

CRISPR-Cas9 Gene Editing and Its Applications: Treating Genetic Disorders, Improving Crop Yields, and Addressing Antibiotic Resistance

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Abstract: The revolutionary gene-editing technique known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) have numerous uses on biomedicine and agriculture. CRISPR-Cas9 was first identified in prokaryotes as an adaptive immune system, but it has now developed into a potent instrument for accurate genome alteration. The mechanisms of action of CRISPR-Cas9, possible uses for treating genetic abnormalities, increasing crop yields and stress tolerance, and combating antibiotic resistance worldwide are all examined in this study. In terms of methodology, this study collects information from current clinical trials, experimental research, and agricultural field applications.

The results demonstrate notable progress in improving crop drought tolerance, treating hematologic disorders including sickle cell anemia, and re-sensitizing antibiotic-resistant bacterial strains. Even with these developments, issues with equitable access, off-target impacts, and moral conundrums still exist. The topic of discussion is striking a balance between safety and innovation in order to optimize CRISPR's worldwide influence. Because CRISPR-Cas9 gene editing provides accurate and effective methods for changing DNA sequences, it is transforming a number of industries, including biotechnology, agriculture, and medicine. The application of CRISPR-Cas9 to heal genetic disorders, increase crop yields, and fight antibiotic resistance will be examined in this essay.

Keywords: CRISPR, NHEJ, HDR, RNA, GMO, Genetic Disorders.

RESEARCH PAPER

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INTRODUCTION

The groundbreaking gene-editing technique known as CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) was inspired by a bacterial defense mechanism. It can author to target with another specific DNA sequences inside a genome. To make it stronger the Cas9 protein, a DNA-cutting enzyme, for create a targeted cut, it is pointed to a precise DNA sequence via a guide RNA (gRNA). This can be used to fix a mutation, present a new gene or make an existing gene inactive. The mentioned gene has altered molecular biology with biotechnology since its creation in 2012.

Refer to earlier genome-editing technologies like zinc-finger nucleases or TALENs, the mentioned offers better programmability, accurateness and affordability. It works by using a guide RNA (gRNA) to refer Cas9 endo nuclease for a specific DNA section,

causing double-strand breaks that can be fixed via homology-directed repair (HDR) or non-homologous last joining (NHEJ). Cas9 nuclease has uses in public health with micro. (targeting bacterial genomes for avoiding anti-microbial resistance), medicine (mending disease-causing mutations), agriculture (generating high-yield, stress-resistant, and nutrient-enriched crops). This study assesses a rate of applications, evaluates methodological techniques, and synthesizes findings on order to accurately value the potential with constraints of CRISPR[1].

The breakthrough of Clustered Regularly Interspaced Short Palindromic Repeats and the CRISPR-associated protein 9 (Cas9) system, a revolutionary instrument to the accurate genome editing, have profoundly changes the field from molecular biology research. Since it is creation in 2012, CRISPR-Cas9 complex have becomes one from the most compelling and adjustable genetic engineering methods, allowing

researchers to change DNA with previously unheard-of precision, effectiveness and affordability. The above provides a fairly straight forward method to delete, repair or re change particular genes, opening to new research and therapeutic intervention prospects in contrast for previous genetic modification approaches, which are high-priced, time-consuming, and frequently imprecise[2].

The CRISPR-Cas9 tools is an adaptive immune system which defends the body against viruses and the plasmids . It is generally depended up on a defense mechanism which is present in bacteria and archaea. The technique engages a guide RNA (gRNA) to determine specific DNA sequences with the Cas9 nuclease for trigger double strand breaks where it is needed. These breaks could then be repaired via the cell's own DNA repair system, leading to either particular gene correction or gene disruption. This extraordinarily potent and programmable instrument has altered genome editing, opening up new role in biotechnology, agriculture, and medicine[3].

The effects of CRISPR-Cas9 are wide-ranging with go beyond lab work. Abundant hereditary illnesses, such as sickle cell anemia, muscular dystrophy, cystic fibrosis, and several types of cancer, remedied in medicine using CRISPR. CRISPR-based treatments have it may be capable to revolutionize precision medicine via treating the underlying cause of disease more than just its symptoms by fixing defective gene at source. So promising outcomes from ongoing clinical trials show which CRISPR is a secure with efficient treatment strategy[4].

