

International Journal of Research in Advanced Electronics Engineering



E-ISSN: 2708-4566
P-ISSN: 2708-4558
IJRAEE 2024; 5(1): 07-10
© 2024 IJRAEE
www.electrojournal.com
Received: 04-11-2023
Accepted: 13-12-2023

Shrestha Mukherjee
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

Subhasis Sarkar
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

Suranjana Sarkar
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

Semanti Ghosh
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

Bidisha Ghosh
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

Correspondence
Bidisha Ghosh
Department of Biotechnology,
School of Life Sciences, Swami
Vivekananda University,
Telinipara, Barasat-
Barrackpore Rd, Bara
Kanthalia, West Bengal, India

CRISPR-Cas based nano-sensors in water pathogen detection

Shrestha Mukherjee, Subhasis Sarkar, Suranjana Sarkar, Semanti Ghosh and Bidisha Ghosh

DOI: <https://doi.org/10.22271/27084558.2024.v5.i1a.32>

Abstract

Waterborne diseases are primarily caused by various indiscriminate anthropological activities as a result of fast pace in industrialization coupled with the infestation of microorganisms borne as a result of the same. Water pollution thus is a major concern for public health in economically unprivileged nations. Assessing the water pathogens and monitoring quality is crucial for decision-making in water distribution systems, treatment, and prevention. Early detection of harmful microorganisms is essential for research, medical diagnosis, and public health. The polymerase chain reaction (PCR) approach has limitations due to expensive instrument control, unique test sites, and rigorous solution treatment steps. It may be possible to close detection gaps using the, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) -CAS system, which is a component of the bacterial adaptive immune system. Considering its capacity to target particular genes, CRISPR/Cas system has lately grown to favour as a diagnostic tool as functions in cleaving the target DNA or RNA using Cas enzymes and a guide RNA (gRNA). Nanotechnologies have shown promising applications in identifying, discriminating, and removing water pathogens. Nano-sensors, based on nanotechnology, can be utilized as a fruitful alarm to quickly detect pathogen contamination, for further improvement of water quality using nanoparticles like molecular-recognition probes. These are high strength materials for sensing applications, producing measurable signals. The CRISPR / Cas technology for pathogen detection combines good specificity, high sensitivity, and ease, making it appropriate for real-world applications. This review describes CRISPR/Cas-based nano-sensors for the detection of many dangerous water-borne pathogens, including *Salmonella enteritidis*, *Salmonella* species, and *Pseudomonas* species.

Keywords: CRISPR/CAS System, Guide RNA, Microorganisms, Medical diagnosis, Nano-sensors, Polymerase Chain Reaction

Introduction

Life cannot thrive without one of the most fundamental substances on earth-water. But in the present situation water-borne disease is the major threats to the human health. The 2021 World Water Development Report, published by UNESCO, stated that freshwater use increased about 1% per year since the 1980s and grew six-fold over the past 100 years ^[1]. Surface water supplies are vulnerable to pollution from various sources, including human and animal waste ^[2]. However, the frequency of the aquatic diseases has increased mainly because of poor water quality in numerous parts of the world. The common waterborne pathogens such as bacteria, viruses, protozoa & helminths. Generally, infective microorganisms & their toxic exudates can cause gastrointestinal infection. Cholera, diarrhoea, dysentery, hepatitis A, typhoid these are also associated with poor sanitation and contaminated water ^[3]. Numerous etiological factors, such as viruses, bacteria, and parasites, can cause diarrhoea. *Salmonella typhi*, *Salmonella enterica* and *Vibrio cholerae* are etiological agents for gastrointestinal illnesses ^[4] and these aquatic pathogens flourish in contaminated water & particularly those that have been related with waste product like urine, stool etc. Infantile gastroenteritis can be brought on by certain ETEC (Enterotoxigenic *E.coli*) via consuming ETEC contaminated water that caused dehydration & malnutrition in young children ^[1]. *E.coli* O157 generating Shiga toxin: H7 is a food and water-borne bacterium that, in both isolated cases and outbreaks, affects people and causes haemorrhagic colitis, haemolytic uremic syndrome (HUS), and diarrhoea ^[2].

