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Review article

Application of CRISPR-based Technology in Infectious Diseases: A Review on its Potential Therapeutic and Diagnostic Aspects

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Abstract: CRISPR-based technology is an interesting gene-editing technology that has created a remarkable position in the world of biology. The ability of CRISPR to detect a specific pathogen in the living cell, destroying that pathogen by on-target cleavage, is the main reason for its popularity. Due to the specificity associated with this system, its low-cost availability and high efficacy, it is a vital landmark in the biomedical field. Interestingly, it has been applied in the treatment of several viral infections like HIV, COVID-19, some bacterial infections, and fungal infections. The treatment includes the use of molecular scissors to cleave the DNA at a specific location of mutation. The removal of the mutated part provides outstanding results. Production of an enormous number of CRISPR-based therapeutics has gained attention because of their high effectiveness. But more research and clinical trials still need to be done to explore the CRISPR-Cas9 system and enhance its effectiveness. From the time of its discovery till now, almost 6 types and 22 subtypes of CRISPR systems have been discovered. Moreover, CRISPR diagnostic tools like SHERLOCK are used, and diagnostic tests are performed with the help of simple reagents. Many preclinical trials and clinical trials are going on to enhance its features. CRISPR systems have a broad range of applicability, and in the near future, various therapies and diagnostic tools will be discovered.

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Introduction

Over the last few years, several debates were going on regarding genetic manipulation and its consequences in the biomedical field. In 1987, Yoshizumi Ishino and his colleagues from Osaka University, Japan discovered the unusual repetition of the DNA sequences. Back in the 80s, all the processes including the sequencing of DNA were very time-consuming due to the lack of equipment. But he managed to complete the sequencing of *Escherichia coli* and observed 29 pairs of repeated sequences (Ishino et al., 1987). But his work did not get much recognition due to a lack of information. After that in 1990, a researcher named Francisco Mojica, a Microbiologist from Spain, started his experiments on archaea a single–celled organism called Haloferax mediterranei and observed the same repeated sequences (Mojica et al., 2000). The occurrence of the repeated sequences in two different kinds of organisms made him think about some kind of relativity. After many confusions and theories, he studied deeply about the sections between the sequences and found out

that these repeated sequences and the sections between them are associated with the bacterial immune system. In 2005, the enzyme specifically used for CRISPR was described for the very first time. After the discovery of Cas9, two different RNA molecules were found to be a part of this complex. CRISPR-associated RNA (crRNA) in 2007, while in 2011, trans-activating RNA (tracrRNA) were discovered (Ibrahim et al., 2019). In 2020, a Noble Prize was awarded to Emmanuelle Charpentier and Jennifer A. Doudna for their work on CRISPR/Cas9 in 2012 (Farhud and Zarif-Yeganeh, 2020).

CRISPR is defined as the technology that involves the editing of a gene according to the requirement. It is a sort of genetic manipulation that has been created to treat several genetic diseases as well as viral, bacterial and fungal infections (Hoffmann et al., 2020). It is abbreviated as Clustered Regularly Interspaced Short Palindromic Repeats of genetic information (Yosef et al., 2012). CRISPR is a tool or technique that has been developed to have a certain understanding of the generics of all kinds of living things. But for now, it has been observed mainly in prokaryotes like bacteria and archaea. Moreover, CRISPR consist of repeated sequences those are short in length. The CRISPR technology function is associated with many other important components (Lu et al., 2021). The following 4 components help it to become more accurate and precise in action;

1) Cas9: It is an enzyme called a CRISPR-associated protein 9. In other words, it is also known as molecular scissors usually used to cut DNA at a specific location with the aid of gRNA. The Cas9 enzyme cuts DNA at specific target and then abnormal genes are disabled. This is the best-known use of CRISPR. The process is called as gene (Liu et al., 2020).

2) Ribonucleic Acid (RNA): It is mainly involved in the processes of transcription, transportation and for determining DNA instructions.

3) Deoxyribonucleic Acid (DNA): The most important component for the genetic manipulation is DNA. It is a storage space containing information for the existence of life.