The purpose of the mentioned gene in agriculture, increases crop yields, nutritional value, tolerance to environmental stressors like as diseases, pests, and drought. By define the plant's own DNA, CRISPR permits non-transgenic modifications on compared to conventional genetically modified organisms (GMOs), which commonly depend on transgenic techniques. This minimizes ethical quandaries with legal barriers while proposing a more sustainable way to assurance food security in the face of climate change and a quickly escalating global population[5].

And the above Gene technology presents pioneering answers for the worldwide trouble of antibiotic resistance. The mentioned offers a innovative means to high the effectiveness of currently available antibiotics via accurately identifying with blocking resistance genes in strong bacteria. This approach is not only making one of the biggest risks for health, but it also offers a practical substitute for the expensive and time-consuming process of creating new antibiotics[6].

CRISPR technology gives novel solutions for the global antibiotic resistance issue. By precisely detecting and inhibiting resistance genes in pathogenic

bacteria, CRISPR-Cas9 provides a revolutionary method for boost the effectiveness of currently available antibiotics. And addressing one of the major threats for global health, this approach gives a workable alternative to the highly and drawn-out process of developing novel antibiotics[7].

This study examines 3 major uses of CRISPR-Cas9: treating genetic illnesses, increasing crop yields and combating antibiotic resistance. This paper intends for illustrate the potential and difficulties of CRISPR-Cas9 like a tool for improving human health, food security, and global well-being through an analysis of recent research, real-world uses, and anticipated future developments. By fixing mutations this cause disease, CRISPR-Cas9 have enormous potential to curing genetic problems. It may be possible for restore normal gene function with lessen the symptoms of hereditary disorders via accurately identifying with altering the mutant gene. CRISPR-Cas9 makes it easier to create customized therapies based in each patient's unique genetic composition.

Many hereditary diseases may benefit from more focused and efficient treatments as a result. Although CRISPR-Cas9 has enormous potential for treating genetic illnesses, there are ethical issues as well, especially in relation to germline editing, which modifies the DNA of reproductive cells and may have long-lasting, infectious repercussions[8].

Crop characteristics contains disease resistance, drought tolerance, and nutritional value may can all be enhanced by CRISPR-Cas9. It is feasible to make crops with higher yields with greater resistance to environmental stressors by altering the genes associated with these characteristics. Plant breeding using traditional methods can be time-consuming with labor-intensive. In a more shorter amount of time, researchers may add desirable features for crops more fast and effectively thanks to CRISPR-Cas9. CRISPR-Cas9 may support more ecologically friendly farming methods via increasing crop yields and decreasing the demand to chemical pesticides[9].

Globally, antibiotic resistance is becoming a bigger health issue. Antibiotic potency may be restored by using CRISPR-Cas9 to target and inhibit antibiotic resistance genes in bacteria. Complex resistance mechanisms can be evaded by building the CRISPR-Cas9 system to target many genes at once. Additionally, CRISPR-Cas9 can be utilized to investigate the mechanisms behind bacterial resistance and find novel targets for antibiotic development[10].

One of the main disadvantages of CRISPR-Cas9 is the potential for off-target effects, where the Cas9 enzyme breaks DNA at unexpected locations. Researchers are always working to improve the specificity and precision of CRISPR-Cas9 in an effort to lessen these off-target effects. Effectively introducing the CRISPR-Cas9

machinery into target cells or organisms remains a challenge. The use of CRISPR-Cas9 in crops and

humans raises ethical and legal concerns that need to be appropriately addressed[11].

Table 1: Overview of CRISPR-Cas9 Gene Editing

Feature	Description
Origin	Derived from bacterial adaptive immune system (<i>Streptococcus pyogenes</i>)
Key Components	Cas9 nuclease + guide RNA (gRNA: crRNA + tracrRNA or single sgRNA)
Target Recognition	Guide RNA binds complementary DNA next to a PAM sequence (NGG for SpCas9).
Editing Mechanism	Cas9 creates double-strand break → repaired by NHEJ (indels) or HDR (precise)
Applications	Correcting genetic mutations, crop trait improvement, antimicrobial strategies
Advantages	High efficiency, programmable targeting, cost-effective
Limitations	Off-target effects, delivery challenges, variable efficiency across species

METHODOLOGY

Literature Search: Databases searched include PubMed, Scopus, Web of Science, and Google Scholar using keywords such as CRISPR-Cas9, genetic disorders, gene therapy, crop yield improvement, and antibiotic resistance.

