ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 06 | June - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN SILICO ANALYSIS OF TARGET GENES AND DESIGNING OF sgRNA FOR CRISPR CONTROL OF CLINICAL METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Medical Microbiology

Preethi Chandran ICMR SRF, DOS in Microbiology, University of Mysore.
Dr. Shubha Gopal* Professor, DOS in Microbiology, University of Mysore. *Corresponding Author

ABSTRACT

Background: Methicillin-resistant Staphylococcus aureus (MRSA) infections pose considerable death and disability among individuals in both community and hospital-associated settings. This is substantial, as MRSA is often resistant to several types of antibiotics and is challenging to treat. Clustered regularly interspaced short palindromic repeats (CRISPR)- CRISPR - associated protein 9 (Cas9) gene editing system is one of the potential and promising strategies for controlling MRSA infections by specifically targeting conserved gene sequences. **Objective:** The study aims to identify the potential target gene sequence and design sgRNA for the CRISPR Cas9 system to intercept clinical Methicillin-resistant Staphylococcus aureus (MRSA). **Methodology:** Bioinformatic analysis of potential target genes such as accessory gene regulator (agrA), staphylococcal protein A (spa), and antibiotic methicillin resistance gene mecA was performed to identify the conserved region. Spacers for the target were designed using an online bioinformatic tool for the construction of sgRNA. **Result:** The conserved sequence of the genes agrA, spa, and mecA of MRSA was chosen as a target for the study. Using the in silico method CHOPCHOP, spacers for each target gene were designed for genome editing to construct CRISPR- based antimicrobial gene knockout modules. **Conclusion:** In this study, we explored the possible potential target genes agrA, spa, and mecA in MRSA and tools for the construction of the sgRNA for the development of CRISPR based antimicrobial module against MRSA.

KEYWORDS

Methicillin-resistant Staphylococcus aureus (MRSA), sgRNA, target gene, CRISPR Cas9.

INTRODUCTION:

Antimicrobial resistance (AMR) is considered to be one of the top 10 worldwide public health dangers to humans, according to WHO (WHO, 2021). AMR has a substantial negative impact on the economy. A protracted illness and extended hospitalization not only increase the risk of mortality and disability but also mandate the use of more expensive medications and place individuals financially at risk.

Staphylococcus aureus, one among the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) pathogens, is considered as one of the high priority pathogens for development of new antibiotics / therapeutics according to WHO (WHO, 2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from both hospital- and community-associated infections have been demonstrated to acquire resistance to multiple antimicrobial drugs. Hence, they pose a peril to global health. This highly alarming situation requires specific attention to develop alternative therapies (Garoy *et al.,* 2019).

Without efficient antimicrobials, the ability of contemporary medicine to treat infections, especially those that arise following major surgery and cancer treatment, would be more susceptible to failure (WHO, 2021).

The CRISPR-Cas9, a prokaryotic adaptive immune system, is one of the most promising novel gene editing method for managing antibiotic-resistant pathogens (Saha *et al*., 2023). Two elements make up engineered CRISPR systems: a guide RNA (gRNA) or single guide RNA (sgRNA) and a CRISPR-associated endonuclease (Cas9 protein) (fig.1).

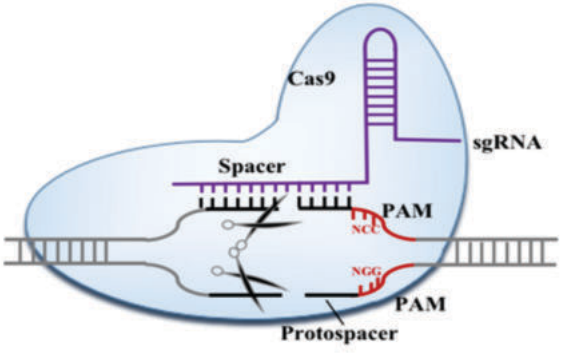

Fig.1: A schematic representation of the components of CRISPR-Cas9.