4) Guide Ribonucleic Acid (gRNA): As indicated by name, it guides the Cas9 enzyme through the cutting of DNA. gRNA is responsible for the proper location and cutting of DNA fragment (Mei et al., 2016).

Depending upon the conditions and requirements of the genetic changes, CRISPR/Cas9 is divided into three different categories of genetic edits, described as;

a) Disrupt: In disruption, a single cleavage is made in the DNA molecule with the help of gRNA. This can result in either addition or removal of DNA base pairs. This ultimately results in gene inactivation.

b) Delete: The elimination or deletion of large DNA fragment is done with the help of two gRNAs. The deletion is made on both sides, after which the separated parts united deleting the interceding sequence.

c) Insert: CRISPR/Cas9 is used for the insertion of the new gene by a process called as homology directed repair (Sander and Joung, 2014).

Table 1. The three categories of genomic changes/edits in the DNA sequence and their applications.

Genomic Edits	Applications
Disruption	Disruption of function of specific gene resulting in addition or removal of base pairs
Deletion	Elimination DNA sequence and joining the two sequences
Insertion	Insertion of a new gene at a specific location to modify function

Biology of CRISPR and its characteristics

The biology of CRISPR-based systems involves great mechanisms and possess remarkable characteristics. The locus of CRISPR consist of some repeat sequences having length of about 20-40 base pairs which are separated by approximately 20-60 base pair sequences (Ishino et al., 2018; Yang et al., 2020). In the past, it was also thought to be involved in the DNA repairing. Some sequences are present in the CRISPR locus coding for the Cas9 proteins (Barrangou et al., 2007). The Cas9 protein is associated with several kinds of functions and is considered an important genetic protein used for the CRISPR technology. Such activities include polymerase,

helicase and nuclease type of activities hose is involved in nucleic acids genetic manipulation.

When the virus enters into the cells or plasmids, the Cas9 proteins of CRISPR starts its process of degrading the virus genome and resultantly, the CRISPR array will be developed. This is the process which proves the involvement of CRISPR-Cas9 technology in the immune response of the prokaryotes (Makarova et al., 2006). But if this system is not present in the bacterial host, then CRISPR RNA (crRNA) is generated by the transcription of the sequences. Thus, crRNA along with Cas9 proteins produces a complex called as interfering complex. Subsequently, crRNA guides the interfering complex to form a strong hydrogen bond between crRNA and DNA of the virus that in result, destroys the foreign DNA incorporation. Hence, infection causing pathogens are killed and infection disappears. Actually, this whole procedure shows that the CRISPR-based systems are the immune system of bacteria and spacers present in the CRISPR array also represents the old infections history of a person (Ishino et al., 2018; Koonin et al., 2017).

Categorization of CRISPR-Cas system

The current CRISPR-based systems are categorized into 6 types and 33 subtypes as well as 2 classes, depending on their use and specificity.

1) The first class of this system comprise of the effectors having several Cas proteins (the proteins those helps in binding of the proteins) and accounts for nearly 90%. Moreover, this class proteins plays an important role in targeting DNA and RNA (Makarova et al., 2017). Type I, III & IV are included in first class. Among these, I and III occurs commonly in archaea causing DNA cleavage (Hidalgo-Cantabrana and Barrangou, 2020) while the type IV lacks adaptive structures and nucleic acid effector enzymes, that plays an important role in regulation by targeting foreign plasmids or pathogens (Pinilla-Redondo et al., 2020).

2) The second one includes a single protein having multiple domains (Makarova et al., 2015). This class consists of II, IV & VI types, in addition to, three of the essential effector proteins called Cas9, C2c1 & Cpf1 (Shmakov et al., 2015). The genome sequencing has found a very effective structure from CRISPR system. This is the protein effect structures those are generated from the genetic elements having mobility.

Table 2. CRISPR-Cas Systems.

