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Exploring the versatility of Magnetic Relaxation Switching (MRS) and CRISPR-based biosensors for the detection of foodborne pathogens; a comprehensive review

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ABSTRACT

Food-borne pathogens such as *E. coli., Salmonella, Listeria monocytogenes, Campylobacter jejuni* are the common ones that pose a significant threat to human health and therefore require quick accurate affordable detection of food pathogens. Traditional pathogen identification procedures in foods are laborious and expensive and require specialized equipments. Magnetic Relaxation Switching (MRS) biosensors, which work by monitoring changes on dispersion, aggregation, or concentration change of magnetic nanoparticle (MNPs), have emerged as an potential alternative. Therefore, MNPs have gained wide applications due to the typical magnetic properties that may efficiently enhance the target, separate, and capture with high sensitivity. However, traditional MRS biosensors are often insensitive and unstable. The review, therefore, focuses on the latest development of CRISPR-Cas12a-assisted MRS biosensors for improving food safety monitoring. The CRISPR-Cas12a system enhances pathogen detection by recognizing and cleaving target sequence with high precision. Lastly, the review sums up the improvements of different biosensors in comparison with conventional biosensors and shows the use of MNPs in the identification process of various pathogens.

1. Introduction

The international commerce of agricultural products has resulted in the recent global spread of microbiological illnesses (Machado-Moreira et al., 2019). The highest priority in recent years has been both food safety and quality. Furthermore, the risk of foodborne illness is heightened due to increased microbial contamination. Various techniques have been investigated for the identification of microbes in food, viz. Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA) (Wei et al., 2021). Despite significant improvements, these approaches are still restricted due to their extremely time-consuming nature, requiring expensive equipment and well-trained operators.

Therefore, it is imperative to create diagnostic techniques that are quicker, more precise, and less expensive (Du et al., 2022). It should come as no surprise that major attempts have been made to create new technologies that can meet these demanding specifications. These include biosensors, which integrate mechanical, electrochemical, or optical transduction techniques with biological molecules as recognition elements (Wilson et al., 2019). Rapid development in biosensors has helped in the identification of various pathogens (Alhadrami, 2018). Biosensors have achieved simple and effective target analysis by first using bio-recognition elements (e.g., antibodies, enzymes, nucleic acids) and these elements then identify target substances and transform them into signals that are easily captured and recognized (e.g., light, electricity, and magnetism).

Food safety is detected using a variety of biosensors, the most common of which is the Magnetic Relaxation Switching (MRS) biosensor (Wei et al., 2024). The various limitations of traditional sensors and the expensive instruments gave rise to various MRS sensors so that the different pathogens can be identified and then coverted into recognized signals (Dong et al., 2021). The basis of MRS is supermagnetic nanoparticles, or SNPs. These monitor a variety of targets and induce aggregation or disaggregation. The state of the target can alter the SMNs,

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which affects the (transverse relaxation time) T_2 of the surrounding water molecules. This property is characteristic of (T_2) molecules or atoms; it is the time taken by MNPs to stabilize after activation by a magnetic field. The degree of aggregation of the SMNs is determined by the number of targets in the sample and coupled to the ΔT_2, which is the change in T_2 value, acting as the signal readout (Xu et al., 2022).

The (Magnetic Relaxation Switching) MRS biosensor can be applied in turbid and light-impermeable environments without a separation and purification process. Main applications include the detection of heavy metals, DNA, antibiotics, bacteria, pesticides, and hormones. MRS biosensors depend on the working mechanism based on MNPs dispersing, aggregating, or changing concentration (Huang et al., 2023).

Nanotechnology is one of the advanced technologies that aremore important in developing, enabling, and having a sustainable impact on both the food and agriculture industries. Nanomaterials have promising prospects in terms of quality and quantity in the manufacture of perishable or semi-perishable functional foods (Nile et al., 2020). Materials with at least 50 % of their nanoparticles having one or more exterior diameters between 1 and 100 nm are called nanomaterials (Modena et al., 2019). MNPs are among the most important nanomaterials that possess magnetic properties. Preparations on a large scale are made from mixes of metals and polymers and pure metals like iron, cobalt, nickel, or their metal oxides (Wu et al., 2019). A fast and efficient method for separating and enriching pathogens in food is using a magnetic separation approach based MNPs (Xiao et al., 2022). MNPs are introduced into the sample where the target was present, and then an external magnetic field is applied. The MNPs interact with the magnetic field and affect T_2 of the water molecule in the surrounding region. It depends on how much of the target molecule is present and bound to the MNPs and how much the variations in T_2 measured by the MRS sensor depend upon it. Immuno-magnetic separation, also known as antibody-based or MNP-based approaches, is combined with several detection systems for separating pathogens and resistant bacteria from food (Caliskan-Aydogan et al., 2023).