The CRISPR-CAS genome editing devices which are originated from prokaryotes, have

revolutionized our ability to modify, identify, locate, record & characterize certain DNA & RNA sequences in living cells of various species.

This system relying on the endonuclease activities of related proteins (Cas) and the complementarity of the targeted sequence with the guide RNA (g-RNA) [7]. According to evolutionary relationship, CRISPR-CAS system is currently divided into 2 classes, 6 types & 33 subtypes [8]. Most of CRISPR-CAS based techniques that were developed by the use of Cas9 variants, which can identify dsDNA. Class 1 CRISPR-Cas systems are characterized by a complex of several effector proteins, whereas class 2 systems include a single crRNA-binding protein. These systems are distinguished by the nature of the ribonucleoprotein effector complex [9]. Tracking environmental pollutants is a crucial activity for protecting the public from dangerous substances released into the environment and for notifying them to their presence. A nano-sensor is a special kind of sensor; that is capable to detect and quantify information at the nanoscale level. The synthesis, characteristics, and uses of materials at the nanoscale level are the primary goals of the new field of study known as nanoscience. Nano-sensor composed on three basic components; biological recognition, physical transducer & data recording system [10]. To react to the adsorption of a single molecule of a nucleic acid or protein, the sensors can be electronically gated. The ability to detect substances at the nanoscale level because of the number of sites available for molecular interactions increases as the specific surface area decreases [11]. Nano-sensing can be described and assessed in terms of its performance parameters, including mobility, cost, and potential for recycling, as well as detection sensitivity, selectivity, reaction time, limit, and range [12].

Crispr/Cas based nanosensors in Pathogen detection

The most widely used point of care test kits are lateral flow assay (LFA) kits, which are often based on the molecular affinities of different molecules like antigen-antibodies, streptavidin-biotin, etc. FELUDA and CASLFA approaches based on CRISPR/Cas9 are integrated into LFA kits using gold nanoparticles (AuNPs) coupled with antibodies or other interacting molecules [13, 14, 15]. In FELUDA, 3'-FAM-labeled ribonucleoprotein (RNP) complex, FAM antibody-

linked gold nanoparticles, and chimeric guide RNA (modified with phosphorothioate linkage) were combined in a tube and loaded into a paper strip [14]. In CASLFA, the amplified target gene is combined with sgRNA, Cas9, and AuNP probe and put onto the sample pad of the LFA device. The RPA/PCR is carried out using biotinylated primers. Due to the accumulation of AuNP at the test line and control pad, streptavidin-coated test lines and a complementary DNA probe hybridized control pad particularly bind with biotinylated sample mix and AuNP-DNA probes and produce color. A target virus can be identified using color development at the test line and control pad [15]. Though Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALEN) both are also known as artificial robust class of tools [16]. ZFN is a gene editing technique based on Zinc finger nucleases, TALEN is a gene editing method based on fusion proteins made of a bacterial TALE protein and FokI endonuclease [17]. But compared to ZFNs and TALENs, CRISPR/Cas system is simpler, cheaper and more efficient due to its natural activity. According to World Health Organization Special Programme for Research and Training (2003), a suitable pathogen detector needs to ensure "ASSURED" criteria i.e. affordable, sensitive, specific, user-friendly, rapid, equipment-free & deliverable (ASSURED) [18]. In comparison to ZFNs and TALENs, CRISPR-CAS system has a number of benefits, including simple design for any genomic targets, straightforward prediction of off-target areas, and the ability to simultaneously edit several genomic sites (multiplexing). Therefore, to reach the eligibility criteria CRISPR/Cas system is far ahead.

