Microbiome applications of CRISPR to the healthcare facility built environment: a systematic scoping review

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Abstract

Hospital-acquired infections (HAIs) remain a substantial challenge for health systems, driven by pathogens that persist in the healthcare-built environment through traits such as biofilm formation, adhesion, and environmental resilience. Modifying the built-environment microbiome to suppress or exclude these traits is a promising strategy to reduce HAI risk. CRISPR technologies enable selective targeting and modification of microbial DNA to alter community composition, suppress pathogen survival, and enhance beneficial traits. Yet, their applications in real-world healthcare settings remain limited, and evidence synthesis is limited. We conducted a systematic scoping review to identify CRISPR applications for microbiome engineering in healthcare built environments and to assess CRISPR-enabled modification of ESKAPEE pathogens relevant to such interventions. Searches across PubMed, Scopus, Embase, and Web of Science yielded no *in situ* CRISPR applications in healthcare facilities, so we broadened our search to CRISPR applications targeting ESKAPEE pathogens to modify traits relevant to healthcare built environments. A second search identified nine eligible laboratory studies. All engineered microorganisms were bacteria, predominantly *Escherichia coli*. CRISPR/Cas9 and CRISPR interference (CRISPRi) were used to modify genes associated with biofilm formation, adhesion, virulence, or regulatory pathways. Biofilm-related traits were most investigated, while pathogen persistence, horizontal gene transfer, and ecological impacts were rarely evaluated. All experiments were conducted at the single-colony or culture scale, with no environmental or pilot-scale testing. This review reveals a substantial gap between the conceptual potential and the demonstrated application of CRISPR-based microbiome engineering in healthcare settings, underscoring the need for ecologically informed models and standardized evaluation metrics to support safe translation into infection-prevention strategies.

Introduction

Hospital-acquired infections (HAIs) are a substantial challenge for health systems, with an estimated prevalence of 7-10% among hospitalized patients per year in high-income countries (World Health Organization, 2024). Multi-drug-resistant pathogens are also on the rise, creating new and emerging risks for health systems worldwide.

The healthcare-built environment, defined as man-made surfaces and fixtures permanently affixed to the healthcare facility (e.g., countertops), and non-invasive, non-living objects within the facility (Fry-Brumit *et al.*, 2026), plays an important role in the transmission of HAIs. Microbial communities persist on surfaces and are transmitted between patients and healthcare workers. ESKAPEE pathogens (*Enterococcus faecium*; *Staphylococcus aureus*; *Klebsiella pneumoniae*; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; *Enterobacter spp.*; *Escherichia coli*) are of particular concern due to their frequent detection in hospital settings, their ability to persist in the environment, and their high burden of antibiotic resistance [reference]. Traits such as biofilm formation, surface adhesion, environmental resilience, and antimicrobial resistance influence their persistence and transmission within healthcare settings. Modifying the built-environment microbiome to suppress or exclude these traits offers a promising approach to reduce HAI risk (Gottel *et al*., 2024).

Novel applications of microbiome engineering technologies, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology, may help reduce the burden of HAIs caused by ESKAPEE and other pathogens. CRISPR and its associated Cas proteins constitute a family of adaptive immune systems found in bacteria and archaea, which function to defend against invading bacteriophages (Barrangou *et al*., 2007; Horvath & Barrangou, 2010). The functional characterization of these systems revealed that they provide adaptive immunity through RNA-guided DNA cleavage (Garneau *et al*., 2010; Jinek *et al*., 2012). This inherent programmability—achieved simply by altering the guide RNA sequence—enabled precision in genetic manipulation, distinguishing CRISPR from prior technologies such as zinc-finger nucleases and TALENs (Gaj *et al*., 2013). At the single-nucleotide level, CRISPR enables precise correction of point mutations when paired with engineered repair templates (Jiang *et al*., 2013). CRISPR can also target multiple genomic loci simultaneously using a single Cas enzyme and multiple guide RNAs (Cong *et al*., 2013). The development of high-fidelity Cas variants, including eSpCas9(1.1), SpCas9-HF1, and HiFi Cas9, has further refined editing precision by reducing off-target effects while preserving on-target activity (Slaymaker *et al*., 2016; Kleinstiver *et al*., 2016; Vakulskas *et al.*, 2018). Beyond DNA editing, CRISPR systems have been adapted for precise RNA targeting. Cas13 enzymes provide programmable RNA knockdown capabilities with single-nucleotide specificity, enabling transient modulation of gene expression at the transcript level and expanding this precision engineering toolkit (Abudayyeh *et al*., 2017). Collectively, these features establish CRISPR as a powerful tool for precisely engineering ESKAPEE pathogens, offering new strategies to attenuate virulence, disrupt microbial functions, and reduce infection potential.

To deliver CRISPR to its intended targets, a variety of delivery modalities have been developed across the field (Lino *et al*., 2018). Viral vectors such as adeno-associated viruses (AAVs) offer high transduction efficiency but face cargo capacity constraints. (Ran *et al*., 2015). Non-viral approaches, including lipid nanoparticles (LNPs), provide transient Cas expression

with reduced immunogenicity (Jiang et al., 2017; Miller *et al*., 2017). Physical methods such as electroporation remain an option for *ex vivo* applications (Stadtmauer *et al*., 2020). For bacterial pathogen engineering, bacteriophages offer unique advantages. Phages naturally infect bacteria with high specificity, penetrate biofilms, and can be engineered to deliver CRISPR constructs directly into pathogenic bacteria *in situ* (Citorik *et al*., 2014; Bikard *et al*., 2014). This method has been successfully deployed to achieve sequence-specific killing of antibiotic-resistant bacteria, including *Staphylococcus aureus* and *Escherichia coli*, in animal models of infection (Yosef *et al*., 2015). Collectively, these delivery strategies position CRISPR as a promising platform for *in situ* engineering of ESKAPEE pathogens and precise manipulation of complex microbial communities.