The sgRNA is a short synthetic RNA made up of the scaffold sequence essential to Cas9 binding and a user-specified spacer of around 20 nucleotides that specify the genomic target that is to be edited. When a single guide RNA (sgRNA) complex forms and binds to spacer sequence that is next to the protospacer adjacent motif (PAM) sequence at the 5' NGG close to the protospacer sequence, the CRISPR/Cas-9 framework allows specific target cleavage of genomic DNA that is guided by Cas9 nuclease. So, by merely changing the spacer for the target sequence in the gRNA, one may modify the genomic target of the Cas9 protein (Hiranniramol *et al.*, 2020).

By directly introducing sgRNA, the technique produces gene knockouts, which cause a DNA double-strand break (DSB) at the specified location. Efficiently analyzed and exploited *in silico* targets for sgRNA expression in MRSA could enable a CRISPR-Cas9 genome editing system for bacteria.

This study aims to identify the conserved sequence of potential target genes of MRSA and to design an effective spacer for the construction of sgRNA using bioinformatic tools. Such constructed sgRNA would enable the development of efficient CRISPR-based antimicrobial modules to tackle MRSA.

MATERIALS AND METHODOLOGY:

Bioinformatic Analysis Of Target Genes:

The genomic sequence of *Staphylococcus aureus* strain - MRSA ATCC 43300 was retrieved from genomic database. The sequence of genes - *agrA, mecA* and *spa* were selected as reference to study the *in-silico* approach for the designing of sgRNA.

sgRNA- spacer designing tool:

There are various *in silico* tools now available to assist with sgRNA selection based on on- and off-target activity considerations. CHOPCHOP is one among the most frequently and efficiently used online tool (Karlapudi et al., 2018; Hwang et al., 2021).

CHOPCHOP:

As a query target, the *agrA*, *spa,* and *mecA* genes were analyzed using online tools like CHOPCHOP in order to select a potential spacer target sequence (Labun *et al*., 2019).

The CHOPCHOP tool (https://chopchop.cbu.uib.no) is most commonly used for the analysis of gRNA sequences and off-target numbers. The CHOPCHOP web server is designed and most broadly utilized for CRISPR-based genome modifications. CHOPCHOP initially requires a nucleotide sequence of a selected gene of interest. We can specifically target the conserved sequences. The CHOPCHOP software automatically analyzes the query sequence and enlists all possible 20bp sequences identified immediately after the PAM

sequence (5'-NGG), providing gRNA scores from best to lowest (Labun *et al*., 2019).

RESULTS:

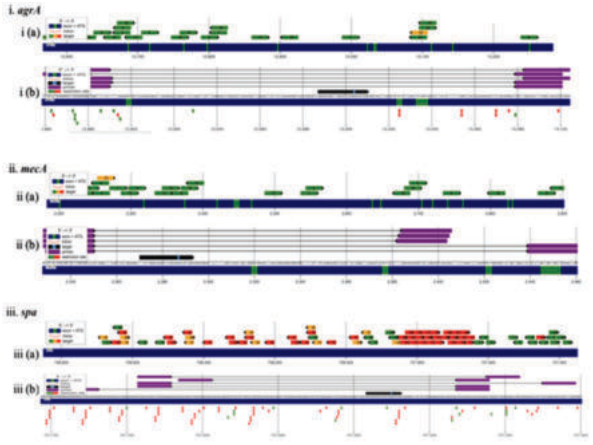

Fig.2: Representation of CHOPCHOP output

Bioinformatic Analysis Of Target Gene For Conserved Region:

The potential target genes (*agrA*, *mecA* and *spa*) were retrieved from NCBI. Obtained target gene nucleotide sequence in fasta format from NCBI search was submitted to CHOPCHOP. The software generates identified 20 mer and PAM sequences in the query gene sequence and determined the upstream starting point, followed by specifying the short spacer sequence for single guide RNA. The software also identifies the oligonucleotides required for the PCR assembly of the sgRNA.

Output results represented in Fig. 2 illustrate quality of spacer for sgRNA visualized in colours.

Results:

illustrated in Cas9 knockout mode with default parameters. The results are displayed across the query genes (i) *agrA*; (ii) *mecA* and (iii) *spa* ; (a) sgRNA target sites within the gene, green (good), yellow (better) colors indicated according to ranking (b) After selecting a specific target spacer sequence result displays the predicted cut site in red, primer options. Different colors indicate the quality of spacer for sgRNA, green indicates good quality

Table 1 provides information regarding target sequence GC content (%), so that the best sequences are selected between 40 and 80%. Primer details to the target site are provided in Table 2.