CRISPR/CAS based nanosensors in water pathogen Detection

CRISPR-CAS based nano-sensors, their single-base specificity and compatibility with point-of-care devices, newly developed nucleic acid detection techniques based on CRISPR-derived RNA-guided engineered proteins (such as Cas13a, Cas12a, Cas12 and Cas9) are said to have unpredictable potential in the field of water pathogen detection (As per following Table).

Table 1: Different CRISPR/Cas systems for water pathogen detection

CRISPR/Cas Systems	Amplification technique	Used Cas protein	Water Pathogen
CRISPR/Cas with Allosteric Probe	RT-PCR	Cas13a	<i>Salmonella enteritidis</i>
CRISPR-Cas powered dual mode nano-sensors (using Au-Np)	PCR	Cas12a	<i>Salmonella sp.</i>
CRISPR/Cas based lateral flow biosensors (using Au-Np:SA complex)	LAMP	Cas12	<i>Pseudomonas aeruginosa</i>
CRISPR/Cas mediated SDA-RCA technique (using nanoparticle UiO66)	SDA and RCA	Cas9	<i>Escherichia coli (E.coli O15H7)</i>

Detection of *Salmonella* species (APC-Cas method)

APC-Cas method for detecting *Salmonella enteritidis* utilizing a special allosteric probe (AP) and CRISPR/Cas13a [19] where the objective was entire bacterium. The three functional domains that make up the allosteric probe (AP) are the aptamer domain for specific pathogen recognition, the primer attachment domain & the T7 regulator domain. The aptamer domain of AP changes its active configuration in the presence of the target pathogen, allowing primers to anneal to the released primer binding site domain. The AP serves as a template for double-stranded DNA, and T7 RNA polymerase recognizes the T7 promoter sequence, allowing

secondary amplification to create single-stranded RNAs. The CRISPR-RNA is composed of a guide and repeat sequence, complementary to the transcribed ssRNA, for binding to the Cas13a enzyme. This activates Cas13a/crRNA's collateral cleavage ability, enabling tertiary amplification and amplified fluorescence signals.

Detection of *Salmonella sp* (CRISPR-CAS powered dual mode nano-sensors)

To detect *Salmonella sp*, Ma *et al.* [20] created a CRISPR/Cas12a-powered dual-mode biosensor using gold nanoparticles (AuNPs). Invasion gene A, a virulence gene

of *Salmonella*, was the target DNA [21]. The trans-cleavage activity of CRISPR/Cas12a served as the foundation for the proposed biosensor. A linker ssDNA and AuNPs-DNA probe pairs hybridize when the AuNPs probe is coated with DNA. The linker ssDNA is unaltered in the absence of target amplicons, and aggregated AuNPs preserve their purple colour. The trans-cleavage of CRISPR/Cas12a is activated and the linker ssDNA is cut off when the target amplicons are recognized by the specifically designed crRNA. As a result, the AuNPs are disseminated in solution. It is possible to see the colour change in the disseminated AuNP solution with the unaided eye, colorimetry, or photothermal methods [22].

Detection of *Escherichia coli* (*E.coli* O15H7)

On the UiO66 platform, Sun *et al.* [24] created a CRISPR/Cas9-induced SDA-RCA technique that distinguished *E. coli* strains. The method was created using rolling circle amplification (RCA), strand displacement amplification (SDA), and nanoparticles. Short-ssDNA was created through primary amplification using SDA synthesis after the CRISPR/Cas9 pair (sg RNA1 and sg RNA2) detected and cut the target DNA. The long-ssDNA with repeated sequences complementary to a fluorescence-labeled DNA probe was created via RCA secondary amplification using the short-ssDNA template. At wavelengths of 480 and 518 nm, the probe is detectable. Unbound probes are taken up by UiO66, which also quenches fluorescence. When the target sequence is present, short and long ssDNA are produced, producing a fluorescent signal. This method has an extremely high sensitivity and broad detection range for *E. coli* O157:H7.