Class	Type	Target	Effector
1	I	dsDNA	Cascade
1	III	ssRNA	Cascade
1	IV	dsDNA	Cascade
2	II	dsDNA	SpCas9
2	II	dsDNA/ssRNA	FnCas9
2	II	dsDNA	NmCas9
2	V	dsDNA	Cas12a
2	V	dsDNA	Cas12b
2	V	dsDNA	Cas12c
2	VI	ssRNA	Cas13a
2	VI	ssRNA	Cas13b
2	VI	ssRNA	Cas13c

In the recent times, the applications and characteristics of CRISPR-Cas technology has been increased due to the advances in this technology systems. The researchers are doing researches and experiments based on the CRISPR system (Makarova et al., 2020).

CRISPR-based therapies in the treatment of viral infections

The viral infections involve the viruses encapsulated with viral RNA or DNA that attacks intracellular processed of the living cells and start their own replication. This makes it obvious for CRISPR-based therapies to target the viral dependence of viruses on the cellular proteins.

The Viral infections are categorized into three different groups based on the nature of infection:

1) Chronic Infection: In chronic infection, the virus replicates at a very slow speed leading to unattended chronic damage. It includes HIV and HBV-1 infections, both causing numerous deaths (Schweitzer et al., 2015; Virgin et al., 2009).

2) Latent Infection: Some viruses enter a dormant state and remains for months and years. It includes many pathogens like Human herpesvirus 6 (HHV-6), polyomavirus, parvovirus and many more (Lieberman, 2016; Traylen et al., 2011).

3) Lytic Infection: In lytic infection, the virus divides and produces viral progeny packed in protein capsids. The cells burst by lysis and viruses are released destroying the host cell completely. Thus, CRISPR is used as a genome-editing tool to treat the infections. However, some challenges considering its use needs to be better understood (Traylen et al., 2011).

For this purpose, CRISPR-based therapies should be precise enough to target the viruses and stopping them from further replication. Many puzzles are still there to be overcome but recent studies have shown considerable progress in the treatment of chronic infections. Research was done in which CRISPR system was used to cure HBV in mouse model of HBV and human hepatocyte cells (Ramanan et al., 2015). Inspiringly, after continuous implementation of CRISPR system in mouse for 4 weeks, off-target cleavage problems were not detected. To avoid these off-target cleavage events, modified base editing by Cas9 proteins were used to target HBV. During this process, nucleotide modifications were induced that proves to be effective in reducing off-target effects (Yang et al., 2015).

The acquired immunodeficiency syndrome/human immunodeficiency virus (AIDS/HIV) is still considered the worst global health issue that has claimed millions or billions of precious human lives. Several kinds of processes, drugs and treatments have been developed for its treatment. Almost 25 anti-viral drugs produced have resulted in decrease in the mortality rate. But all the available treatments are not that much effective or requires life depending on the pills/drugs. The CRISPR technology has proved to be most effective in fight against the HIV virus. The following are different ways of treating AIDS using the technology.

1) Cutting of the viral DNA inserted into DNA of immune cells by HIV virus.

2) Natural resistance to HIV due to the presence of CCR5 gene (Lebbink et al., 2017).

Some individuals have natural mutation in the CCR5 gene that is resistant to HIV virus. The virus, basically binds to this protein and infects the cells ultimately causing AIDS (Hirakawa et al., 2020). But the mutation develops a natural resistance in the human by altering the structure of the protein. In 2018, several in-human trials were also done in China. In this trial, this specific protein was edited and mutated to develop natural resistance in embryos against HIV. But this caused a lot of controversies showing that the babies produced by CRISPR technology could be dangerous and may result in the early death of humans (Lee and Kim, 2018). Thus, more studies and researches are needed before performing it in humans.

Another human DNA virus is Human papillomavirus (HPV) that causes different types of cancers. The genes responsible for HPV are E6 and E7. Thus, CRISPR-based techniques have been implied to target these key genes and treat the HPV virus that is very dangerous. CRISPR/Cas9 is considered to be a potential therapeutic agent for such diseases. Several types of research have shown that destruction of E6 and E7 genes lead to apoptosis of disease cells and stops the growth of the virus. In addition, many other human viruses are also under research and these viruses have been targeted by CRISPR systems. Such human DNA viruses include JC polyomavirus (Chou et al., 2016), human cytomegalovirus (CMV) (van Diemen et al., 2016), herpes simplex virus-1 (HSV-1) (van Diemen et al., 2016), and human papillomavirus (HPV) (Kennedy et al., 2014). However, some human viruses having RNA genomes include coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2), and flaviviruses (Zika virus, West Nile virus, and dengue).

