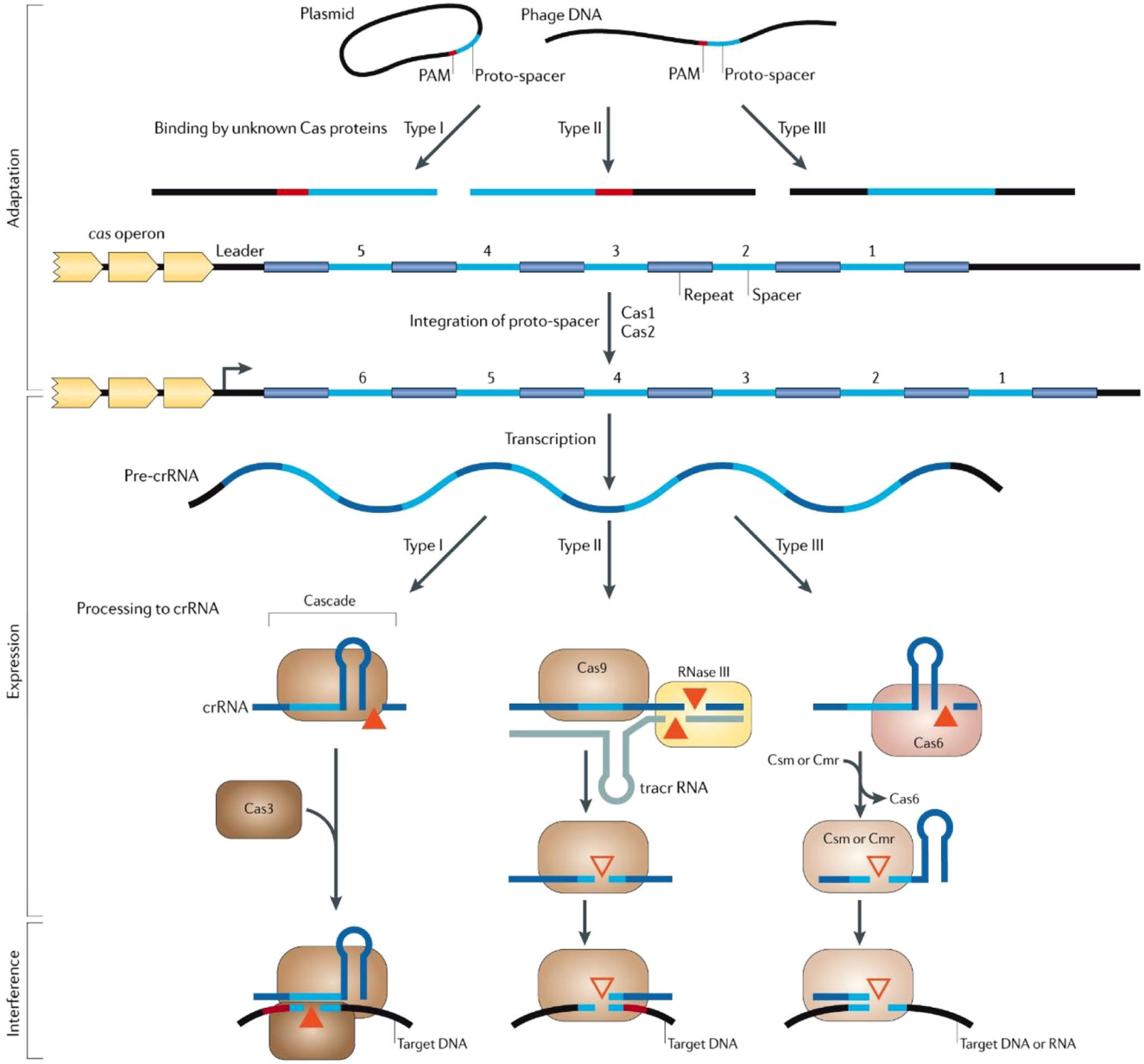

Fig. 1 Three types of the CRISPR/Cas system's mechanism against invading genetic elements [9]. All three types are summarised as adaptation, expression and interference stages. During the adaptation stage, short segment of homologous to plasmid or phage are integrated into the leader side of the CRISPR locus (approximately 30 bp). Protospacer adjacent motifs (PAMs) have selected spacer precursors of invading DNA by Cas 1 and Cas 2. During expression, long primary transcript of a CRISPR containing the spacer is expressed (pre-crRNA) and is processed into short crRNAs (cascade in *Escherichia coli* as type I, tracr RNA in *Streptococcus pyogenes* as type II and Cas6 in *Pyrococcus fusiosus* as type III). During the interference step, foreign DNA or RNA is cleaved into the protospacer sequence. The crRNA guides the complexes of Cas proteins to the plasmid or phage sequences that match the spacers

sequences [15, 16]. In this stage, Cas 1 and Cas 2 are the initial candidates of proteins, which play a key role in this process [1, 4].

(2) In the expression stage, a long primary transcript of a CRISPR containing the spacer is expressed (pre-crRNA) and processed into short crRNAs [4, 17]. During the process of this stage, endoribonuclease have catalysed crRNAs that operate as a subunit of a large complex in *Escherichia coli* known as a CRISPR-associated complex for antiviral defence cascade (type I CRISPR/Cas system) [4, 17], a trans-encoded small RNA (tracrRNA) in *Streptococcus pyogenes* (type II CRISPR/Cas system) [18] and a single enzyme archaea in *Pyrococcus fusiosus* (Cas 6) (type III CRISPR/Cas system) [19–21]. In *S. pyogenes*, tracrRNA is catalysed by RNase III in the presence of Cas 9 (also known as Csn1) [18]. Cas 9 plays a key role in CRISPR encoded interference and mutation analysis, showing that interference by Cas 9 is based on Ruvc/RNaseH- and McrA/HNH-motifs [8, 22, 23].