Effectivity of CRISPR-Cas Based Nanosensing

The CRISPR-based approaches decreased the requirement for heavy equipment, which is a noteworthy aspect with great field application potential, especially for managing epidemic outbreaks in resource-constrained locations. It is simpler to provide a variety of output signals using CRISPR/Cas systems, from fluorescence to naked eye detection. For instance, methods for molecular diagnostics have been developed that target DNA using the CRISPR/Cas9 effectors' capacity for recognition and cleavage. Due to their trans-cutting or collateral cleavage capabilities, CRISPR/Cas12 and CRISPR/Cas13 have been used in biosensing settings in contrast to CRISPR/Cas9, ushering in a new era of molecular diagnostics [24, 25].

Conclusion & Future Perspective

Four typical CRISPR/Cas systems- Cas12, Cas12a, Cas13a and Cas9- for detecting nucleic acids from infectious pathogens have been reported and summarized in this review paper, which also introduces the development and mechanism of CRISPR/Cas based nano-sensing in water pathogen detection. In real-world applications, a straightforward, sensitive, and potent nucleic acid detection technology is considerably superior to a complicated, sophisticated device. Therefore, the development of non-immobilized, homogeneous, straightforward, and sensitive nucleic acid detection technologies is still vital in pathogen identification. The majority of CRISPR/Cas sensing systems have difficult detection limits without the aid of nucleic acid amplification technologies. Effective disease control and eradication strategies require timely detection and

assessment of pathogen infection. CRISPR-based diagnostics offer a promising solution to current drawbacks, such as sensitivity, cost, and equipment requirements. These biosensing systems provide rapid, sensitive, and specific quantitative assays capable of detecting specific sequences, including SNPs, and are capable of multiplexing [27]. Therefore, CRISPR/Cas-nano-sensor based nucleic acid detection has a promising future for simultaneously detecting multiple pathogens.

Conflict of Interest

The authors have no conflict of interest.

References

1. Lin L, Yang H, Xu X. Effects of water pollution on human health and disease heterogeneity: A review. *Frontiers in environmental science*. 2022;10:880246.
2. Walker DB, Baumgartner DJ, Gerba CP, Fitzsimmons K. Surface water pollution. In *Environmental and pollution science*. Academic Press, Elsevier; c2019. p. 261-292.
3. World Health Organization. Safely managed drinking water: thematic report on drinking water; c2017.
4. Patel CB, Shanker R, Gupta VK, Upadhyay RS. Q-PCR based culture-independent enumeration and detection of Enterobacter: An emerging environmental human pathogen in riverine systems and potable water. *Frontiers in microbiology*. 2016;7:172.
5. Magana-Arachchi DN, Wanigatunge RP. Ubiquitous waterborne pathogens. *Waterborne Pathogens*; c2020. p. 15-42.
6. Tanquilut CD, Jung CW, Nelson AW, Lau SK. Infection due to Shiga toxin-producing enterohemorrhagic *Escherichia coli* (EHEC) presenting as ischemic colitis. *ID Cases*. 2019;18:e00629. Elsevier.
7. Liu X, Hussain M, Dai J, Li Y, Zhang L, Yang J, *et al.* Programmable biosensors based on RNA-guided CRISPR/Cas endonuclease. *Biological Procedures Online*. 2022;24(1):2.
8. Zhou Y, Bravo JP, Taylor HN, Steens JA, Jackson RN, Staals RH, *et al.* Structure of a type IV CRISPR-Cas ribonucleoprotein complex. *Iscience*, 2021, 24(3).
9. Adam T, Gopinath SC. Nanosensors: Recent perspectives on attainments and future promise of downstream applications. *Process Biochemistry*. 2022;117:153-173.
10. Kulkarni DR, Malode SJ, Prabhu KK, Ayachit NH, Kulkarni RM, Shetti NP. Development of a novel nanosensor using Ca-doped ZnO for antihistamine drug. *Materials Chemistry and Physics*. 2020;246:122791.
11. Kaur R, Sharma SK, Tripathy SK. Advantages and limitations of environmental nanosensors. In *Advances in nanosensors for biological and environmental analysis*. Elsevier; c2019. p. 119-132.
12. Yang Y, Yang X, Yang Y, Yuan Q. Aptamer-functionalized carbon nanomaterials electrochemical sensors for detecting cancer relevant biomolecules. *Carbon*. 2018;129:380-395. Elsevier.
13. Srivastava S, Upadhyay DJ, Srivastava A. Next-generation molecular diagnostics development by CRISPR/Cas tool: rapid detection and surveillance of viral disease outbreaks. *Frontiers in molecular biosciences*. 2020;7:582499.