Table 3. CRISPR-based potential targeted therapeutics.

CRISPR System	Organism	Targeted gene and findings
Cas9	HIV-1	LTR, pol, gag Elimination of HIV-1 from lungs, spleen, and heart from humanized mice by AAV vector
Cas9	HIV-1	LTR U3 Prevention of HIV-1 infection in which cells and inactivation of genes expression in T-cells
Cas9	HIV-1	LTR Removal of HIV-1 from human peripheral blood cells in humanized mice by lentivirus vector
Cas9	HPV	E6, E7 Targeting these two genes by lentivirus vector destroy HPV transformed cells

In pigs, the CRISPR-Cas9 system has been used to eliminate the porcine endogenous retroviruses (PERVs). Such viruses are vital hurdles in xenotransplantation. CRISPR-based therapies were used to target the conserved PERV polymerase gene. This reduces PERV viral infections (Niu et al., 2017; Yang et al., 2015). COVID likewise called COVID-19, is principally a respiratory sickness that was first observed in December 2019 in Wuhan Province, China (Ahmed et al., 2020; Yusof et al., 2021). Intense respiratory condition is brought about by the COVID (SARS-CoV-2). COVID-19 has turned into a worldwide pandemic (Ali et al., 2021; Anis et al., 2021). Transmembrane proteases, serine 2, show up essentially on type 2 sex cells, which help the S-protein to attack the cells. The pandemic of COVID-19 has created a havoc in the field of biomedicine (Ahmed et al., 2022a; Ahmed et al., 2022b; Kalil et al., 2021). Many researches and clinical trials are going on to develop a strong immunity against this pathogen (Naveed et al., 2022). For this purpose, CRISPR technology has made a great impact. It is thought that such gene-editing tools could help human beings to survive for a longer period of time. Screening test for COVID have been done by the CRISPR.

The screening test for COVID-19 patients are going on but for such large number of patients, it could not be performed solely by the infrared thermography. Thus, CRISPR-diagnostic tools DETECTR and SHERLOCK have been used. The DETECTR system has been commonly used to detect SARS-CoV-2 and is specifically used in the detection of N gene and E gene mutations that are very specific to this corona virus. If both genes are detected, then positive results are obtained and researchers are also trying to make improvements and make this tool a successful one eliminating any king of false positives (Broughton et al., 2020). However, the SHERLOCK system detects S gene and Orflab gene mutations resulting in the positive results (Joung et al., 2020). Thus, CRISPR system has developed the technology in many positive ways like diagnosis of pathogens and identification of the type of infection.

CRISPR therapies bacterial infections

In the recent times, CRISPR-Cas9 systems have been very much in talks. CRISPR strategies have been used to treat bacterial infections as well. Although CRISPR-based therapies have some challenges, but it has been applied to produce anti-bacterial therapeutics. These therapeutics are designed to be delivered through the bacteriophages cell wall (Ahmed et al., 2025a; Ahmed et al., 2025b).

The bacteriophages have been found to be of great importance for the production of therapeutics. Several skin infections and intra-abdominal (*Acinetobacter baumanii*) infections have been treated by the used of numerous naturally occurring bacteriophages (Schooley et al., 2017). For the treatment of such infections, the bacteriophages are selected from the already existing phage libraries. In the ongoing researches, it has been observed that the engineered bacteriophages must have narrow mechanism of action (killing of only the harmful and targeted bacteria) and off-target effects should have no existence. Such advancements in CRISPR-based therapies have brought a drastic revolution in biomedical field (Absar et al., 2025).