Table 1: The interpretation of CHOPCHOP result providing the list of possible target sites, GC% and efficiency with the best target site as rank one.

agrA						
Rank	**Target sequence**	**Genomic location**	**Strand**	**GC content (%)**	**Self-complementarity**	**Efficiency (%)**
1	GAAACGATTGAGTTAAAACGTGG	S00000008_CO17:12988	+	35	0	74.56
2	ATTGTAAGCATGACCCAGTTGG	S00000008_C017:12790	+	45	0	63.05
3	ATAAGGATTATCAGTTGCGAGGG	S00000008_C017:12668	-	35	0	60.85
4	GCTAAAAATATGAATGACATAGG	S00000008_CO17:12706	+	25	0	60.22
5	CATAAGGATTATCAGTTGCGAGG	S00000008_C017:12669	-	40	0	59.98
mecA						
Rank	**Target sequence**	**Genomic location**	**Strand**	**GC content (%)**	**Self-complementarity**	**Efficiency (%)**
1	TTATGGACAAATCGCAAACGTGG	S00000008_C004:2271	+	40	1	64.72
2	TATAACATATAGCGATATCGAGG	500000008_C004:2225	+	30	1	64.40
3	TATAGCGATATCGAGGCCCGTGG	S00000008_C004:2232	+	55	1	63.35
4	ACAAAAGAAAAAGAGGCTCAAGG	S00000008_C004:2544	+	35	0	61.67
5	AATCTTCACGACTAAATCCACGG	S00000008_C004:2249	-	35	0	61.00
spa						
Rank	**Target sequence**	**Genomic location**	**Strand**	**GC content (%)**	**Self-complementarity**	**Efficiency (%)**
1	GATAAAAACATGATCAAACCTGG	MRSA Scaffold1:727521	-	30	0	60.54
2	GGTACATTACTTATATTGGTGG	MRSA_Scaffold1:728766	-	35	0	58.53
3	GGAGTACATTGTAAACCTGG	MRSA Scaffold1:727617	-	45	0	58.40
4	TCAACAACAAGTTCTTGACCAGG	MRSA_Scaffold1:727503	+	40	1	58.43
5	TTAAACGCTGATCAACGTAATGG	MRSA Scaffold1: 728664	-	35	0	56.62

Table 2: Details Of Oligonucleotides Or Primer Adjacent To PAM Sequence.

agrA							
Pair	**Left primer coordinates**	**Left primer**	**Left primer Tm**	**Right primer coordinates**	**Right primer**	**Right primer Tm**	**Product size**
1	S00000008_C017:12869-12891	AAGTTGCAGCGATGGATTTTAT	60.0	S00000008_C017:13080-13102	ACGGTTATCTAAATGGGCAATG	60.1	233
2	S00000008_C017:12870-12892	AGTTGCAGCGATGGATTTTATT	60.0	S00000008_C017:13080-13102	ACGGTTATCTAAATGGGCAATG	60.1	232
3	S00000008_C017:12870-12892	AGTTGCAGCGATGGATTTTATT	60.0	S00000008_C017:13084-13106	TTTGACGGTTATCTAAATGGGC	60.2	236
4	S00000008_C017:12860-12882	TTGTCTACAAAGTTGCAGCGAT	59.9	S00000008_C017:13080-13102	ACGGTTATCTAAATGGGCAATG	60.1	242
5	S00000008_C017:12869-12891	AAGTTGCAGCGATGGATTTTAT	60.0	S00000008_C017:13084-13106	TTTGACGGTTATCTAAATGGGC	60.2	237
mecA							
Pair	**Left primer coordinates**	**Left primer**	**Left primer Tm**	**Right primer coordinates**	**Right primer**	**Right primer Tm**	**Product size**
1	S00000008_C004:2230-2251	CATATAGCGATATCGAGGCCC	60.8	S00000008_C004:2440-2462	TCAGTTGCATCATCATCAGACA	60.3	232
2	S00000008_C004:2229-2251	ACATATAGCGATATCGAGGCCC	61.5	S00000008_C004:2440-2462	TCAGTTGCATCATCATCAGACA	60.3	233
3	S00000008_C004:2229-2251	ACATATAGCGATATCGAGGCCC	61.5	S00000008_C004:2383-2405	GTGACTTCGACACCTTTTTCAA	59.3	176
4	S00000008_C004:2229-2251	ACATATAGCGATATCGAGGCCC	61.5	S00000008_C004:2384-2406	TGTGACTTCGACACCTTTTTCA	60.7	177
5	S00000008_C004:2229-2251	ACATATAGCGATATCGAGGCCC	61.5	S00000008_C004:2385-2407	TTGTGACTTCGACACCTTTTTC	59.3	178