CRISPR technology enables selective targeting and modification of microbial DNA, allowing researchers to alter community composition, suppress pathogen survival, and enhance beneficial traits. It offers a promising strategy for infection control by selectively reshaping microbial communities rather than broadly eliminating them, thereby enabling a next-generation, targeted approach to reducing healthcare-associated infections (HAIs) (Bikard *et al*, 2014; Citorik *et al*, 2014).

While CRISPR may be a promising solution for infection control in healthcare settings, there are few real-world applications to date, and most evidence comes from lab-based experiments rather than real-world settings.

To address this, we conducted a systematic scoping review to explore the following questions:

1. How has CRISPR been applied for microbiome engineering in healthcare built environments?
2. How has CRISPR been applied for genetic engineering of ESKAPEE pathogens to modify traits that are relevant to microbiome engineering in healthcare built environments, including field, *in vivo*, and benchtop studies?

Methods

Aims and scope

To address these questions, we conducted a two-phase systematic scoping review. The first phase explored research question 1 by examining *in situ* applications of CRISPR in healthcare facilities. However, we anticipated that there would be few relevant studies addressing research question 1 because CRISPR-based microbiome engineering in healthcare-built environments is a relatively new and highly specialized field. Therefore, we designed a second search strategy to explore research question 2, examining a broader body of literature from both healthcare and non-healthcare settings in which CRISPR has been used to genetically manipulate microbial traits relevant to microbiome engineering in healthcare-built environments. For the

second search, we considered CRISPR-enabled modifications to ESKAPEE pathogens to achieve the bacterial traits listed in Table 1.

For research question 1, we considered *in situ* applications of CRISPR technology for built environment microbiome engineering in healthcare facility settings, which we define as the man-made physical settings where healthcare delivery occurs—including hospitals, clinics, long-term care facilities, operating and patient rooms, plus their supporting infrastructure such as ventilation systems, plumbing, and high-contact surfaces. This understanding is consistent with definitions of the built environment in the literature, which include human-constructed structures and the indoor infrastructure that houses microbial ecosystems (National Academies of Sciences, Engineering, and Medicine, 2017; Gilbert & Stephens, 2018).

For research question 2, we considered three experimental contexts: (1) field tests conducted in simulated healthcare environments where microbiome engineering interventions are applied and assessed under simulated conditions; (2) *in vivo* tests conducted under controlled laboratory conditions using host or animal models to assess microbial interactions, genetic modifications, and the effectiveness of interventions within living organisms; and (3) benchtop work refers to small-scale, laboratory-based experimental setups (e.g., bioreactors, 96-well plates). Together, these categories span from controlled laboratory experimentation to applied, real-world testing, enabling evaluation of both feasibility and translational potential (Gilbert & Stephens, 2018; Sheth *et al*., 2016).

For research question 2, we considered modification of ESKAPEE pathogens, because these organisms are recognized as high-priority, multidrug-resistant pathogens of global concern (Tacconelli *et al*., 2018). Key microbial traits that influence the persistence, resistance, transmission, and virulence of ESKAPEE pathogens in healthcare environments are central targets for microbiome engineering and offer a promising avenue for reducing infection potential. Existing systematic reviews have evaluated the use of CRISPR technologies to modify antimicrobial resistance genes in pathogenic organisms (Souza *et al*., 2025; Okesanya *et al*., 2025). For this review, we examined the traits outlined in Table 1 as the most salient for microbiome engineering for infection prevention and control in healthcare built environments.

Table 1. CRISPR-enabled modifications of ESKAPEE pathogens relevant to the control of healthcare-acquired infections that were examined in this systematic scoping review

Bacterial trait	***Definition***	***Example indicators***
Biofilm formation and adhesion traits	Ability to form microbial biofilms on surfaces; ability to attach to a certain surface or cell.	Reduction in biofilm biomass or coverage; fewer biofilm-associated pathogens. Reduction in the ability to attach to a certain surface or cell.
Pathogen persistence	Ability of pathogenic bacteria to colonize in the healthcare environment, independent of mechanisms	Reduction of ESKAPEE pathogens.

	related to adhesion or biofilm formation.	
Horizontal gene transfer	The ability to transfer genetic material among bacteria.	Reduced rate of plasmid transfer or acquisition of new genes; decreased recovery/loss of modified gene function.
Pathogenicity and virulence traits	Bacterial pathogenic potential beyond the specific adhesion or biofilm-related virulence factors already described, including toxin production or other mechanisms that contribute to disease.	Decrease in toxin production or other virulence factors.
Enhancement of *in situ* CRISPR engineering in the built environment	Improvements in the efficiency, stability, or functional impact of CRISPR-based interventions directly within microbial communities in built environments.	Detection or increased abundance of CRISPR-edited strains; successful gene edits in target microbes; persistence or activity of engineered microbes *in situ*; reduction in unintended off-target effects.

Outcomes of interest

To assess research question 1 on CRISPR applied to healthcare-related bacteria or the healthcare microbiome, we examined changes to the composition or quantity of microbes in the built environment as the primary outcomes (Table 2).

Table 2. Measurable outcomes used to evaluate CRISPR applications in healthcare-related bacteria and the healthcare microbiome.