(3) During the interference stage, foreign DNAs or RNAs are cleaved into the protospacer sequence [11, 13, 14]. RuvC/RNaseH-motif is a characteristic protein that exhibits wide spectra of nucleolytic function and acts on both DNAs and RNAs [24]. McrA/HNH-motif includes many nucleases. This motif acts on double-stranded DNAs, including restriction enzymes, colistin, homing endonucleases, transposes and DNA packaging factors [25]. The crRNA guides the complexes of Cas proteins to the plasmid or phage sequences that match the spacers [4]. Moreover, PAMs seem to play a key role in the interference process and are required for efficient interference [16, 26].

There are several Cas proteins that possess DNase and/or RNase activity. Diverse Cas proteins could take part in either one or multiple stages of the CRISPR/Cas system action, and most of these proteins probably act as protein complexes [11]. Two universal core Cas proteins are Cas 1 and Cas 2 [27, 28]. Cas 1 is a metal-dependent DNase with no specificity, which integrates the spacer DNA within the CRISPR cassette [27]. Also, Cas 2 is a metal-dependent endoribonuclease [28]. The role of Cas 2 in the CRISPR/Cas system's mechanism has remained unclear [28]. Various classifications of CRISPR/Cas systems are defined in different studies. In Haft et al.'s and Makarova et al.'s studies, CRISPR/Cas systems are classified into CASS1–CASS8 on the basis of Cas 1 phylogeny and on the composition and architecture of the Cas operons [8, 22]. Makarova et al. suggested that CRISPR/Cas systems are classified into three types on the basis of the constitution of Cas 1 and Cas 2 genes into the core of CRISPR/Cas systems [9]. The type 1 CRISPR/Cas system contains Cas 3 genes and other genes encoding proteins that probably produce cascade-like complexes with various compositions [9]. Cas 3 is a large protein with separate DNase and ATP-dependent helicase activity [9]. Type 2 contains *cas 9* genes [9]. Cas 9 is a very large, single protein that generates crRNA and cleaves the target DNA [29, 30]. Type 3 contains polymerase and repeat-associated mysterious proteins (RAMP) modules [9]. RAMP generates a large superfamily of Cas proteins. RAMP contains a characteristic glycine-rich loop and at least one RRM (RNA recognition motif; also known as a ferredoxin-fold domain [8].