spa							
Pair	Left primer coordinates	Left primer	Left primer Tm	Right primer coordinates	Right primer	Right primer Tm	Product size
1	MRSA Scaffold1:727720-727742	GGCAACAAACCTGGTAAAGAAG	60.0	MRSA_Scaffold1:727461-727483	TGAGCTTTGTTAGCATCCAT	60.0	281
2	MRSA Scaffold1:727672-727694	GGCAACAAACCTGGTAAAGAAG	60.0	MRSA_Scaffold1:727461-727483	TGAGCTTTGTTAGCATCCAT	60.0	233
3	MRSA Scaffold1:727672-727694	GGCAACAAACCTGGTAAAGAAG	60.0	MRSA_Scaffold1:727404-727426	CCACCAAATACAGTTGTACCGA	59.8	290
4	MRSA Scaffold1:727645-727667	AACAAGCCTGTAAAGAAGACG	59.8	MRSA_Scaffold1:727461-727483	TGAGCTTTGTTAGCATCCAT	60.0	206
5	MRSA Scaffold1:727672-727694	GGCAACAAACCTGGTAAAGAAG	60.0	MRSA_Scaffold1:727441-727463	CACCAGTTTCTAATGCTTG	59.7	253

DISCUSSION:

Staphylococcus aureus, though a normal component of our skin flora, remains a prominent cause of infections in both community and hospital-associated facilities. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections increase the risk of death by 64% compared to drug-sensitive infections. MRSA is not only recognised as a nosocomial bacteria but also as the primary contributor to hospital- and community-acquired infections (HA-MRSA and CA-MRSA). Worldwide, the morbidity of CA-MRSA infections has increased. MRSA is on a high-priority list of the most adverse antibiotic-resistant bacteria that cause community- and hospital-acquired diseases, according to the World Health Organization (Tacconelli *et al*., 2018, WHO-2021).

The increasing worldwide proliferation of multi- and pan-resistant bacteria, commonly referred to as "super bugs," causes diseases that cannot be treated with current antimicrobial medications like antibiotics, which is highly alarming (Esposito and De Simone *et al*., 2017).

One potential strategy for controlling MRSA infections is to employ the CRISPR-Cas9 gene editing system to specifically target and eliminate MRSA. The CRISPR-based screenings rely greatly on the sgRNA's design. To guide the Cas9 or dCas9 proteins to genomic targets in a CRISPR system, a sgRNA carries a spacer sequence complementary to the targeted DNA sequence. Any DNA sequence of 20 nucleotides can serve as the genomic target as long as the sequence is distinct and conserved, and the target should be present right next to a protospacer adjacent motif (PAM) (Binnie *et al.,* 2021).

Also, it is crucial to analyze the potential target genes required to design an efficient spacer for sgRNA. The genome of MRSA was analyzed, and the sequence of three genes—the accessory gene regulator (*agr*) system that is responsible for regulating the expression of several genes that code for virulence factors; the antibiotic methicillin resistance gene (*mecA)*; and staphylococcal protein A (*spa*), an important virulence factor of *S. aureus*—was retrieved in fasta format from Genbank, NCBI (Gao et al., 2004; Tan et al., 2018). The retrieved gene sequence were analyzed for the spacer design. The target sequence, which should be 20 nucleotide gene sequence immediately upstream of a protospacer adjacent motif (PAM), and is distinct and conserved from the rest of the genome, are considered in the potential search of genomic targets. The CHOPCHOP tool has the most advanced features, with 10 - 20% of user utility functions which aid in primer designing for sgRNA (Karlapudi et al., 2018).