Effectiveness outcomes	***Definition***	***Example indicators***
Composition	Balance of pathogenic/beneficial microbes.	Relative abundance of pathogens/beneficial microbes; diversity indices; reduction in ESKAPEE pathogens.
Quantity	Total amount/concentration of microbes.	Colony-forming units (CFU); microbial load measured by qPCR or sequencing read counts.
Virulence	Changes in the pathogenic potential or disease-causing ability	Expression or abundance of virulence genes; reduction in toxin production; decrease in

	of microorganisms within the community.	virulence-associated factors (e.g., adhesion, toxin genes); reduced pathogenicity.

To address research question 2, we examined whether CRISPR applications have been used to modify the traits of interest outlined in Table 1 in ESKAPEE pathogens. We considered the modification successful based on the following outcomes: the number of genes modified, changes in gene expression, and changes in the target phenotype (Table 3).

Table 3. Measurable outcomes used to evaluate the success of CRISPR-mediated trait modification in ESKAPEE pathogens.

Effectiveness outcomes	***Definition***	***Example indicators***
Number of genes that have been modified	The total count of targeted genes successfully altered using CRISPR or other genetic engineering approaches.	Number of edited genes; proportion of target genes successfully modified.
The change of gene expression	The measurable effect of genetic modification on gene activity or cellular phenotype.	Change in gene expression (e.g., mRNA levels); changes in protein activity or cell behavior.
The change of target phenotype	The measurable effect of genetic modification on the target phenotype, such as biofilm formation or virulence.	Change in expression product; changes in product compositions.

Search strategy

For research question 1, we searched PubMed, Scopus, Embase, and Web of Science databases for three clusters of terms: (1) CRISPR technology, (2) ESKAPEE pathogens, and (3) healthcare terms. We applied Boolean logic, such that at least one term from each cluster must be included.

For research question 2, we searched the same databases for three clusters of terms (1) CRISPR technology, (2) ESKAPEE pathogens, and (3) key traits of interest (as outlined in Table 1). We included a fourth cluster of exclusion terms for clinical diagnostics and treatment, as well as for plants (e.g., agricultural CRISPR applications). We applied Boolean logic such that search results must contain at least one term from each of the three target clusters and no terms from the fourth cluster.

Study screening

Inclusion and exclusion criteria

Studies were included if they employed CRISPR systems to edit the genomes of ESKAPEE bacteria, for example, through gene knockouts, knockdowns, or knock-ins. Studies focused on the identification and classification of CRISPR systems, the development of CRISPR technology itself (e.g., improving efficiency or reducing off-target effects without bacterial applications), applications of CRISPR in plants or human cells, or organisms outside the ESKAPEE group were excluded.

For RQ1, studies were included if they applied CRISPR in a healthcare setting and reported on effects on microbial abundance, microbiome composition, or virulence attenuation. For RQ2, studies were included when CRISPR was used to modify bacterial traits listed in Table 1. In contrast, as stated above, this review did not include studies that focus solely or primarily on modifying AMR genes or related traits.

Regarding settings, for RQ1, eligible studies were conducted in the healthcare built environment, including hospitals, clinics, and other healthcare facilities. Exclusions are studies conducted in settings whose primary purpose was nonmedical, such as schools, residential group homes, or temporary and mobile facilities established for vaccination campaigns. For RQ2, studies were included if they were conducted in simulated built environments, *in vivo* models, or benchtop experiments.

Title/abstract screening

Two reviewers independently screened each study at the title-abstract level. If screening decisions conflicted, the two reviewers conferred to determine whether to include the item. We used the Covidence software to screen studies.

Full-text data extraction and synthesis

Data were extracted using an Excel form. We extracted the following information: citation details; study setting (e.g., geographic location); study design; CRISPR-based microbiome-engineering intervention details; control or comparison conditions; and measures of effectiveness, genetic outcomes, and phenotypic changes.

We did not anticipate sufficient homogeneity in study designs and effectiveness measures to conduct a meaningful meta-analysis. We presented the data using narrative synthesis.

For RQ1, we reported CRISPR interventions targeting microbial communities in healthcare settings. Key items of interest included the CRISPR strategies employed, microbial targets (pathogenic and beneficial), changes in microbial load or community composition, reductions in virulence, and effects on horizontal gene transfer. For RQ2, we focused on CRISPR-enabled modifications of bacterial traits that influence healthcare microbiomes, as described above. Data on RQ2 included both quantitative and qualitative outcomes, such as the number of genes modified, changes in gene expression, and/or bacterial traits. For both RQ1 and RQ2, tables were used to summarize outcomes where possible, while narrative descriptions provided context for less standardized measures.

To address the research questions, we used a combination of descriptive statistics and qualitative synthesis. Descriptive statistics were calculated across key metadata categories, including:

- Types of engineered microbes (e.g., laboratory strains vs. built environment isolates)
- Types of healthcare, environmental, or benchtop settings studied, and level of experimental population (e.g., culture from a single colony, defined microbiome, built environment microbiome).
- Distribution of CRISPR applications (e.g., pathogen suppression, biofilm reduction, modulation of horizontal gene transfer).
- Proportion of studies reporting quantitative indicators (e.g., percent biofilm reduction, number of off-target edits).