The transfer of antimicrobial resistance and virulence genes by the foreign DNA is the main reason for the emergence of increased resistant and pathogenic bacterial strains [31, 32]. The ability of CRISPR/Cas systems to trigger virulence and antibiotic resistance genes in plasmids or in phages has been demonstrated [13]. Sequence analysis of pathogenic bacteria demonstrated a correlation between the accumulation of antibiotic resistance genes and the absence of the CRISPR/Cas locus. These results suggest that the CRISPR locus presents a barrier to the transfer of traits under selective pressure during infection [32, 33]. This review evaluates the role of CRISPR/Cas systems in bacterial pathogens. To this end, we tend to investigate CRISPR/Cas, targeted bacteria, pathogenicity and antibiotic resistance. Further, articles on the role of CRISPR/Cas in pathogenicity and interference with the acquisition of antibiotic resistance in these targeted bacteria were studied from 1997 to 2017.

Streptococcus spp.

Streptococcus pneumoniae displays the ability to insert new genetic materials via horizontal gene transfer and for direct uptake of exogenous DNA or DNA transformation [32, 34, 35]. In response to the immune system attack, *S. pneumoniae* can alter their surface polysaccharide capsule and avoid capsule-specific antibodies, and, also, acquire new capsule-encoding genes and establish a successful infection [32, 35]. Capsule switching in *S. pneumoniae* is a main mechanism for the rapid evolution in this organism [36]. Bikard et al. demonstrated that, at low frequencies, *S. pneumoniae* can lose CRISPR/Cas function and acquire capsule genes [32]. The presence of capsule in *S. pneumoniae* is necessary for a successful infection [32]. The results of Bikard et al.'s study showed that CRISPR/Cas interference can prevent the appearance of novel pathogenic strains, because it is a strong selective pressure for antibiotic resistance or virulence factor [32]. They transferred the CRISPR1 locus from *S. pyogenes* to *S. pneumoniae* [32] and illustrated that the CRISPR loci can prevent transformation in bacteria that possess natural transformation between competent bacteria [32]. Bikard et al., Edgar and Qimron, and Gudbergsdottir et al. suggested that CRISPR/Cas systems and genetic materials cannot simultaneously coexist in the same bacterial cells [32, 37, 38]. Bikard et al. demonstrated that the capsule gene of *S. pneumoniae* is the target of the CRISPR1 locus and these loci can prevent the transfer of these genes to non-capsulated *S. pneumoniae* and mice can survive the infection [32]. Marraffini and Sontheimer demonstrated that CRISPR loci can prevent plasmid transformation and conjugation in *Staphylococcus epidermidis* [2].

spc1 is homologous with the *nickase* gene that is present in staphylococcal conjugation plasmids, including MRSA (methicillin-resistant *S. aureus*) and VRSA (vancomycin-resistant *S. aureus*) [39, 40]. *Staphylococcus epidermidis* Rb62a strains contain a CRISPR locus that contains spacer 1 (*spc1*) [41]. Marraffini and Sontheimer demonstrated that insertion of a self-splicing intron into plasmid that possesses the *nickase* gene interferes with the target plasmid directly [2]. These results showed that CRISPR interference would limit the spread of antibiotic resistance plasmid genes such as MRSA and VRSA and virulence factor between staphylococcal strains, if the system interference could be manipulated in clinical treatment. In another study, Barrangou et al. demonstrated a correlation between the CRISPR locus and phage resistance in