The MRS biosensors are attractive for food safety as it detects pathogens in food but the main problem with the traditional MRS is that they are not sensitive and stable enough. CRISPR (clustered regularly interspaced short palindromic repeats) is a new technology that is often preferred over MRS when the ultra-sensitive detection of pathogens is needed, for example, in clinical diagnostics or food safety applications, where trace-level targets such as drug-resistant bacteria must be rapidly and accurately detected. Therefore, the CRISPR-Cas12a system was introduced into an MRS biosensor (Wei et al., 2023). CRISPR-based biosensors are highly sensitive and specific, carrying a high degree of specificity to detect single-base mismatches as well as allowing amplification-free detection to depress the possible contamination. They must be carefully designed to avoid off-target effects, though, and are restricted to nucleic acid targets (Shen et al., 2022). CRISPR-based biosensors take advantage of the programmable recognition capability of CRISPR-associated (Cas) proteins like Cas12 and Cas13 to identify particular nucleic acid sequences of pathogens (Gootenberg et al., 2017). The CRISPR-Cas system is deployed and applied for genome editing since it is sensitive to recognising and activating cleavage of particular DNA and RNA regions (Aman et al., 2020). Recent researches has shown the possibility of using CRISPR-based biosensors to identify different pathogens in food (Chakraborty et al., 2022). The overall sensitivity and specificity of the detection system can be improved by functionalizing MNPs with CRISPR components to enable simultaneous target capture and signal amplification. These technologies are also relatively affordable and portable, allowing them to be used for on-site testing in resource-limited environments, thereby,filling a significant void in food safety procedures (Wang et al., 2024).

This comprehensive review emphasizes the current developments, challenges, and potential future directions as it reviews the adaptability of MRS and CRISPR-based biosensors for the detection of foodborne pathogens. We cover the basic concepts of these technologies, how they are applied to detect various types of pathogens, and how they might be integrated with other sensing platforms. This study provides a thorough understanding of how MRS and CRISPR-based biosensors can revolutionize food safety monitoring and help prevent foodborne diseases by exploring the latest available studies.

2. Mechanism of MRS

The mechanism of magnetic relaxation is based on MNPs and their interaction with water molecules and magnetic centres in which the major role is that of the magnetic characteristics of MNPs (Zhou et al., 2019). The relaxation mechanisms underlying MRS sensors highlight the impact of target and particle sizes on the spin-spin relaxation time (T_2) of water molecules. Relaxation describes the process of a system returning to equilibrium after a disturbance and the equilibrium stage is considered the most stable state of a system following a disturbance, the relaxation time constant shows how quickly a system returns to its equilibrium condition. A quicker return to balance is indicated by a shorter relaxation time. Types of relaxation are T_1 (Longitudinal Relaxation) and T_2 (Transverse Relaxation) (Wei et al., 2021). Compared to NPs existing in their dispersed condition, the aggregated form of the NPs dephases the spins of the surrounding protons of water molecules more effectively which causes a decrease in the spin-spin relaxation time, T_2 (Table 1) (Rezaei et al., 2024). Under the influence of an external magnetic field (B_o), tiny fraction of the proton nuclei align parallel to (B_o). Proton nuclei that are aligned, begin to process at the Larmor frequency (ω_o) (Zhang et al., 2017). As protons spin around the z-axis at a precession rate known as the Larmor frequency(ω_o), a net magnetization (M_z) is created along the direction of the magnetic field (z-axis). When the nuclei receive an RF (radiofrequency) pulse at the same precession frequency, some protons in the lower energy state absorb the RF energy and flip to the higher state. Protons also start to precess in phase (synchronize) with one another. As a result, a transverse magnetization perpendicular to the static magnetic field is produced, representing M_{xy}. When the radiofrequency pulse is removed, the excited nuclei return to their equilibrium distribution. There are two types of relaxation longitudinal (T_1) and transverse (T_2). T_1 relaxation, sometimes called spin-lattice relaxation, occurs when antiparallel protons return to the parallel state while giving up energy to molecules in the surrounding environment (lattice). The amount of time required to regain 63 % of the initial longitudinal magnetization is known as the T_1 relaxation time. T_2 is the amount of time it takes for transverse magnetization(M_{xy}) to decrease to 37 % of its starting value (Huang et al., 2021; Nikolaev et al., 2023) (Fig. 1). Present research continuously specifies that the biggest challenge in attaining MNP uniformity—particularly uniform size, shape, and dispersion—therefore it directly affects their magnetic properties and biological activity. For example, traditional co-precipitation and thermal decomposition processes likely to produce extensive sizes and uneven morphologies, which result in batch-to-batch variation and agglomeration problems (Adeeyo et al., 2025; Lak et al., 2021). Physical processes such as ball milling and lithography also have a trouble with uniformity, which typically results in defects or polydispersity. Aggregation caused by high surface energy and insufficient effective surface stabilization worsen the issue, degrading colloidal and magnetic homogeneity. These obstacles have been addressed by the introduction of microfluidic synthesis, which allows for fine control of reaction conditions (flow rate, mixing, and temperature) to give improved reproducibility and narrower size distributions (Niculescu et al., 2022). In addition, sophisticated surface functionalization and core-shell morphologies (e.g., silica or polymer coatings) have proved to prevent aggregation, balance crystal structure, and maintain magnetic uniformity (Sanz et al., 2015; Muthukumaran and Philip, 2024). These synergistic approaches are increasingly settling uniformity issues, making it achievable to develop more reproducible and scalable production of MNPs.