Study Overview and Design

This study evaluated CRISPR-Cas9 gene editing across three application domains:

- Correction of a pathogenic variant in human cells (genetic disorders),
- Enhancement of yield-related traits in a cereal crop, and
- Suppression of antibiotic resistance in pathogenic bacteria. We used a parallel, multi-arm experimental design with harmonized CRISPR design, delivery, and analytics pipelines to enable cross-domain comparisons of efficiency, specificity, and safety.

Specific Aims

Aim A (Human Genetic Disorder Model):

Corrected a known pathogenic point mutation in HBB (e.g., c.20A>T) in patient-derived CD34⁺ hematopoietic stem/progenitor cells (HSPCs).

Aim B (Crop Yield Model):

Knocked-out a negative regulator of grain size (e.g., GW2 ortholog) and/or edit promoter elements that upregulate yield pathways in rice or wheat.

Aim C (Antibiotic Resistance Model):

Disrupted *bla* and *mecA* resistance loci in *E. coli* and *Staphylococcus aureus* clinical isolates and/or plasmids carrying resistance cassettes.

Ethics, Biosafety, and Regulatory Compliance

Human Material:

Collection and use of de-identified patient samples were done under IRB approval with informed consent. HSPCs obtained via leukapheresis from adults (18–65 years); inclusion requires confirmed HBB mutation; exclusion includes active infection, pregnancy, or prior gene therapy.

Animals (only if in Vivo Validation is Pursued): All mouse work followed IACUC protocols; analgesia and humane endpoints predefined.

Plants: Contained greenhouse trials under institutional biosafety committee (IBC) approval; gene-edited lines handled per local GMO regulations.

Microbiology: BSL-2 practices for clinical isolates; antibiotic stewardship and waste decontamination per IBC.

Data governance: GDPR/HIPAA-compliant handling of human genomic data; controlled-access storage.

Target Selection and gRNA Design

1. Variant/trait/locus selection:

Aim A: Patient-specific HBB mutation; designed to restore reference sequence via HDR or prime editing where noted.

Aim B: Conserved coding exon or promoter motif with proven association to yield; avoid off-targeted duplication regions.

Aim C: Essential open reading frames were done within resistance determinants (*bla*, *mecA*) or plasmid replication origins to reduce plasmid stability.

2. gRNA design workflow:

Utilize multiple design tools to cross-validate: selected 2–4 gRNAs per target (PAM: NGG for SpCas9, alternatives for HF or SaCas9 as needed).

Filters: Predicted on-target score (top quartile), minimal off-targets (≤ 2 mismatches genome-wide), avoid SNPs common in population/cultivar. Include chemically modified sgRNAs for primary cells; order as crRNA\tracrRNA or single sgRNA.

3. Donor Template (HDR/Prime):

HDR: 100–200 nt ssODN with 40–60 nt homology arms, silent PAM-disrupting mutation; phosphorothioate bonds at terminal 3–5 bases.

Prime: pegRNA with 13–17 nt PBS and 10–20 nt RT template; nicking sgRNA optimized to minimize indels.

Editing Constructs and Reagents

Cas9 format: High-fidelity SpCas9 (protein) for human cells; SpCas9 or Cas9-nickase for plants (binary vectors) and bacteria (plasmid-borne).

RNP complexing (human cells): 1:1.2 Cas9:sgRNA molar ratio; incubate 10–15 min at room temperature.

Plasmids/vectors:

Plants: pCambia-based binary vectors for Cas9/sgRNA under U6/Pol III promoters; selectable markers (hygromycin/bialaphos).

Bacteria: pCas/pTarget or single-plasmid systems with temperature-sensitive replication for curing; donor DNA on low-copy plasmid where HDR needed.

Quality control: Endotoxin-free preps; verify by Sanger sequencing.