S. thermophilus [1]. Their results showed that plaque formation was reduced and, also, some phages were unable to infect the phage-resistant mutants of *S. thermophilus* [1]. They observed that the addition of two spacers causes this mutant [1]. Also, the two spacers, S1 and S2, were reported to be responsible for virulent phage variants and, also, two distinct small nucleotide proteins were identified in the spacer 1 sequence [1]. The results of this study demonstrated that *cas 5* inactivation causes the phage susceptibility, because it contains an HNH type nuclease motif (McrA/HNH-motif) that acts as a nuclease [1]. Also, Cas 7 acts as an enzyme that synthesises and/or inserts new spacers and is unable to generate phage-resistant mutants [1].

Escherichia coli

In *E. coli* species, CRISPR's map has been observed in two loci: CRISPR 1 and 2 determined at 62 min (including subtype I-E of *cas* genes) and CRISPR 3 and 4 determined at 20 min (including subtype I-F of *cas* genes) on the chromosome [42, 43]. CRISPR 1 and 2 are large numbers of CRISPR repeats in *E. coli* strains [44]. Yosef et al. demonstrated that CRISPR activity against lambda prophage is associated with the high-temperature protein G (HtpG) in *E. coli* and is essential for CRISPR activity in *E. coli* [45]. Inactivity of CRISPR related to HtpG deficiency can be suppressed by the Cas 3 protein [45]. The steady-state level of Cas 3 overexpression is enhanced following HtpG expression [45]. The results of Touchon et al.'s study demonstrated that the activity of CRISPR and the presence or absence of *cas* genes are not associated with the presence of integrons and plasmids, as well as antibiotic resistance in *E. coli* [44]. Their results are inconsistent with phage resistance and other reports that concern other species [7, 33, 37, 46]. The results showed that subtype I-E *cas* genes were present in both susceptible and resistant strains or in both producing and non-producing beta-lactamases strains, and the subtype I-E did not differ between these strains [44]. However, subtype I-F *cas* genes were more prevalent among susceptible strains and, also, these strains were almost devoid of CRISPR systems [44]. This study suggested that, if such activity inhibits transfer, plasmids and integrons acquisition followed by multi-resistant acquisition should be higher for strains that do not possess active CRISPR [44].

Toro et al.'s study investigated 194 strains of Shiga toxin-producing *E. coli* (STEC) and identified CRISPR 1 and CRISPR 2 in all the strains; however, in their study, both CRISPR 3 and CRISPR 4 were only identified in one isolate and 193 strains exhibited a short, combined array, and identified 3353 spacers [47]. Their results demonstrated that there was no correlation between virulence genes and the presence of subtype I-E *cas* genes, but the presence of spacers causes negative reduction of potential pathogenicity [47]. The results of their study also demonstrated that strains with only one *stx* gene had higher spacers than those having multiple *stx* genes [47]. These results were consistent with the described role of the CRISPR/Cas system in limiting the genetic material acquisition [7, 33, 37, 46].

In another study, Sapranauskas et al. demonstrated that the CRISPR/Cas system of *S. thermophilus* can be transferred into *E. coli* and protects this organism against invasive phages, plasmid transformation and makes a safer organism [23]. Also, their study demonstrated that mutations within the PAM and its vicinity authorise plasmids to evade the CRISPR-encoded immunity [23].

Another application of CRISPR is the detection of *E. coli* serotypes. Studies demonstrated that specific CRISPR polymorphisms can be distinguished in the O:H serotypes of STEC, such as O157:H7, O145:H8, O104:H4, O103:H2, O45:H2, O26:H11 etc. by real-time polymerase chain reaction (PCR) [47–50]. But there are numerous cross-reactions between primers of some of the same H antigen serotypes, such as cross-reactions between primers of O145:H28 and O28:H28 or between O103:H2, O45:H2, O128:H2 and O145:H2 [47, 48, 50].

Pseudomonas spp.