MNP plays a crucial role in MRS sensors by acting as a signal source

Table 1
Pathogen detection using different nanoparticles and biosensors.

Pathogen	Nanoparticles used	Method used	Detection range	Time	Reference
Salmonella	MNP	Phage based MRS biosensor	10^3 –10^6 CFU/mL		Huang et al., 2023.
Salmonella	–	MS-MRS	100 CFU/mL	30 mins	Chen et al., 2015
Salmonella	MNP	UPC-MRS	53 CFU/mL	10 mins	Wen et al., 2023
Salmonella	MNP	CRISPR assisted MRS	1.3×10^2 CFU/mL	–	Shen et al., 2022
Salmonella	Fe_3O_4	NMR biosensor	10^5 CFU/mL	160 mins	Zou et al., 2019
Salmonella	Superparamagnetic nanoparticle	(TD-NMR) biosensor	2.3×10^3 CFU/mL	90 mins	Jin et al., 2020
Salmonella	CoFe-MOFs-graphene nanocomposites	Electrochemical immune-sensor	1.2×10^2 CFU/mL	–	Feng et al., 2021
Pseudomonas aeruginosa	Super magnetic iron oxide nanoparticle	MRSw aptasensor	50 CFU/mL	45mins	Jia et al., 2017
Vibrio parahaemolyticus	hollow mesoporous silica microspheres (HMSMs)	MRS	$10 \sim 1.0 \times 10^8$ CFU/mL	30 mins	Chen et al., 2023
Vibrio parahaemolyticus	AuNPs	MRS			Chen et al., 2025
Listeria monocytogenes	Au@Pt nanoparticles	MRS DNA sensor	30 CFU/mL	–	Wu et al., 2022
Listeria monocytogenes	MNP	One-step and DNA amplification-free MRS	50 CFU/mL	2hrs	Qi et al., 2021
V. alginolyticus	Fe_3O_4@SiO_2-AptVA	MRS	26 CFU/mL	15mins	Wang et al., 2021
Aspergillus flavus	MNP	MRS	0.02–200 ng/mL	–	Lu et al., 2023
Noro-virus	Fe_3O_4@Au	MRS	0.38 and 3.45 pg/mL.	–	Wang et al., 2023