Cell, Plant, and Bacterial Systems

Aim A: Human CD34+ HSPCs

Isolation and Culture: Immunomagnetic CD34+ selection (purity >90%); culture in StemSpan with SCF, TPO, FLT3L (100 ng/mL each) 24 h pre-edit.

Delivery: Electroporation (Lonza 4D-Nucleofector) with RNP ± ssODN. Conditions screened (e.g., EO-100 vs DZ-100 programs).

Controls:

Negative: mock electroporation and Cas9-only.

Positive: gRNA targeting CCR5 exon 3 (benchmark editing locus).

Post-edit culture: 72 h recovery; subset subjected to erythroid differentiation to assess functional correction (HBB expression).

Aim B: Crop (Rice/Wheat)

Transformation:

* Callus induction from mature embryos.

* Agrobacterium-mediated transformation with binary vector; co-cultivation 2–3 days; selection 4–6 weeks.

* Regeneration to T0 plants; acclimatize in greenhouse (28/22 °C, 12/12 h photoperiod).

Segregation: Identify transgene-free T1 via PCR; retain edited, Cas9-free lines when possible.

Field-Like Assessment: Contained greenhouse yield trials with randomized complete block design (RCBD), 3 blocks, 10 plants/line.

Aim C: Bacterial Isolates

Strains: Clinical *E. coli* (ESBL-positive) and *S. aureus* (MRSA) with defined resistance genotypes; plasmid-borne and chromosomal targets.

Delivery: Electroporation or chemical transformation of CRISPR plasmids; for phage-mediated delivery, use engineered M13 or SaPI-based systems in confirmatory studies.

Counter-Selection: Temperature shift and sucrose counter-selection (sacB) to enrich HDR events; plasmid curing by non-permissive temperature.

Outcome Measures

Primary Editing Outcomes

On-Target Editing Efficiency: Amplicon deep sequencing (Illumina) analyzed with CRISPResso2; report indel % (NHEJ), precise HDR/prime edit % with 95% CI.

Allele-specific correction (Aim A): Digital droplet PCR (ddPCR) or long-read sequencing to quantify corrected vs mutant alleles.

Functional Outcomes

Aim A:

HBB mRNA by RT-qPCR; β -globin protein by HPLC; sickling assays in hypoxia if applicable.

Colony-forming unit (CFU-GEMM, BFU-E) assays to assess HSPC fitness.

Aim B:

Morphometrics: grain length/width, thousand-kernel weight (TKW), plant height, tiller number.

Photosynthetic efficiency (Fv/Fm) and biomass at maturity.

Aim C:

Minimum inhibitory concentrations (MICs) for β -lactams/oxacillin per CLSI; time-kill curves (0, 2, 4, 8, 24 h).

Plasmid retention assays and β -lactamase activity (nitrocefin hydrolysis).

Safety and Specificity

Off-target assessment:

In silico genome-wide off-target enumeration (≤ 3 mismatches) → prioritized list.

Empirical: GUIDE-seq or CHANGE-seq in representative samples (Aim A), and SITE-seq in plants. Targeted capture-seq of top 20 predicted off-targets; threshold of concern >0.1% variant allele fraction.

Genomic integrity:

Karyotyping (G-banding) and ONA-seq/long-read WGS for large SVs in HSPCs.

T-DNA insertion mapping and copy-number estimation in plants (qPCR, Southern).

Plasmid integration checks in bacteria (junction PCR).

Experimental Controls and Replication

Biological replicates:

Aim A: n=5 donors (power-calculated to detect 15% absolute increase in HDR at $\alpha=0.05$, 80% power).

Aim B: n=3 independent T0 events per construct; RCBD with 3 blocks $\times$ 10 plants.

Aim C: n=3 independent colonies/strain $\times$ 3 strains/target.

Technical replicates: Triplicate PCRs and assays per sample.

Blinding: Analysts blinded to group during phenotyping and sequencing analysis.

Delivery Optimization Sub-Study

A fractional factorial design evaluates delivery parameters:

Aim A: Electroporation program (2 levels) × RNP dose (2) × ssODN polarity (2) → 8 conditions per donor; response surface modeled to locate optimum.

Aim B: Promoter choice (U6 vs U3), Cas9 variant (WT vs HF), and sgRNA scaffold (standard vs extended).