For studies reporting quantitative outcomes, we summarize key statistics such as:

- Mean and range of changes in target gene expression.
- Mean and range of microbial control measures (e.g., survival reduction, biofilm inhibition, HGT reduction)

Study quality assessment

Table 1. Study quality assessment for a systematic scoping review on CRISPR applications to the healthcare built environment

	Allocation bias. How were participants/study sites allocated to receive the intervention?	Follow-up bias. What proportion of study sites were present at the time of follow-up?	How has CRISPR technology been verified to be successful?	Outcome assessment [effectiveness outcomes]. How were microbial contamination outcomes assessed?	Confounding in analysis. Have test/in vivo/ benchtop conditions and confounding variables been balanced?	Reproducibility for benchtop studies. Is the experiment reproducible?
High risk of bias	0 = study sites allocated to exposure by convenience or other intentional/unbalanced techniques, or no control is present (e.g., before and after studies)	0 = less than 60% of participants/study sites enrolled at follow-up	0 = no attempt in verification	0 = No quantitative culture or microbial viability measure was reported or assessed	0 = no attempt to balance confounders (unadjusted analysis)	0 = no biological replicates, experimental conditions/operations/outcomes are not reproducible
Medium risk of bias	1 = study sites allocated to exposure using quasi-experimental designs to balance	1 = 60-80% of participants/study sites lost to follow-up	1 = verified by maker gene expression	1 = present/absence of culture or microbial	1= some confounders adjusted, but substantial confounders	1 = over 50% experiments have biological replicates, experimental

	confounders (e.g., matching, step-wedge)			viability data were reported	not accounted for	conditions/operations/outcomes are partially reproducible
Low risk of bias	2 = study sites randomly allocated to exposure	2 = <80% of study sites lost to follow-up	2 = verified by sequencing; detection of expression of target gene by qPCR or western blot; other quantitative assays for detection of related traits.	2 = CFU or other metrics that assess relative levels of contamination are reported	2=all key confounders adjusted (quasi-experimental design) or addressed through randomization (RCTs)	2 = over 90% experiments have biological replicates, experimental conditions/operations/outcomes are highly reproducible

Results

Characteristics of included studies

Our first search yielded 1,014 unique studies. After screening, no studies met the inclusion criteria. The primary reason no studies were identified was the absence of direct application: no studies were found that applied CRISPR directly in the built environment to control the EXKAPEE pathogen. Although no studies met the inclusion criteria, several related studies were subsequently identified in the RQ2 search.

Our second search yielded 3,230 unique studies. After screening, we included 9 eligible studies (Figure 1). We report on the results of nine studies that met the inclusion criteria.

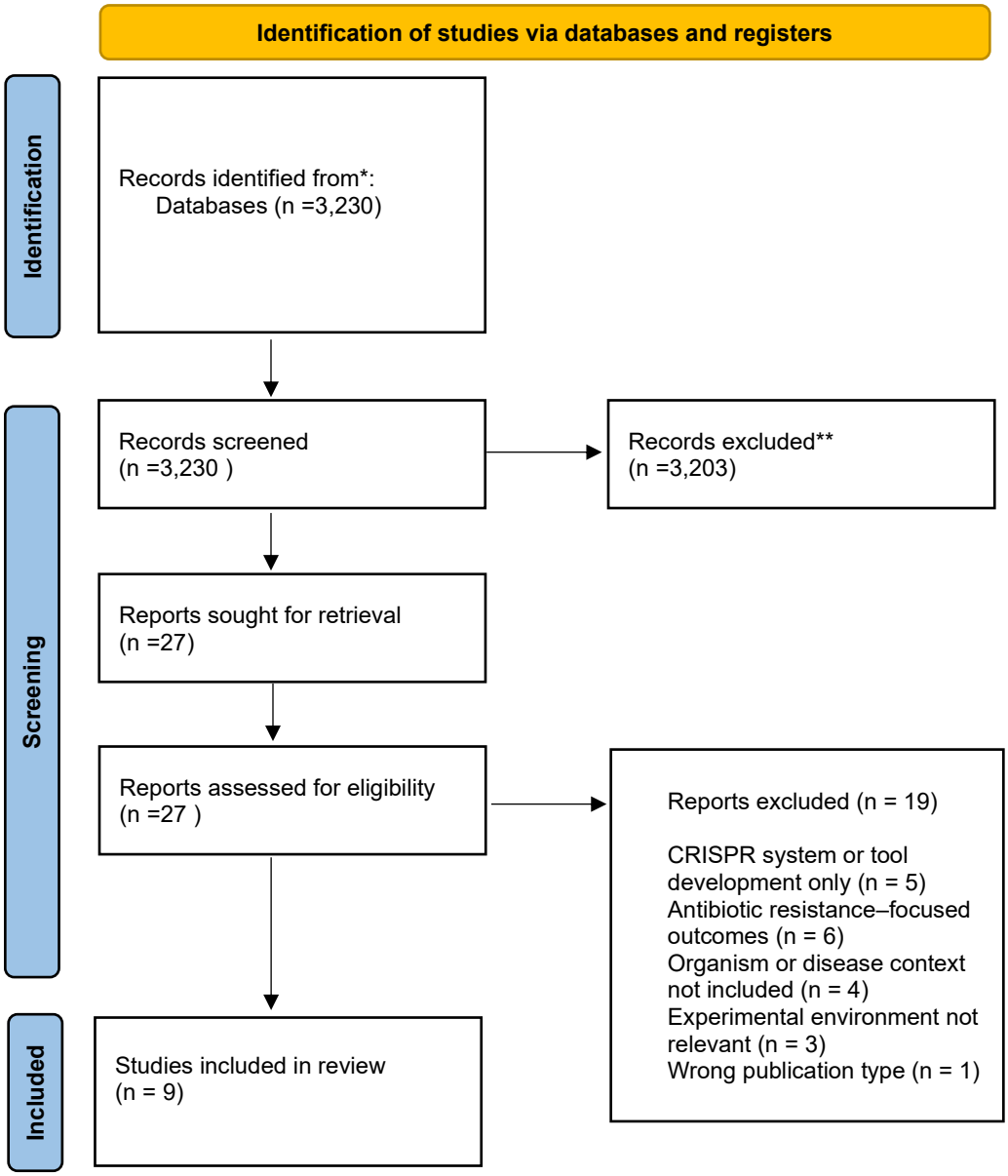

Figure 1. PRISMA flow diagram for a systematic review of Microbiome applications of CRISPR to built environments in healthcare facilities