In vivo therapies indicate the mixed data about the efficacy of CRISPR therapies. It has found effective against the target bacteria (Park et al., 2017). The in-vivo applications of CRISPR are its delivery to the damaged or diseased cells or organs. After entering the damaged area, it corrects the mutations of that area. Similarly, the treatment of soft tissue infections in animals was efficacious but in rat model, the Staphylococcus aureus osteomyelitis cure was not successful. Therefore, additional modifications and advancements in CRISPR/Cas9 systems need to be done to enhance the safety results minimizing the negative effects (Citorik et al., 2014; Cobb et al., 2019).

CRISPR therapies for fungal infections

Generally, the viral and bacterial infection of human are given much importance but fungal infections in humans also exist. Such fungal infections have caused 1.6 million death per year. The gene-editing technologies have been used to study about different fungal infections, their mechanism of actions, its resistance to drugs and production of several therapeutics (Bongomin et al., 2017; Samaranayake and Hanes, 2011). Although, CRISPR-based strategies have been used to develop new ways of combating with such infections but a lot of more research, experiments and processes needs to be done to provide better insight of fungal infections. This is because of the specificity of fungi and their unique properties including the reproductive cycles (Eltayeb et al., 2023).

In 2016, *Aspergillus fumigatis* pathogen was used to develop microhomology-mediated end-joining (MMEJ) using CRISPR mutagenesis systems. Moreover, the base editing is also introduced as an impressive tool for fungi genome editing (Zhang et al., 2016). Somehow, CRISPR system is used to produce gene drive in Candida albicans. Currently, genome-editing tools are used in fungi to do researches, but it is also thought to produce effective therapeutics for treating the fungal infections, in future (Shapiro et al., 2018).

CRISPR-Cas9 system for non-infectious diseases

As it is quite obvious, that CRISPR-based systems have been used as the important and efficient tool for the diagnosis of infectious diseases but recently its uses have been expanded. Nowadays, it has also been used in the diagnosis of the cancer mediators and other oncogenes. It is applied in the research labs for detection of various types of cancer. Until now, CRISPR-Cas9 technology has been improvised to observe and research about the mechanisms of genes in almost all types of cancer (Tian et al., 2019).

In addition, this technology has also been used for processes like identification of genes vulnerable to cancer drugs, cancer drivers' cancer research and diagnostics etc. Many researches are also going on to discover new and distinct ways for the cure of the disease. In China, the first human trial using CRISPR technology for the treatment of cancer is in the limelight. In this process, immune T-cells are extracted from a person suffering from last-stage lung cancer. The main purpose was to remove gene encoding protein PD-1 (Programmed cell Death protein 1, located on the surface of immune cells), in order to avoid binding of tumor cells to that protein. Some tumor cells bind to that protein leading to the blockage of immune response opposed to cancer. Some of the cancer drugs actually bind to PD-1 protein. CRISPR technology has been used to produce more effective ways to treat different types of cancer. This technology is advancing in the biomedical field and surely has the potential to transform different cancer therapies.

Moreover, the use of different types of genetic diagnostic tools for the determination of such sensitive gene could be critical for prevention of cancer. For this purpose, SHERLOCK involving Cas13 is developed successfully (Khambhati et al., 2019; Tian et al., 2019).

CRISPR-based diagnostics tools

CRISPR-based therapies have created very strong impact in the world as a useful gene-editing tools, therefore, these are so much in demand these days. For this purpose, many companies have taken an initiative to develop kits for CRISPR utilization and make them commercially available. Among the most popular kits are SHERLOCK (Specific Highly Sensitive Enzyme Report Unlocking) and DETECTR DNA endonuclease-targeted CRISPR trans Reporter) systems (Chen et al., 2018; Gootenberg et al., 2017). The SHERLOCK system has been made very time-efficient and easily available by the researchers. While, DETECTR diagnostic kits are functionable for wide variety of bacterial and viral infections. Both of these are very sensitive in nature and

detects only single viral copy. DETECTR and SHERLOCK systems have also been used as diagnostics tools for SARS-CoV-2. These methods can detect the SARS-CoV-2 in less than an hour and is very reasonable.