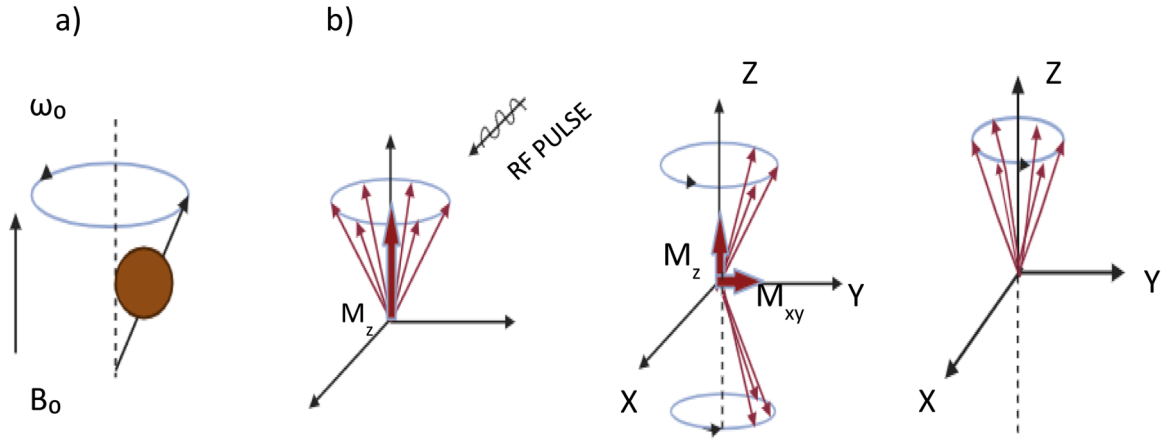

Fig. 1: a). Under an external magnetic field (B_0), protons align either parallel or antiparallel and undergo precession b) Protons get excited when RF pulses are introduced, and they relax when the RF pulses are removed.

that affects the relaxation of the water molecule. Larger MNP enhances relaxation time, different shapes can also influence the relaxation properties, and surface coating of MNP influences the water interaction (Zhou et al., 2019). MNPs have a major impact on T_1 and T_2 relaxation times. Changes in T_2 relaxation behaviour can result from MNP aggregation or disaggregation. Measuring the amount of target analyte present is possible since the change in T_2 (ΔT_2) is proportionate. There are two types of MRS: Type I and Type II, which vary by the size of the magnetic nanoparticles. In the case of Type I systems, it shows a decrease in T_2 with aggregation, while Type II system shows an increase in T_2 during aggregation. These varied behaviours are explained by the outer sphere relaxation theory which requires the parameters t_D (Translational Diffusion Time), describing how long a typical water molecule takes to diffuse around a particle and Dw (Difference in Angular Frequencies) that describes how a magnetic field will differ near the surface of a water particle compared to the bulk water for a proton along with the motional average condition. This theory explains how the interaction of water protons with the magnetic field produced by MNPs influences T_2 relaxation. This theory has explained the relation between the size of MNP, aggregation, and T_2 relaxation. The outer sphere relaxation hypothesis occurs when the motional average requirement ($D_w t_D < 1$) is met, then, in this case, the sphere's size increases, and the relaxation rate R_2 (= $1/T_2$) increases. If the motional average requirement is not met ($D_w t_D > 1$), then in this case R_2 (= $1/T_2$) decreases during MP aggregates. Two models can explain MNP behaviour in the presence of analytes: the static dephasing model (SD) and the motional averaging model (MA). SD model shows that a static dephasing effect and an increase in T_2 result from MNP aggregation, defeating thermal randomization (SD, $D_w t_D > 1$) whereas, in MA model, the MNPs aggregate in a motional averaging model (MA, $D_w t_D < 1$), unable to overcome the thermal randomization. Water molecules diffuse at a pace that allows them to average out the magnetic field that results from MNP accumulation and therefore the T_2 decreases. The connection between the aggregation of MNPs and the diffusional motion of water molecules determines the kind of system (kind I or Type II). Comprehending these correlations is essential in developing and enhancing MNP-based nanosensors for diverse uses (Zhang et al., 2017; Wei et al., 2021; Hu et al., 2020) (Fig. 2**).**

Target-induced aggregation (or disaggregation) of SMNs is the basis for the use of Magnetic Relaxation Switching (MRS) sensors, which are used to measure a variety of targets (Liu et al., 2020). When the target molecule binds with the receptors, nanoparticles combine and influence the nearby water molecules. It leaves a difference in these T_2 relaxation times of the water molecules around the magnetic field. The unique features of magnetic nanoparticles have popularized them for pathogen detection. These nanoparticles can be constructed with different functional groups, including aptamers or antibodies to make them capable of selective binding of specific bacteria. For the detection of target pathogens in food, the use of antibody-conjugated MNPs will be made (Mocan et al., 2017).