Lysogenic conversion promotes the continued persistence of the bacteriophage genome within the host bacteria population. Lysogenic conversion mediates secretion of virulence factors in some bacteria, such as improved adhesion to epithelial cells in *Pseudomonas aeruginosa* PAO1 infected with bacteriophage FIZ15 [51], altered lipopolysaccharide profile in *P. aeruginosa* infected with phage D3 [52] and production of cholera toxin in *Vibrio cholera* infected with phage CTXφ [53]. Zegans et al. in their study demonstrated that infection of *P. aeruginosa* by bacteriophage DMS3 causes lysogenic strains that do not form biofilm or are unable to undergo swarming motility [54]. Also, they showed that CRISPRs play a necessary role for restored biofilm formation and swarming motility, and five of six *cas* genes are required for the restoration of biofilm formation and swarming motility in these strains [54]. In another study, Heussler et al. showed that the PAM and DMS3 protospacer plays an important role in CRISPR-dependent loss of both biofilm formation and swarming motility in *P. aeruginosa*, and do not require addition of DMS3 phage sequences [55]. Also, their results revealed that the interaction between the DMS3 protospacer and the CRISPR system induces expression of SOS-regulated phage-related genes, such as *pyocin* operon, via RecA activation after the activity of nuclease Cas 3 [55]. These results demonstrated that loss of both biofilm formation and swarming motility has indirect effects of CRISPR self-

targeting [55] and may be useful for the reduction of inflammation of *P. aeruginosa* by DMS3 impression, if it can inhibit the CRISPR/Cas activity.

Nine distinct families of phage proteins have been described to possess anti-CRISPR/Cas activity [56–60]. These proteins are classified into nine groups and are termed as A–I [58]. Homologs of these anti-CRISPR proteins were detected only within the *Pseudomonas* genus [57]. Pawluk et al. revealed that four anti-CRISPR/Cas genes are present in different types of *Pseudomonas* prophages [57]. Studies demonstrated that five families of anti-CRISPR/Cas proteins found in phages and other mobile genetic elements inhibit the type I-F CRISPR/Cas systems of both *Pectobacterium atrosepticum* and *P. aeruginosa*, and a specific dual anti-CRISPR/Cas protein ($AcrF6_{Pae}$) inactivates both type I-E and I-F CRISPR/Cas systems [56–58].

Three mechanisms of anti-CRISPR/Cas proteins have been detected in *P. aeruginosa* [59]. Two anti-CRISPR/Cas proteins (AcrF1 and AcrF2) interact with various DNA-binding protein subunits (Csy complex subunits), use non-steric and steric modes of inhibition and, likewise, barricade the DNA-binding activity of the CRISP/Cas complex [59, 61, 62]. Binding to the Cas 3 and inhibiting its recruitment to the DNA-bound CRISPR/Cas system is the third mechanism of anti-CRISPR/Cas proteins [59]. There are two primary quorum-sensing auto-inducer (AI) receptors in *P. aeruginosa*, known as LasIR and RhlIR [63–66]. Quorum-sensing activates *cas* gene expression to increase foreign DNA-targeted CRISPR/Cas to promote CRISPR adaptation [67]. The type I-F CRISPR/Cas system provides phage resistance in *P. aeruginosa* [68, 69]. Høyland-Kroghsbo et al. showed that the administration of pro- and anti-QS components can regulate CRISPR/Cas activity in *P. aeruginosa* PA14 [67], which possesses the type I-F CRISPR/Cas system [70]. Also, they suggested that quorum-sensing inhibitor could be used to suppress the CRISPR/Cas system in order to elevate medical application, such as phage therapies [67].