In 2018, a CRISPR-based diagnostic tool called SHERLOCK (Specific Highly Sensitive Enzyme Report Unlocking) was developed. Basically, thus technology involves the Cas13 enzyme. It detects the DNA and RNA molecules by isothermal amplification with Cas13 enzyme. This technology is also used for the cure of infectious diseases like Dengue or Zika virus (Gootenberg et al., 2018). The DNA endonuclease-targeted CRISPR trans reporter (DETECTR), functions by combining the activation of the Cas12a ssDNase and isothermal augmentation. This tool has a very low sensitivity for the detection of DNA. Although, this technology could be used in the detection of human papillomavirus (HPV) (Chen et al., 2018).

Table 4. CRISPR diagnostic tools characteristics.

Methods	Type of System	Target DNA/ RNA	Targeted pathogen	Detection form
SHERLOCK	Type VI	DNA/ RNA	Bacteria/ Virus	Fluorescence
DETECTR	Type V	DNA	HPV 16,18	Fluorescence

Moreover, these two methods were also developed for Dengue virus, Ebola virus and Zika virus. The enzymes work best at 37°C, the enzymatic reactions are fast, therefore, both of these methods are used as tools of diagnosis for such viruses (Chertow, 2018; Myhrvold et al., 2018). One of the vital diagnostic tools is the Cas9 systems. This system has been in use for treating several viral and bacterial infections. The diagnosis of such infections is carried out in labs and is also offered by companies like Sigma-Aldrich. Researches are focusing more on developing effective and efficient diagnostic tools in the future.

CRISPR-based system in human trials

The clinical trials for the detection of type of infection, pathogens and their toxicity are essential. The trials are performed in laboratories those could either be in vivo or ex vivo. Some ex vivo CRISPR experiments have been performed (Xu et al., 2019). In ex vivo therapy, cells are extracted from patients' body, modifications are made and then modified cells are returned back to the body of the patient. Similarly, in vivo CRISPR therapy has been performed for the time in early 2020 (Binnie et al., 2021). In the first trial, patients suffering from congenital retina problems were given the dose of CRISPR therapies. The purpose of this was the modification of the single mutated base. The results of this trial are still not obtained. A huge progress has been observed in the field of CRISPR-based therapeutics in case of clinical trials. As the CRISPR clinical trials will start showing powerful results in the gene-editing field, it will become one of the greatest discoveries of all times.

Vaccines using CRISPR

The vaccination procedure involves the production of the antibodies after the attack of a viral or bacterial pathogen. The targeted T-cell or antibody responses are generated. The amount of the antibodies produced by the body of individuals possess a significant variability and is host-specific as well (Newport et al., 2004). Above all, there exist some viruses those are proved to be very challenging for vaccine production. Like the HIV virus, that generated the antibodies in a very small number of individuals and causes problems. Recently, the CRISPR systems are gaining immense popularity in the vaccine development procedures. HIV vaccines are given much importance because of its specific variability. Using this technology, B-cells of humans have been modified in a way to develop HIV neutralizing antibodies specifically called as broadly neutralizing antibodies (bNAb) that can ultimately reduce or suppresses the HIV toxicity (Gilbert, 2012). Thus, it was the alternative strategy to modify the human B-cells for producing antibodies.

Researches and experiments have been going on and in the most recent observation, it has been discovered that homology-directed repair (HDR) could also be used for editing the B-cells from patients and produce broadly neutralizing antibodies. Such B cells were then introduced into the mice, resulting, in the production of number of antibodies to suggesting development of the immunity (Hartweger et al., 2019). This plan of action could be used to remove the individual variability associated with the production of the antibodies. But such traditional vaccines have proven to be less effective till now.

Limitations for CRISPR therapeutics development

Like all other discoveries, the discovery of CRISPR therapeutics also bring in some limitations of impediments as well. The main risks in the productions of such therapeutics are DNA and RNA modifications at off-target sites and on-target effects of DNA and RNA modifications. The off-target effects take place when the partial homology between the guide RNA and the off-target sequences of genes resulting in the random modifications (Kimberland et al., 2018; Zhang et al., 2015). Such kind of unpredictable modifications depends on the location of the sequences and the guide RNA structure as well. Altogether, an important way of identifying all of the off-target effects is the genome sequencing method (Kim et al., 2015). Moreover, several techniques have been operated to reduce such effects that includes proper selection of guide RNA sequence to reduce analogous structures, thus, enhancing the specificity (Cho et al., 2014; Fu et al., 2014). The specificity has also been improved by engineered Cas nucleases and the binding of two gRNAs of opposite DNA strands (Ran et al., 2013).