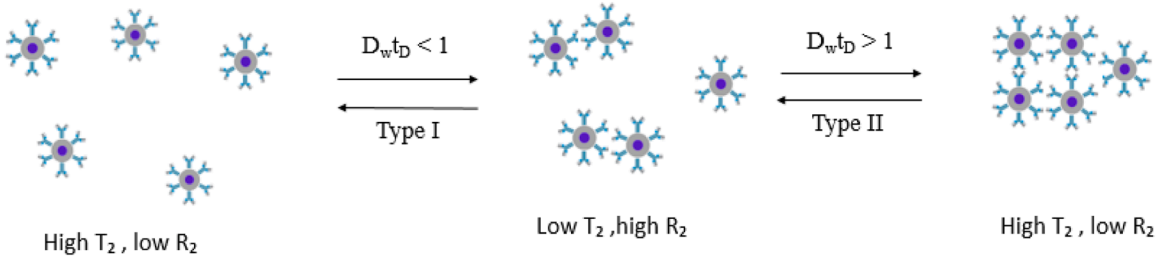

Fig. 2. Diagrammatic representation of MRS-based nanosensor.

MNP material, containing superparamagnetic iron oxide (SPIO) materials, has been widely applied in biochemical detection as a result of their special properties, which include super-paramagnetism, good monodispersity, high saturation magnetization, and efficient separation (Kang et al., 2022). The mean size, shape, and physicochemical features of MNPs all have a significant influence on their characteristics. Supermagnetism is the term used to describe this oscillation (Farinha et al., 2021). It is a unique property of small magnetic particles that exhibit both supermagnetic and paramagnetic characteristics (Fig. 3). In contrast to ferromagnetic particles, superparamagnetic particles do not have a permanent magnetic moment. Both are composed of ferromagnetic or ferromagnetic materials, but in the absence of an external field, the small size and thermal fluctuations of superparamagnetic particles allow their magnetic domains to randomly orient, resulting in a net magnetic moment of zero. They briefly align when a field is added, but when the field is removed, the alignment is lost. They are useful for MR biosensing applications because of this characteristic as well as their capacity to improve T_2 relaxation (Li et al., 2020) (Fig. 4).

2.1. Impact of MNP size on T_2 relaxivity

Atoms on the outer surface of MNPs have a different electrical configuration than those on the inner surface, which causes spin canting. The spin-canted spins at the boundary and the long-range ordered magnetic spins in the middle of MNPs have an impact on their magnetic moment. The size-dependent magnetic moment, T_2 relaxivity, and crystallinity influence the thickness of the spin-canting layer in maghemite (Zhou et al., 2019). Unpaired electrons in transition metal ions can affect the T_2 relaxation time of water protons. Unpaired electrons of nanoparticles cause a stronger reduction in T_2 (Wu et al., 2021). T_2 relaxation describes the process by which the magnetisation of a magnetic nanoparticle returns to equilibrium after being disturbed. Every dimension, every shape, and each composition of particles influences relaxation (Kostevšek, 2020). When water protons undergo precessing spins in an external magnetic field (B_o), magnetic resonance signals are produced (Rhodes, 2017). In designing MRS systems, one should understand the dependence of T_2 signal and nanoparticles aggregation state. The most commonly applied transverse T_2 contrast agents are iron oxide nanoparticles. MNP size and form have a great impact on T_2 relaxation. Larger particles carry higher magnetic moments and therefore generally exhibit longer T_2 relaxation periods (Vuong et al., 2017). The Outer-Sphere Diffusive Theory at MAR (Motional Average Regime) explains how interactions between water protons and the magnetic field produced by MNPs (magnetic nanoparticles) in the surrounding environment (outer sphere) impact T_2 relaxation. Superparamagnetic NPs' relaxivity (r_2) can be increased via coating optimization or the magnetic characteristics of the NP (Kostevšek, 2020). Differently shaped nanoparticles have unique effective radii, which are crucial for determining the transverse relaxation rates, as well as the intensity of stray fields and local field inhomogeneity caused by NPs. These factors ultimately lead to various water diffusion processes (Yang et al., 2017a).