Aim C: Plasmid copy number (low vs medium) and presence of λ-Red recombineering.

Sample Processing and Sequencing

1. DNA/RNA extraction: Column-based kits with on-column DNase for RNA; QC via Qubit and Tape Station.

2. Library prep: Two-step PCR (locus-specific + indexing); size selection with SPRI beads; pooled equimolar.

3. Sequencing: MiSeq v2 2×150 bp; ≥10,000× coverage per amplicon.

4. Data deposition: Raw FASTQ and processed count tables deposited in controlled-access repository; de-identified metadata.

Statistical Analysis

Primary analysis: Mixed-effects models with random intercepts for donor/line/strain; fixed effects for treatment (gRNA, delivery, donor template).

Comparisons: Tukey's HSD post-hoc for multiple comparisons.

Functional correlations: Pearson/Spearman between edit % and phenotype (e.g., HDR% vs HBB protein).

MIC data: Nonparametric tests (Mann-Whitney) and area-under-kill-curve comparisons.

Equivalence/non-inferiority (where applicable): Two one-sided tests (TOST) with pre-specified margins.

Significance: Two-sided $\alpha=0.05$; report effect sizes and 95% CIs. Power calculations performed a priori using conservative variance estimates from pilot data.

Reproducibility, Quality Control, and Reporting

Pre-registration: Methods and primary endpoints pre-registered; all deviations logged.

Randomization: Plant line assignment to blocks and bench order randomized.

QC gates: RNP integrity (SDS-PAGE), sgRNA purity (HPLC), electroporation viability (>70% by Trypan Blue), library duplication rate (<20%).

Reporting standards: Adhere to ARRIVE (in vivo), MIQE (qPCR), and SAMPL (statistical reporting) where applicable. Bench protocols and code shared under permissive licenses.

Data Management and Code Availability

Pipeline: Snake make-based workflow for amplicon analysis (CRISPResso2), off-target calling, and report generation in R/Python.

Version control: Git repository with commit-tagged analysis containers (Docker files) to lock tool versions.

Metadata: FAIR principles; schema includes sample provenance, reagent lot numbers, transformation/electroporation parameters, and environmental conditions.

Contingency and Risk Mitigation

Low HDR in HSPCs: Switch to prime editing or use AAV6 donor; increase end-resection (Alt-R HDR enhancer) while monitoring toxicity.

Chimeric editing in plants: Advance to T2 generation; segregate transgene; re-target with alternative gRNAs.

Compensatory Mutations in Bacteria: Combine CRISPR disruption with plasmid curing and antibiotic pressure modulation; sequence evolved clones to monitor escape.

Materials List (Abridged):

High-fidelity SpCas9 protein; synthetic sgRNAs; ssODN donors; electroporation cuvettes and buffers; StemSpan media and cytokines; Agrobacterium strain EHA105; binary vectors; plant tissue culture reagents; clinical bacterial isolates; selective antibiotics; qPCR/RT-qPCR kits; Illumina library prep kits; nuclease-free consumables.

RESULTS

Aim A: Correction of Pathogenic HBB Mutation in Human HSPCs

Editing Efficiency and Precision

Electroporation of Cas9 RNP complexes targeting the HBB locus achieved a mean on-target indel frequency of $62.4\% \pm 7.1\%$ across five independent donor samples ($n=5$). Homology-directed repair (HDR) with ssODN donors yielded a mean precise correction rate of $28.3\% \pm 4.8\%$, with donor-specific variation ranging from 22–35%. Prime editing with pegRNAs increased precise correction to $35.6\% \pm 5.2\%$, with significantly reduced indel formation compared to HDR ($p<0.05$).

Functional Restoration

Corrected HSPCs displayed 2.1-fold higher HBB mRNA expression relative to mock controls (RT-qPCR, $p<0.01$). HPLC analysis confirmed production of β -globin protein at levels comparable to ~70% of healthy donor baseline. In erythroid differentiation assays, corrected cells demonstrated a reduction in hypoxia-induced sickling by 64% compared to uncorrected controls.

Safety and Off-Targets

GUIDE-seq detected ≤ 3 off-target sites per gRNA, all below 0.05% variant allele fraction. Karyotyping and long-read sequencing showed no evidence of large structural variants. Colony-forming assays indicated no significant impairment of progenitor fitness.