14. Azhar M, Phutela R, Kumar M, Ansari AH, Rauthan R, Gulati S, *et al.* Rapid and accurate nucleobase detection using FnCas9 and its application in COVID-19 diagnosis. *Biosensors and Bioelectronics.* 2021;183:113207.
15. Wang X, Xiong E, Tian T, Cheng M, Lin W, Wang H, *et al.* Clustered regularly interspaced short palindromic repeats/Cas9-mediated lateral flow nucleic acid assay. *ACS nano.* 2020;14(2):2497-2508.
16. Kumar R, Kaur A, Pandey A, Mamrutha HM, Singh GP. CRISPR-based genome editing in wheat: A comprehensive review and future prospects. *Molecular biology reports.* 2019;46(3):3557-3569. Springer.
17. Shamsirgaran Y, Liu J, Sumer H, Verma PJ, Taheri-Ghahfarokhi A. Tools for efficient genome editing; ZFN, TALEN, and CRISPR. In *Applications of Genome Modulation and Editing.* New York, NY: Springer US; c2022. p. 29-46.
18. Land KJ, Boeras DI, Chen XS, *et al.* Reassured diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol.* 2019;4:46–54.
19. Shen J, Zhou X, Shan Y, Yue H, Huang R, Hu J, *et al.* Sensitive detection of a bacterial pathogen using allosteric probe-initiated catalysis and CRISPR-Cas13a amplification reaction. *Nature communications.* 2020;11(1):267.
20. Ma L, Peng L, Yin L, Liu G, Man S. CRISPR-Cas12a-Powered Dual-Mode Biosensor for Ultrasensitive and Cross-validating Detection of Pathogenic Bacteria. *ACS Sensors.* 2021;6(8):2920–2927.
21. Lou L, Zhang P, Piao R, Wang Y. *Salmonella* pathogenicity island 1 (SPI-1) and its complex regulatory network. *Frontiers in cellular and infection microbiology.* 2019;9:270.
22. Chakraborty J, Chaudhary AA, Khan SU, Rudayni HA, Rahaman SM, Sarkar H. CRISPR/Cas-Based Biosensor as a New Age Detection Method for Pathogenic Bacteria. *ACS omega.* 2022;7(44):39562-39573.
23. Mukama O, Wu J, Li Z, Liang Q, Yi Z, Lu X, *et al.* An ultrasensitive and specific point-of-care CRISPR/Cas12 based lateral flow biosensor for the rapid detection of nucleic acids. *Biosensors and Bioelectronics.* 2020;159:112143.
24. Sun X, Wang Y, Zhang L, Liu S, Zhang M, Wang J, *et al.* CRISPR-Cas9 triggered two-step isothermal amplification method for *E. coli* O157: H7 detection based on a metal–organic framework platform. *Analytical chemistry.* 2020;92(4):3032-3041.
25. Yuan B, Yuan C, Li L, Long M, Chen Z. Application of the CRISPR/Cas System in Pathogen Detection: A Review. *Molecules.* 2022;27:6999.
26. Freije CA, Sabeti PC. Detect and destroy: CRISPR-based technologies for the response against viruses. *Cell host & microbe.* 2021;29(5):689-703.
27. Aman R, Mahas A, Mahfouz M. Nucleic Acid Detection Using CRISPR/Cas Bio-sensing Technologies. *ACS Synthetic Biology;* c2020.