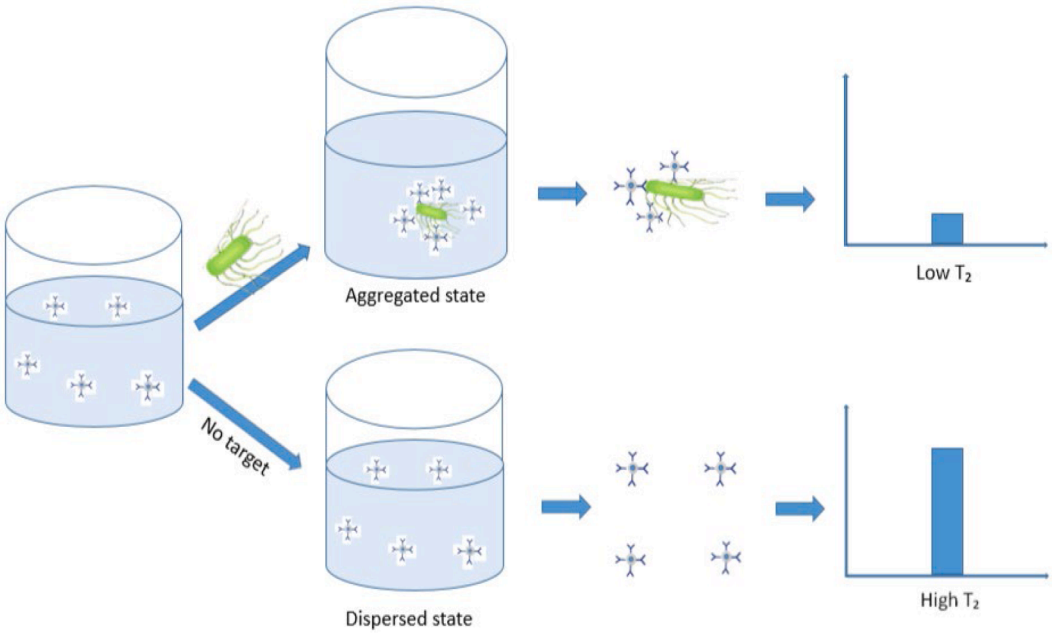

Fig. 3. Pathogen identification based on the transition of nanoparticles between scattered and clustered forms.