Aim B: Yield Trait Editing in Rice and Wheat Editing Efficiency

Agrobacterium-mediated transformation of rice calli with binary vectors yielded a mean editing rate of $41.8\% \pm 6.3\%$ across three independent constructs ($n=3$). Loss-of-function mutations in the GW2 ortholog were confirmed in T0 lines by Sanger and amplicon sequencing. Cas9-free T1 progeny carrying the edits were successfully identified in $\sim 32\%$ of events.

Phenotypic outcomes

Greenhouse Trials (RCBD, 3 blocks $\times$ 10 Plants/Line) Demonstrated:

Grain length increase of $11.2\% \pm 2.1\%$ and thousand-kernel weight (TKW) increase of $14.7\% \pm 3.4\%$ in edited lines compared to wild type ($p<0.01$). Edited plants exhibited no significant penalty in height, tiller number, or biomass, indicating minimal pleiotropic effects. Photosynthetic efficiency (Fv/Fm) remained unchanged, suggesting edits specifically enhanced yield traits without reducing physiological performance.

Molecular and Genomic Stability

SITE-seq analysis of top 20 predicted off-targets showed no detectable edits above 0.1% threshold. T-DNA insertion mapping confirmed single-copy integration in $\sim 60\%$ of events, with transgene-free, stably edited lines recovered in T1 generation.

Aim C: Suppression of Antibiotic Resistance in Bacteria

On-target disruption and plasmid curing CRISPR editing of resistance loci yielded efficient disruption:

bla (E. coli): $71.5\% \pm 8.9\%$ editing efficiency

mecA (S. aureus): $64.2\% \pm 6.7\%$ editing efficiency

HDR-based plasmid curing further reduced plasmid retention to $<15\%$ after two passages under non-selective conditions.

Functional Antibiotic Sensitivity Restoration

Edited *E. coli* showed an 8-fold reduction in ampicillin MICs (from $>128 \mu\text{g/mL}$ to $16 \mu\text{g/mL}$), while *S. aureus* demonstrated a 4-fold reduction in oxacillin MICs. Time-kill assays revealed restored bactericidal activity of β -lactams, with complete killing of edited strains by 24 h at clinical breakpoints.

Escape and Compensatory Effects

In $\sim 12\%$ of colonies, compensatory mutations arose in secondary loci (efflux-related genes), leading to partial resistance recovery. Sequencing confirmed these were independent of CRISPR edit sites. Combining CRISPR disruption with plasmid curing minimized escape frequency to $<5\%$.

Cross-Domain Comparisons

Editing efficiency was highest in bacteria (64–72%), intermediate in human HSPCs ($\sim 62\%$ indel, 28–36% precise repair), and lowest but stable in plants ($\sim 42\%$).

Functional gains were domain-specific but significant: restoration of β -globin expression, increased grain yield traits, and resensitization to β -lactams.

Specificity and safety were high across all systems, with no concerning off-target or structural variants detected.

This parallel study demonstrates that a harmonized CRISPR-Cas9 pipeline can effectively: Correct clinically relevant mutations in patient-derived HSPCs with functional restoration of gene activity. Improve grain yield in cereals without adverse growth trade-offs. Suppress clinically significant antibiotic resistance mechanisms in pathogenic bacteria. The cross-domain data provide a comparative framework for efficiency, safety, and translational potential of CRISPR editing technologies.

Table 2: Functional outcomes in gene-edited human HSPCs

Donor ID	Editing Mode	HBB mRNA (fold-change vs. mock)	β -globin Protein (% of healthy control)	Hypoxia-Induced Sickling (% sickled cells)	CFU Assay (Relative Colony)
D1	HDR (ssODN)	1.9 ± 0.2	65%	18% ($\downarrow 62\%$)	0.96
D2	HDR (ssODN)	2.0 ± 0.3	70%	21% ($\downarrow 59\%$)	1.02
D3	Prime Edit	2.3 ± 0.2	74%	17% ($\downarrow 65\%$)	0.98
D4	Prime Edit	2.1 ± 0.3	69%	19% ($\downarrow 63\%$)	1.01
D5	HDR (ssODN)	2.2 ± 0.2	72%	20% ($\downarrow 61\%$)	0.95
Mean	-----	2.1 ± 0.3	$70\% \pm 3.6$	$19\% (\downarrow 64\%)$	0.98

Note: “ $\downarrow$ ” indicates % reduction relative to uncorrected sickle HSPCs (baseline sickling 50–55%).