Table 2. Characteristics of studies identified in a systematic review of CRISPR applications to microbiome engineering

Study ID	**Year**	**Species Engineered**	**Strains Engineered**	**Clinical isolates?**
Alshammari *et al*., 2023	2023	*Escherichia coli*	*E. coli* ATCC 25922b	
Azam & Khan, 2022	2022	*Escherichia coli*	*E. coli Nissle* 1917, E.coli K12	
Azam *et al*., 2020	2020	*Escherichia coli*	*E. coli* MG1655	
Zuberi *et al*, 2022	2022	*Escherichia coli*	E. coli (AK118, AK53, AK70, AK81 and AK105)	√
Zhou *et al*., 2025	2025	*Escherichia coli*	*E. coli* BW25113	
Zhan *et al*., 2025	2025	*Klebsiella pneumoniae*	*K.pneumoniae* ATCC43816	
Ou *et al*., 2022	2022	*Escherichia coli*	*E. coli* Nissle 1917	
Gupta *et al*., 2021	2021	*Escherichia coli*	*E. coli* CFT073	
Qu *et al*., 2019	2019	*Pseudomonas aeruginosa*	*P. aeruginosa PA14*	

Across all included studies, engineered microorganisms were exclusively bacterial, with E. coli as the predominant host organism. Seven of the nine studies (77.8%) employed *E. coli* as the primary engineering chassis. The remaining studies included one investigation utilizing *K. pneumoniae* and one study examining *P. aeruginosa*.

Among studies using *E. coli*, a broad range of laboratory, reference, and probiotic strains were reported. These included commonly used strains such as ATCC 25922, Nissle 1917, K-12 derivatives (including MG1655 and BW25113), and CFT073. Some studies incorporated multiple strains within a single experimental framework, whereas others relied on a single reference strain. Overall, substantial heterogeneity in strain selection was observed across studies, even within the same bacterial species.

Only one study explicitly reported the use of clinical isolates, involving multiple *E. coli* strains (AK118, AK53, AK70, AK81, and AK105). In contrast, the remaining studies primarily employed established laboratory or reference strains, and most did not specify whether they used freshly obtained clinical isolates.

From an experimental scale perspective, all included studies (n = 9, 100%) conducted experiments at the single-colony or laboratory culture scale. Experimental validation was

performed exclusively under controlled laboratory conditions. No studies reported evaluations at pilot, environmental, or field scales.

CRISPR engineering design

Table 3 CRISPR Engineering design

Study ID	**CRISPR Tool Used**	**# of gene modified**	**Gene that has been modified**	**Delivery Method**	**Verification Method**
Alshammari *et al*., 2023	CRISPR/Cas9	3	*fimH, luxS, and bolA*	Electroporation	PCR amplification+gel seperation; Sanger sequencing
Azam & Khan, 2022	CRISPRi	1	*csgD*	Transformation	quantitative real-time PCR (qRT-PCR)
Azam *et al*., 2020	CRISPRi	1	*bolA*	Transformation	quantitative real-time PCR (qRT-PCR)
Zuberi *et al*, 2022	CRISPRi	1	*OmpR*	Transformation	quantitative real-time PCR (qRT-PCR)
Zhou *et al*., 2025	CRISPR/Cas9	1	*aiiO*	Electro transformation	length analysis and sequencing of the gene fragments
Zhan *et al*., 2025	CRISPR/Cas9	1	*RstA*	Transformation	quantitative real-time PCR (qRT-PCR)
Ou *et al*., 2022	CRISPR/Cas9	1	*CheZ*	Eletrotransformation	PCR length analysis
Gupta *et al*., 2021	CRISPR/Cas9	1	*papG*	Carbon quantum dots (CQD)	quantitative real-time PCR (qRT-PCR)
Qu *et al*., 2019	CRISPRi	1	*exsA*	Conjugation	quantitative real-time PCR (qRT-PCR)

Across the nine included studies, CRISPR-based genetic engineering approaches were used to modify specific bacterial genes associated with virulence, biofilm formation, or regulatory pathways (Table). Two primary CRISPR modalities were reported: CRISPR/Cas9-

mediated genome editing and CRISPR interference (CRISPRi). CRISPR/Cas9 was employed in five of the nine studies (55.6%), whereas CRISPRi systems were used in four studies (44.4%). Studies using CRISPR/Cas9 primarily aimed to introduce targeted gene disruptions or edits, whereas CRISPRi approaches were used to repress gene expression without permanent genomic modification. Most studies targeted a single gene (8/9 studies, 88.9%). Only one study reported simultaneous modification of multiple genes, targeting fimH, luxS, and bolA. The genes modified across studies included regulators and virulence-associated factors such as csgD, bolA, ompR, aiiO, rstA, cheZ, papG, and exsA. Target selection varied substantially among studies, with no gene targeted by more than one study except bolA, which appeared in two investigations.

Multiple delivery approaches were reported for introducing CRISPR components into bacterial hosts. Transformation-based methods were the most used, including standard transformation and electroporation (electrotransformation) techniques. One study utilized conjugation for plasmid delivery, while another applied carbon quantum dots (CQDs) as a nanoparticle-mediated delivery strategy. Overall, delivery methods varied considerably across studies, reflecting differences in host strains and experimental design.

Verification strategies were consistently reported across studies. Quantitative real-time PCR (qRT-PCR) was the most frequently used validation method (6/9 studies, 66.7%), particularly in CRISPRi experiments assessing transcriptional repression. PCR-based length analysis and gel electrophoresis were commonly used to confirm genome edits, and some studies further validated the modifications by sequencing or by assessing the length of amplified DNA fragments. These approaches collectively confirmed successful gene modification or transcriptional regulation following CRISPR intervention.