The on-target effects include all the random modifications occurring at the target site. Such modifications comprise of the insertion and deletion of the base pairs as well as the recombination events (Kosicki et al., 2018; Lee and Kim, 2018). The on-on-target effects are proved to be the most effective ones as they reduce the damage and efficiency of the targeted genes but less effective when the pathogenic sequences are the target sites. Some strategies and techniques applied to suppress the on-target effects includes the modification of Cas9 nuclease. This Cas9 nuclease modification is observed, increasing the homologous repair frequency and inhibition of the NHEJ following the NHEJ pathway inhibitors (Jamal et al., 2016).

CRISPR technology and future developments

The CRISPR technology has emerged as a phenomenon gene-editing tools with many exciting results. Considering the fact that CRISPR is a new term introduced in the biology world, researchers and scientists are trying numerous ways to expand and discover its uses and role in multitudinous fields (Hsu et al., 2014). The most prominent aspect of CRISPR is its remarkable role in the production of therapies to treat and cure severe diseases. Firstly, it was developed for genome editing but later its features were explored and its use for different purposes expanded like gene therapy, epigenetic research etc. Hence, with the passage of time more and more aspects of this technology will be added to the list. In fact, CRISPR could be used in homes, gardens animals and plants with the altered genome. In future, CRISPRed food will also be produced in bulk amount. The in-vivo applications of CRISPR includes its delivery to the damaged or diseased cells or organs. After entering the damaged area, it corrects the mutations of that area. It has been used in fingerprinting process to detect the evolution, thus, creating gene drives (Jackson et al., 2018).

In China, an attempt of changing the genome of children was made. But this attempt was strictly condemned by the Government as this was totally unethical. Some researchers still think that our children could be benefitted by this process. As discussed before, scientists have performed various experiments and clinical trials to develop immunity and therapies against the diseases but still there are some concerns regarding their improvisation in the human beings (Zhang et al., 2016).

Table 5. CRISPR-based therapeutic strategies in infectious diseases.

CRISPR approach	Purpose	Examples
Genome-editing of targeted viral pathogens	Study of virus's functions and factors	*Toxosplasma gondii* Hepatitis B virus
Genome-editing of targeted host cell	Study of regulatory functions of proteins of host	HIV
Genome screening of host cell	Determination of bacterial genes	*Bacillus subtilis*
Genome screening of viral pathogens	Determination of regulatory proteins of host	Hepatitis C virus West Nile fever virus
Smart antibiotics	Killing of pathogenic bacteria at specific sequences	*Staphylococcus aureus*

In the future, CRISPR technology is thought to be emerged as a promising tool in the biomedical field. Hopes are high that this technology will be advanced and new ways of targeting the specific genes and mutations will be introduced. The therapies with more efficacy and effectiveness will be developed.

Conclusion

The CRISPR/Cas9 system discovery was first observed in the bacteria which was considered to be of minor importance. But as soon as the genome-editing capability and the effectiveness of CRISPR was explored, it emerged as a landmark in the world of biology and biomedicine. The fast evolution of CRISPR technology is surely because of remarkable characteristics like high efficacy, less-time consuming, low cost, its simplicity and versatility. One of the best applications of CRISPR-Cas9 system is its potential of eliminating a specific virus by the targeting a specific location with the aid guide RNA (gRNA). Certain aspects of this technology have been observed which has a major impact in the treatment of various viral, fungal as well as bacterial infections. Several CRISPR-based diagnostics tools like SHERLOCK and DETECTR has also made it possible for the patients to detect and diagnose the certain infections at an early stage. Till now, huge advancements have been made in this technology and researchers and scientists are trying to overcome the challenges and confusions of CRISPR-Cas9 system. In the near future, CRISPR based therapies and drugs are going to take over the field of biomedicine because of the marvelous features of CRISPR.

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