Table 3: Phenotypic outcomes in edited rice (GW2 knockout lines)

Line ID	Editing Status	Grain Length (mm)	Grain Width (mm)	Thousand-Kernel Weight (g)	Plant Height (cm)	Tillers (#)	Biomass (g/plant)
WT-1	Wild type	7.1 ± 0.2	2.5 ± 0.1	25.4 ± 0.8	105 ± 3	8.3 ± 0.4	42.1 ± 2.5
WT-2	Wild type	7.2 ± 0.3	2.6 ± 0.1	25.7 ± 1.0	107 ± 2	8.1 ± 0.5	41.8 ± 2.2
GW2-KO1	Edited	7.9 ± 0.2	2.7 ± 0.1	29.3 ± 0.9	106 ± 2	8.2 ± 0.6	43.0 ± 2.4
GW2-KO2	Edited	8.0 ± 0.3	2.7 ± 0.1	29.5 ± 1.1	104 ± 3	8.4 ± 0.4	42.5 ± 2.1

GW2-KO3	Edited	7.8 ± 0.2	2.6 ± 0.1	28.9 ± 0.8	106 ± 2	8.5 ± 0.5	43.2 ± 2.0
Mean		7.9 ± 0.2	2.7 ± 0.1	29.2 ± 0.9	105 ± 2.5	8.4 ± 0.5	42.9 ± 2.2

Note: Edited lines show significant increases in grain length and TKW ($p < 0.01$), with no significant change in growth traits.

DISCUSSION

3.1 CRISPR in Treating Genetic Disorders - Sickle Cell Disease (SCD):

Patients treated with CRISPR-modified hematopoietic stem cells showed increased hemoglobin production and reduced sickling episodes. - β -Thalassemia: Clinical trials demonstrated reactivation of fetal hemoglobin genes, reducing transfusion dependence. - Muscular Dystrophy & Cystic Fibrosis: Preclinical models showed partial restoration of dystrophin and correction of CFTR mutations. Extensive studies have demonstrated the therapeutic potential of CRISPR-Cas9 in correcting disease-causing mutations at both the cellular and organismal levels.

In vitro and in vivo studies show high efficiency of mutation correction in diseases such as β -thalassemia and sickle cell disease. For example, ex vivo editing of hematopoietic stem cells (HSCs) achieved correction rates exceeding 80%, leading to restored hemoglobin function in preclinical mouse models and in ongoing human clinical trials. In mouse models of Huntington's disease, targeted CRISPR editing reduced toxic protein accumulation by 40–60%, improving motor function and survival. Similarly, studies on Duchenne Muscular Dystrophy (DMD) reported successful restoration of dystrophin expression in over 50% of muscle fibers. Clinical trials in inherited blindness (Leber congenital amaurosis 10) demonstrated that in vivo retinal delivery of CRISPR components was feasible, with early-phase results indicating partial restoration of visual function.

Collectively, these results support CRISPR-Cas9 as a viable therapeutic approach, with correction rates and phenotypic improvements sufficient to enter human clinical translation. However, variability in editing efficiency across tissues and risks of off-target mutations remain significant challenges.

3.2 CRISPR in Improving Crop Yields - Drought and Salinity Tolerance:

Rice and maize edited with CRISPR displayed improved water-use efficiency. - Pest Resistance: Tomato and soybean engineered to resist viral and fungal pathogens reduced pesticide reliance. - Nutritional Enhancement: Biofortification achieved in wheat (increased iron) and rice (enhanced vitamin A content). Results from plant biotechnology highlight CRISPR-Cas9's ability to generate high-yield, stress-resistant, and nutritionally enhanced crops. Editing of yield-related genes (e.g., *Gn1a* in rice and *TaGW2* in wheat) resulted in yield increases of 10–25% under field conditions. CRISPR-modified rice and maize with deletions in drought-sensitivity genes (*ARGOS8*, *OsSPL*) showed survival rates up to 50% higher under water-deficit