Outcomes measured

Table 4 Outcome measured in studies included in a systematic scoping review of CRISPR applications

Study ID	The change in expression of target gene(s)	Biofilm formation	Adhesion Traits	Horizontal gene transfer	Virulence	Enhancement of *in situ* CRISPR engineering in the built environment
Alshammari *et al*., 2023		√	√			
Azam & Khan, 2022	√	√				
Azam *et al*., 2020	√	√				
Zuberi *et al*, 2022	√	√				
Zhou *et al*., 2025		√				

Zhan *et al*., 2025		√			√	
Ou *et al*., 2022			√			
Gupta *et al*., 2021	√	√	√		√	
Qu *et al*., 2019	√				√	

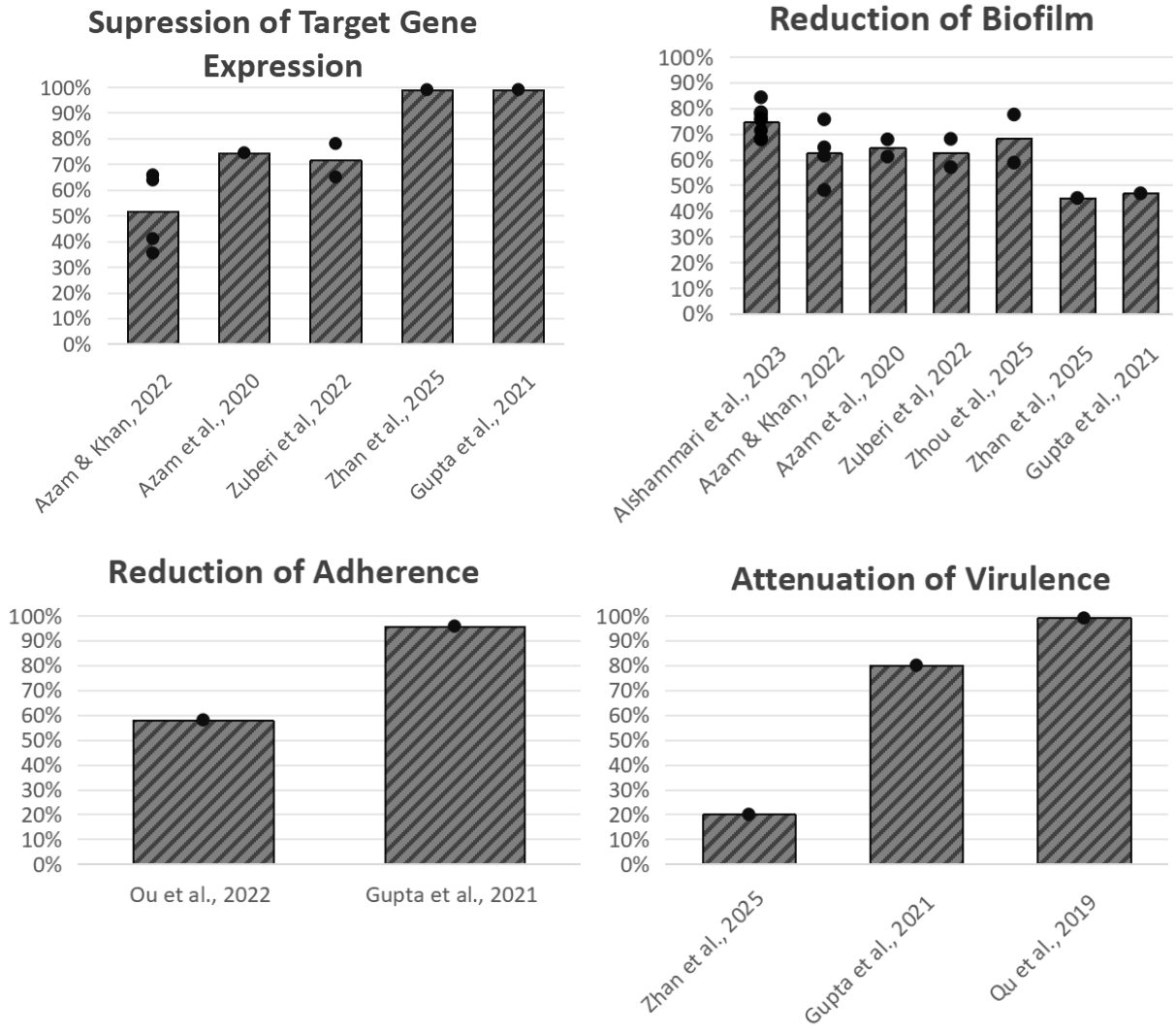

Figure 1: **Summary of quantitative outcomes across independent studies.** The figure summarizes the reported outcomes, including suppression of gene expression, reduction of biofilm formation, decreased adherence, and attenuation of virulence, presented as four

independent panels. In each panel, black dots indicate individual data points extracted from the original studies, expressed as percentage reduction relative to the corresponding control. Columns represent the main value from all data points in each study.

Analysis of quantitative outcomes across the included studies revealed substantial variation in the effects of CRISPR-based interventions on microbial traits in built-environment contexts. Suppression of target gene expression ranged from approximately 50% to 100% across studies, with Zhan *et al*. (2025) and Gupta *et al*. (2021) reporting the highest reductions. This result was mainly reported in CRISPRi-related studies. A reduction in biofilm formation was reported in 7 studies, with the largest decreases reported by Alshammari et al. (2023), reaching 70–80%. Gupta et al. (2021) and Zhan et al. (2025) reported the lowest biofilm suppression, with reductions of 45%-50%. A reduction in microbial adherence was reported in two studies: Ou et al. (2022) reported a 58% reduction, and Gupta et al. (2021) achieved over 90% suppression. Attenuation of virulence varied widely, from ~20% in Zhan et al. (2025) to 99% in Ou et al. (2019), indicating substantial differences in the ability of CRISPR-based interventions to reduce pathogenic potential across microbial targets. However, this difference could also be related to target choice.