Other bacteria

Analysis of the chromosome of *Legionella pneumophila* strain 130b showed that this organism possesses subtype II-B CRISPR/Cas locus [71], and a locus similar to it is found in both the plasmid and chromosome of *L. pneumophila* strain Paris [72, 73]. This locus contains an array with 60 repeats, 58 unique spacers, Cas 9, Cas 1, Cas 2 and Cas 4 [71]. Analysis by reverse transcriptase PCR (RT-PCR) revealed that the CRISPR/Cas locus is expressed during infection of *Acanthamoeba* and *Hartmannella* as aquatic amoeba and macrophages, as well as during extracellular growth in both minimal and rich media [71]. Also, levels of Cas 1 and Cas 2, measured by quantitative RT-PCR, are enhanced during intracellular growth [71]. Gunderson and Cianciotto demonstrated that Cas mutants were impaired for infection of *Acanthamoeba* and *Hartmannella* species but could grow normally in macrophages [71]. Also, they revealed that Cas 2 plays a role in the transmission of Legionnaires' disease [71]. Their results suggested that CRISPR/Cas loci may have relevant physiological functions and are not related to horizontal gene transfer in this organism [71]. Infection of *L. pneumophila* in amoeba and, subsequently, in humans may decrease by mutation in Cas 2.

In *Neisseria meningitidis*, the CRISPR/Cas system could interfere with natural transformation [74]. Zhang et al. demonstrated that the type II-C CRISPR/Cas system of *N. meningitidis* requires Cas 9 and not Cas 1 and Cas 2 for interference of natural transformation [74]. Also, in this type of system, pre-crRNA processing is not required for interference activity [74]. RNase III-catalysed pre-crRNA processing occurs within the bacterial cell; however, it is unnecessary for interference. The tracrRNA cleavage by crRNA-programmed Cas 9 [74] was observed in vitro in Jinek et al.'s study [75]. In human genome engineering of pluripotent stem cells, Hou et al., in their study, demonstrated that *N. meningitidis* Cas 9 distinguishes a 5′-NNNNGATT-3′ PAM [76]. They observed that the CRISPR/Cas system of *N. meningitidis* is able to increase the sequence contexts amenable to RNA-directed genome editing [76]. Their results demonstrated homology-directed repair (HDR) of efficient targeting of an endogenous gene in three human pluripotent stem cell lines by using a distinct CRISPR/Cas system from *N. meningitidis* [76]. Yet, in another research, Lee et al. demonstrated that the CRISPR/Cas 9 system of *N. meningitidis* may represent a safer alternative than the CRISPR/Cas system of *S. pyogenes* for precision genome engineering applications [77] Table 1.

Vibrio cholerae contains a genomic island with VPI-1 (*Vibrio* pathogenicity island-1) and possesses the CRISPR/Cas system and type VI secretion system (T6SS) [82]. Box et al. showed that the transfer of genomic island-24 to the El tor biotype of *V. cholerae* via transformation can enable CRISPR/Cas-mediated resistance to phage CP-T1 [78]. They also demonstrated that CRISPR targets must be attended by a 3′ PAM for affective interference [78]. Transferring the CRISPR/Cas system from the classical biotype to the El tor biotype is functional in providing resistance to bacteriophage infection, and this system can be used as an effective gadget for the editing of lytic phage genomes of *V. cholerae* [78]. In *V. parahaemolyticus*, there are associations between the presence of virulence factor and the CRISPR/Cas system. The results of the studies by Sampson et al. and Sun et al. demonstrated that type II CRISPR/Cas plays a role in virulence [79,