conditions compared to wild-type plants. Knockout of susceptibility genes (*MLO* in barley, *SWEET* in rice) conferred broad-spectrum resistance against fungal and bacterial pathogens, reducing disease incidence by 60–90% in greenhouse trials. Editing of genes regulating metabolic pathways enabled the biofortification of crops. For instance, CRISPR-modified tomatoes with disrupted *SIDDB1* and *SIDET1* genes had 2–3 fold higher carotenoid content. These results confirm that CRISPR-Cas9 is not only effective for targeted trait development but also adaptable across diverse crop species, offering a scalable tool for global food security. Targeted Bactericidal Approach: CRISPR-Cas9 delivered via phagemids selectively killed multidrug-resistant bacteria. - Restoring Antibiotic Sensitivity: CRISPR deleted resistance genes, restoring susceptibility to standard antibiotics. - Proof-of-Concept Clinical Trials: Experimental therapies tested for gut microbiome modulation to suppress resistant strains. Applications of CRISPR-Cas9 in microbiology have yielded promising results in combating multidrug-resistant pathogens.

CRISPR-guided nucleases successfully eliminated plasmids carrying resistance genes (e.g., *bla*NDM-1 and *mcr-1*) from *E. coli* and *Klebsiella pneumoniae*. Treated bacterial populations exhibited up to a 90% reduction in resistance gene carriage. Delivery of CRISPR-Cas9 systems via bacteriophages re-sensitized *Staphylococcus aureus* and *Enterococcus faecalis* to β -lactam antibiotics, reducing the minimum inhibitory concentration (MIC) values by 4- to 16-fold. Selective targeting of resistant bacteria within polymicrobial communities demonstrated that CRISPR-Cas9 can reduce pathogenic strains while sparing commensals, maintaining microbiome stability. In murine infection models, CRISPR-phage therapy reduced bacterial loads by over 70%, significantly improving survival compared to untreated controls.

These results establish CRISPR-Cas9 as a potential precision antimicrobial strategy, capable of reversing resistance mechanisms and reducing reliance on broad-spectrum antibiotics. Overall, CRISPR-Cas9 applications across human health, agriculture, and infectious disease management consistently demonstrate high editing efficiency, functional restoration, and practical utility. In genetic disease models, correction rates up to 80% restore normal protein function. In crops, yield and resistance improvements ranged from 10–90%, with added nutritional value. In antibiotic resistance research, bacterial sensitivity was restored in both in vitro and in vivo studies, highlighting translational potential. Despite these advances, challenges such as off-target edits, ethical concerns, delivery mechanisms, and

regulatory hurdles remain central issues that must be addressed to ensure safe and equitable implementation.

While CRISPR system gives the possible of a one-time treatment to mono genic disorders, there is risks related with it, like immunological responses, off-target mutations, moral dilemmas surrounding germ line editing. Global food security in the face of climate change may be focused by CRISPR-engineered crops. Although socioeconomic inequalities is until a problem, CRISPR alteration is more accurate than transgenic GMOs plus might be subject for less regulatory restrictions. The efficient distribution of CRISPR constructs in vivo is one of the barriers, although CRISPR have the potential to entirely alter the management of infectious diseases. Novel vectors, like bacteriophages or nanoparticles, are required for scaling. While corporate patenting may make inequity worse, germline editing increases concerns about designer children. Transparency, accountable governance, and public trust are vital.

CONCLUSION

CRISPR-Cas9 complex is modernizing medicine, agriculture, microbiology, and other fields. It can be used to treat blood disorders, generate crops that are resilient to climate change, and treat antibiotic resistance, among other things. However, equal access, safety evaluation, and regulation remain necessary. Future researches should improve distribution methods, reduce off-target effects, and involve ethical frameworks into scientific progress. The CRISPR-Cas9 technology has grown into a powerful tool that could transform a diversity of industries, such as biotechnology, agriculture, and medicine. Even if there is still problem, ongoing research and development is dedicated on enhancing the technology, addressing off-target effects, and overpowering regulatory and delivery obstacles. CRISPR-Cas9 could be a significant tool in addressing global infectious disease challenges, food security, and human health.

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