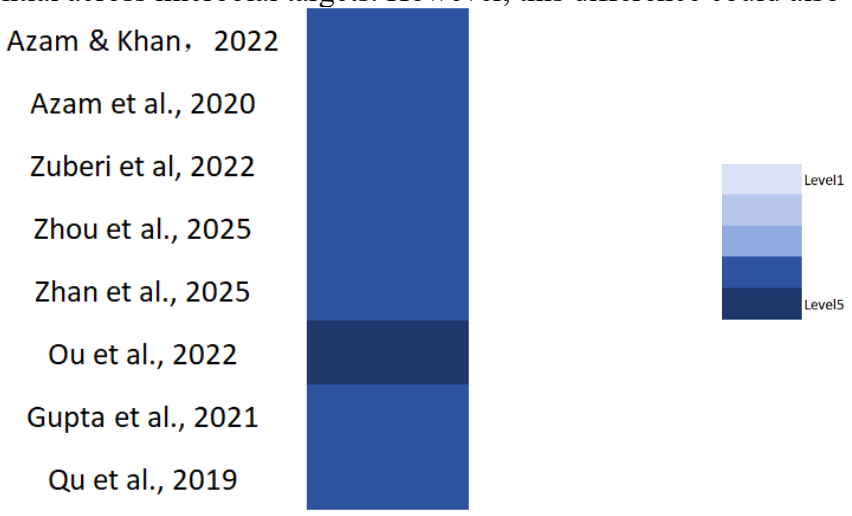

Figure 2: Evaluation of experimental design. Studies were assigned to 5 different levels based on their experimental designs. Level 1: No control, single experimental group, no statistical analysis; Level 2: Wild type group as control, single experimental group, no statistical analysis; Level 3: Wild type group as control, multiply experimental groups, no statistical analysis; Level 4: Wild type group as control, multiply experimental groups with statistical analysis; and Level 5: Wild type and complemented strain as control, multiply experimental groups with statistical analysis

All included studies were evaluated for experimental design using a five-level scale. Most studies were classified as Level 4, indicating the presence of a wild-type control, multiple experimental groups, and statistical analysis, but no complemented strain control. The exception was Ou et al. (2022), which achieved Level 5 and included wild-type and complemented-strain controls.

Discussion

We conducted a systematic review to assess the application of CRISPR for microbiome engineering in healthcare and built environments, and to examine how CRISPR has been used to genetically engineer ESKAPEE pathogens to modify traits relevant to microbiome engineering, including field, *in vivo*, and benchtop studies. We hypothesized that there would be few search results and therefore designed two search strategies. The first search strategy yielded zero studies. The second search yielded nine studies. In the second search, we found that most studies were conducted on Escherichia coli at the single-colony level.

This systematic review uncovers a substantial gap between the theoretical potential of CRISPR-based technologies and their demonstrated application for pathogen control in the built environment, particularly in hospital settings. While CRISPR has been used for microbial manipulation in laboratory and *in vivo* contexts, its use for controlling ESKAPEE pathogens within complex, real-world built environments remains largely unexplored. This absence of applied studies underscores the need to contextualize CRISPR-based interventions within the ecological and functional realities of hospital microbiomes. However, as an emerging technology, CRISPR-based technology shows rapid development and promising application prospects for pathogen control in healthcare facility built environments.

From the perspective of CRISPR technology development, the field is rapidly expanding beyond the foundational Cas9 nuclease to diverse novel systems, artificial intelligence-driven design, and synthetic biology platforms. Expanding the CRISPR toolbox offers more possibilities for broader real-world applications. Cas12a (Cpf1) recognizes T-rich PAM sequences common in bacterial genomes. It processes its own guide RNA arrays, thereby simplifying multiplexed editing to target multiple virulence factors or engineer complex pathways (Zetsche *et al*., 2015). Large serine recombinases (LSRs) enable scarless integration of large genetic payloads when paired with CRISPR, facilitating the installation of biosynthetic gene clusters, synthetic circuits, or biocontainment switches in bacterial genomes (Durrant *et al*., 2023). Cascade-Cas3, a Type I CRISPR system, processively degrades long DNA stretches, making it uniquely suited for eliminating large antibiotic resistance islands or virulence clusters in pathogens (Csörgő *et al*., 2020; Dolan *et al*., 2019). Beyond nucleases, precision editing tools such as base editing and prime editing enable targeted genetic modifications without double-strand breaks. Base editors catalyze direct single-nucleotide conversions, offering a powerful approach to introduce attenuating mutations or correct pathogen-associated sequences (Komor *et al*., 2016; Gaudelli *et al*., 2017). Prime editing extends this capability by enabling search-and-replace edits, including small insertions, deletions, and all point mutations, without requiring donor DNA templates (Anzalone *et al*., 2019). For RNA targeting, Cas7-11 combines the programmable targeting of Cas13 with the processivity of Cas7, enabling precise RNA knockdown with reduced off-target effects (Özcan *et al*., 2021). Compact systems such as CasMINI and CasΦ (Cas12j) offer hypercompact platforms (<500 amino acids) that simplify delivery into challenging environments (Xu *et al*., 2021; Pausch *et al*., 2020). CRISPR-associated transposases (CAST) enable RNA-guided DNA integration without double-strand breaks, providing a programmable platform for large-scale genomic insertions (Strecker *et al*., 2019). Together, these emerging systems continuously expand the toolkits available for ESKAPEE pathogen engineering.