Table 1 The CRISPR/Cas locus application in different bacteria

Organism	Application[a]	Reference
Streptococcus spp.	*S. pneumoniae*: prevention of the transferring of capsule genes from capsulated strains to uncapsulated strains	[3]
	S. thermophilus: reduction of plaque formation against bacteriophages, nability of phages infection	
	S. pyogenes: genome engineering	
Staphylococcus epidermidis	Interference with spread of antibiotic resistance plasmid such as MRSA[b] and VRSA[c]	[2]
Escherichia coli	Suppression of lambda phage, reduction of genetic elements acquisition and follow as multi-resistant acquisition, negative reduction of potential pathogenicity, detection of serotypes	[44, 45, 47–50]
Pseudomonas aeruginosa	Loss of biofilm formation, loss of swarming motility, induction of SOS-regulated phage-related genes expression, *Pseudomonas* pro-phages possess anti-CRISPR/Cas proteins, phage resistance	[54, 55, 57]
Legionella pneumophila	Expression during the infection of amoeba, macrophages and extracellular growth, transmission of Legionnaires' disease, physiological function	[71]
Neisseria meningitidis	Interference with natural transformation, increasing the sequence contexts amenable to RNA-directed genome editing in human pluripotent stem cells	[53]
Vibrio cholerae	Resistance to phage CP-T1, editing of lytic phage genomes	[78]
Vibrio parahaemolyticus	Monitoring in clinical and food samples, detection of virulence factor as a marker	[79]
Francisella novicida	Suppression of bacterial lipoproteins	[80]
Campylobacter jejuni	Reduction of virulence factor, viral defence, reduction of Guillain–Barré syndrome	[81]

[a] All introduced applications are current developments for the mentioned pathogens

[b] Methicillin-resistant *Staphylococcus aureus*

[c] Vancomycin-resistant *Staphylococcus aureus*

80]. Sun et al. also demonstrated that the CRISPR/Cas system provides new methods for monitoring *V. parahaemolyticus* in clinical and food samples and, also, is used as a marker for the detection of virulence factors, such as thermostable direct hemolysin and clonal complex 3 in *V. parahaemolyticus* [79]. Sampson et al. demonstrated that the Cas 9 protein of *Francisella novicida* can use CRISPR/Cas-associated RNA (scaRNA) to suppress a bacterial lipoprotein that is encoded by an endogenous transcript [80]. This lipoprotein is a target of a proinflammatory innate immune response and is armed in fighting pathogens [83, 84]. Their results demonstrated that CRISPR/Cas-mediated gene regulation may contribute during the interaction of *F. novicida* with eukaryotic cell hosts [84].

Guillain–Barré syndrome is a severe sub-acute, post-infection disease occurring after infection with pathogenic bacteria, such as *Campylobacter jejuni*, in the human peripheral nervous system [85]. This syndrome has been provoked by molecular mimicry between *C. jejuni* sialyltransferase (CstII) and ganglioside epitopes in the human peripheral nerves [86, 87]. Mutation in *cas* genes (*cas1*, *cas2* and *csn1*) and CRISPR degeneration of *C. jejuni* are correlated with *C. jejuni* sialyltransferase (CstII) [81]. Louwen et al. demonstrated that inactivation of the type II CRISPR/Cas system, particularly *csn1*, may reduce virulence in Cst-II-positive *C. jejuni* strains [81]. Their results showed a novel correlation between virulence, viral defence and Guillain–Barré syndrome in *C. jejuni* [81].

Conclusion

These results showed that the CRISPR/Cas system may prevent the emergence of virulence both in vitro and in vivo. This system may be a strong selective pressure for the acquisition of antibiotic resistance and virulence factor in bacterial pathogens. This system is used as an adaptive immune system of bacteria against invading foreign DNAs and, also, to block the acquisition of antibiotic resistance and virulence factors, detect serotypes, indirect effects of CRISPR self-targeting, associate with physiological functions, associate with infection in humans at the transmission stage, interfere with natural transformation, edit genomes in genome engineering and monitor foodborne pathogens. Particularly, there are some issues required to be investigated, such as the association between the CRISPR/Cas system and its host, limiting susceptibility to invasive mobile genetic elements, ability to uptake exogenous DNA, expression of virulence genes in foreign DNAs and Cas-9 application for viral immunity in human infection, transcriptional control, genome editing etc.. This system defines

future tools for infection control and bacterial genetic manipulations.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This is a literature review study and, therefore, ethical approval is not required.

Informed consent For this type of study, formal consent is not required.

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