A clear lack of understanding regarding the application of sgRNA on-target design tools in genomes other than those of humans and mice is evident when taking into account the current research situation. Utilizing the various web tools mentioned will ultimately lead to more precise and effective gene editing. Molecular biologists may readily create several cloned cell lines using this gene knockout technique employing CRISPR technology, and they can modify Cas9 to produce desired traits. Due to its simplicity and specificity, CRISPR-Cas9 offers advantages for functional genomic research applications. A crucial step for the CRISPR system is provided by *in silico* sgRNA creation, which also enables CRISPR investigations to benefit from bioinformatics and computational methods.

The design of in silico sgRNAs that are highly effective on target and have fewer off-target effects will need to be improved. Personalized sgRNA design is a significant future direction for in silico sgRNA design because all of the currently available tools are made for general sgRNA creation without taking into account cell-type heterogeneity. In the future, developing error-prone, disease-free organisms and new medications and cures at the gene level will benefit from continued work to assess this technology with regard to prokaryotic and eukaryotic model systems.

Acknowledgement

Authors thank Indian Council of Medical Research for providing Senior Research Fellowship to Chandran Preethi (AMR/Fellowship/9/2019/ECD-II).

Disclosure Statement:

The authors declare no conflicts of interest.

REFERENCES:

1. Binnie A, Fernandes E, Almeida-Lousada H, de Mello RA, Castelo-Branco P. CRISPR-based strategies in infectious disease diagnosis and therapy. Infection. 2021 Jun;49(3):377-385. doi: 10.1007/s15010-020-01554-w. Epub 2021 Jan 3. PMID: 33393066; PMCID: PMC7779109.
2. Esposito S, De Simone G. Update on the main MDR pathogens: prevalence and treatment options. Infez Med. 2017;25(4):301–10.
3. Gao J, Stewart GC. Regulatory elements of the Staphylococcus aureus protein A (Spa) promoter. J Bacteriol. 2004 Jun;186(12):3738-48. doi: 10.1128/JB.186.12.3738-3748.2004. PMID: 15175287; PMCID: PMC419937
4. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, Kiflay R, Tesfu T. Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019 Feb 6;2019:8321834. doi: 10.1155/2019/8321834. PMID: 30881532; PMCID: PMC6381584.
5. Hiranniramol K, Chen Y, Liu W, Wang X. Generalizable sgRNA design for improved CRISPR/Cas9 editing efficiency. Bioinformatics. 2020 May 1;36(9):2684-2689. doi: 10.1093/bioinformatics/btaa041. PMID: 31971562; PMCID: PMC7203743.
6. Hwang, G.-H.; Song, B.; Bae, S. Current widely-used web-based tools for CRISPR nucleases, base editors, and prime editors. Gene Genome Ed. 2021, 1, 100004.
7. Karlapudi AP, T C V, Tammineedi J, Srirama K, Kanumuri L, Prabhakar Kodali V. In silico sgRNA tool design for CRISPR control of quorum sensing in Acinetobacter species. Genes Dis. 2018 Apr 11;5(2):123-129. doi: 10.1016/j.gendis.2018.03.004. PMID: 30258941; PMCID: PMC6146548.
8. Labun, K., Montague, T. G., Krause, M., Torres Cleuren, Y. N., Tjeldnes, H., & Valen, E. CHOPCHOP v3: expanding the CRISPR web toolbox beyond genome editing. Nucleic Acids Research (2019).
9. Saha U, Gondi R, Patil A, Saroj SD. CRISPR in Modulating Antibiotic Resistance of ESKAPE Pathogens. Mol Biotechnol. 2023 Jan;65(1):1-16. doi: 10.1007/s12033-022-00543-8. Epub 2022 Aug 8. PMID: 35939207.
10. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27.
11. Tan L, Li SR, Jiang B, Hu XM, Li S. Therapeutic Targeting of the Staphylococcus aureus Accessory Gene Regulator (agr) System. Front Microbiol. 2018 Jan 25;9:55. doi: 10.3389/fmicb.2018.00055. PMID: 29422887; PMCID: PMC5789755.
12. World Health Organization. 2021. WHO antimicrobial resistance. World Health Organization, Geneva, Switzerland: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.