Meanwhile, artificial intelligence is accelerating CRISPR-based engineering across multiple fronts. Evo2, a foundational genomic language model trained on massive datasets across all domains of life, enables prediction of mutation effects, design of functional sequences, and optimization of guide RNAs for bacterial targets (Brixi *et al*., 2026). Beyond Evo2, machine learning models such as DeepCRISPR and Azimuth improve guide RNA efficiency and off-target prediction (Chuai *et al*., 2018; Doench *et al*., 2016). And deep learning approaches facilitate the discovery of novel CRISPR systems from metagenomic datasets (Harrington *et al*., 2018; Pausch *et al*., 2020). These AI-driven tools are bridging CRISPR, a laboratory technique, with a computationally guided discipline.

Notably, synthetic biology is enabling CRISPR-based engineering at unprecedented scale and complexity. High-throughput CRISPR screens using pooled libraries enable systematic interrogation of genomes to identify essential genes, virulence factors, and drug targets (Shalem *et al*., 2014, Gilbert *et al*., 2014, Rousset *et al*., 2018). CRISPR interference (CRISPRi) and activation (CRISPRa) libraries further expand functional genomics capabilities by enabling tunable gene regulation (Gilbert *et al*., 2014). *De novo* phage rebooting, synthesis, and refactoring of entire phage genomes enable the creation of designer phages with customized host range, attenuated virulence, and built-in CRISPR systems (Chan *et al*., 2023). These synthetic biology approaches are helping to establish CRISPR-based interventions as promising platforms for antimicrobial development. Together with CRISPR toolboxes and AI technology, these advances are reshaping the landscape of ESKAPEE pathogen engineering, offering new opportunities for precision, scalability, and real-world application.

From the perspective of promoting the application of CRISPR technology in healthcare facility built environments, there are gaps and challenges to address. Hospital-built environments host diverse microbial communities that occupy distinct ecological niches, including sinks and drains, high-touch surfaces, medical equipment, and ventilation systems. These environments support both opportunistic pathogens and non-pathogenic or potentially beneficial microbes, and they differ substantially in moisture, nutrient availability, and disturbance regimes. However, the studies identified in this review largely focus on isolated organisms or simplified laboratory systems, limiting insight into how CRISPR-based strategies might perform in heterogeneous, multispecies environments. Future research would benefit from explicitly accounting for these ecological complexities when designing and evaluating CRISPR interventions.

One key gap identified by this review is the lack of standardized physical or experimental models that simulate hospital-relevant built environments. The development of reproducible models incorporating realistic surfaces, airflow or water systems, and environmental conditions would enable more meaningful evaluation of CRISPR-based pathogen control strategies. Such models could serve as intermediate platforms between benchtop studies and *in situ* deployment, facilitating cross-study comparisons and improving translational relevance.

In addition, there is little consensus in the current literature regarding appropriate metrics for evaluating the success of CRISPR-based interventions in the built environment. Most studies emphasize molecular or organism-level outcomes, such as gene disruption efficiency, while overlooking broader functional endpoints, including pathogen persistence, biofilm dynamics, and impacts on resident microbial communities. Establishing standardized evaluation criteria would improve comparability across studies and clarify which CRISPR applications are most relevant to real-world pathogen control.

Moreover, biocontainment and long-term control of engineered microbes also emerge as critical unresolved challenges. Although several studies discuss genetic safeguards in principle, few empirically assess containment performance under environmentally relevant conditions. Future work may continue to evaluate biocontainment strategies—such as kill switches, auxotrophy, or environmental dependence—in the context of built-environment stressors, including nutrient limitation, horizontal gene transfer, and selective pressure.

Finally, ethical, regulatory, and societal considerations remain largely absent from the literature. The deployment of CRISPR-based technologies in shared human environments raises questions related to safety, consent, ecological impact, and governance that are distinct from those associated with laboratory or clinical applications. Integrating regulatory perspectives and stakeholder engagement into early-stage research may help guide responsible development and identify acceptable use cases for CRISPR-based pathogen control in hospitals.

Taken together, the findings of this systematic review suggest that CRISPR-based approaches to pathogen control in the built environment remain at an early conceptual stage. Advancing this field will require a shift from proof-of-concept studies toward ecologically informed, standardized, and ethically grounded research frameworks. Addressing these gaps will be essential to promoting the safe and effective translation of CRISPR technologies into strategies to mitigate hospital-acquired infections and antimicrobial resistance.

This review has several limitations. The search strategy may have missed relevant studies if CRISPR applications were not consistently indexed, described, or labeled in databases. The search strategy may have been limited by database selection, potentially excluding relevant papers not captured by the search. Because no *in situ* studies were identified, our findings rely entirely on laboratory-based work, which limits their generalizability to real-world healthcare settings. Heterogeneity in experimental designs, strains, CRISPR tools, and outcome measures prevented meaningful comparison across studies. Fourth, most studies lacked ecological context, such as multispecies communities or realistic built-environment conditions, restricting insight into translational feasibility. Finally, because we excluded studies focused solely on AMR gene editing and non-ESKAPEE organisms, emerging applications outside this scope may not be represented.

Conclusion

CRISPR-based microbiome engineering remains largely untested in real healthcare environments. Existing evidence is limited to laboratory studies targeting individual microbial traits, highlighting the need for ecologically informed research, standardized evaluation frameworks, and realistic built-environment models to enable safe and effective translation into infection-prevention strategies.

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