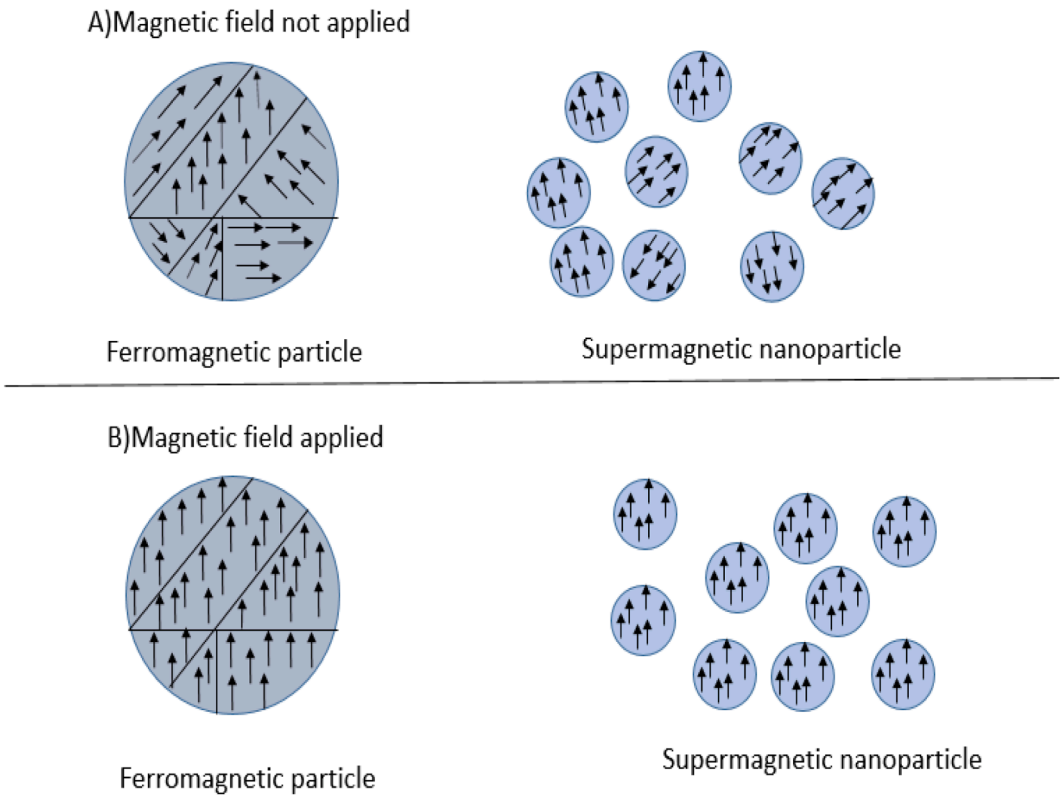

Fig. 4. Comparison of ferromagnetic and superparamagnetic particles; (A) Without the presence of external magnetic field (B) With the presence of external magnetic field.

2.2. Impact of MNP size on T_1 relaxivity

T_1 Relaxation Enhancement is explained by the Solomon Bloembergen Morgan (SBM) theory for paramagnetic molecules. Since MNPs have a strong magnetic field, there may be a large outer-sphere contribution to T_1 relaxation. The measurement of T_1 relaxation may be adversely affected by strong T_2 dephasing. Weak transverse field fluctuations make them generally inefficient for T_1 contrast, especially in high magnetic fields. Size reduction raises surface energy, necessitating management to maintain colloidal stability (Zhou et al., 2019). Strong T_1 relaxation of the proton spins results from changes in the magnetic moment, which cause the water protons to experience fluctuating magnetic fields. T_1 relaxation is strongly influenced by magnetic nanoparticle size and nature. It is among the major factors that influence magnetic characteristics (Zhang et al., 2019). Larger particles have higher magnetic moments, which causes them to have longer T_1 relaxation durations. It has been proven that in comparison to other magnetic materials, iron oxide nanoparticles (IONPs) have longer T_1 relaxation durations. Huge T_1 relaxation can only be achieved with a big fluctuation field amplitude and a relaxation frequency that is near the proton resonance frequency (Zhou, 2019). The relaxation of T_1 is primarily dependent on direct dipole-dipole interactions between metals and water molecules; thus, the atoms within a nanocrystal have a very small role in the relaxation process. From this angle, small-sized nanoparticles (NPs) are preferred due to their large surface-to-bulk ratio. Surface modification is typically required to maximize colloidal stability and promote the interaction between metal and water on the outer layer of nanocrystals (Zhang et al., 2017).

3. Detection of pathogens using MRS

Bacteria are among the most prevalent organisms on earth that have evolved to survive in many types of ecological niches. They are considered advantageous to their host as they play an important role in the food, agriculture, pharmaceutical, and petroleum industries, among other industries however, they are also considered harmful as they show some toxic effects on humans and other living creatures. Among the most significant procedures in the identification and prevention of health, safety, and well-being crises is the testing and quick detection of pathogenic organisms (Rajapaksha et al., 2019). Provided that bacteria and other diseases multiply rapidly over time, the best way to stop food-borne illnesses is to identify bacteria quickly while contamination is first occurring (Bertrand, 2019). Thus, the identification of harmful bacteria is essential, particularly for young patients, the elderly, and those receiving immunosuppressive treatment or having impaired immune systems (Altintas et al., 2018). Modern molecular biology tools such as PCR-based procedures, biosensors, and nucleic acid-based tests (NAT) are replacing traditional methods in the identification of certain pathogens in food. These methods have low detection limits, high-performance costs, and sample pre-treatment in addition to excellent sensitivity and reliability (Umesha & Manukumar, 2018). Nanotechnology can be used to minimize the amount of pollutants in soil, water, and air (Nasrollahzadeh et al., 2019). Nanomaterial has a large surface-to-volume ratio which results in high efficiency and reactivity. Some examples of nanomaterials include Cu, Au, Ag, etc. (Biswas et al., 2023). MNPs are a nanoscale material of wide reputation in many fields including health, energy, engineering, and environmental applications (Ali et al., 2021). MNP / MP are one of the nanomaterials useful for a wide range of applications. Their unique and attractive physicochemical properties allow adding MNPs to electrochemical immune-sensing so that it captures and pre-concentrates the target analyte and makes incubation and washing steps possible via quick and easy separation using an external magnetic field, thus substantially neutralizing the interference of the sample matrix (Pastucha et al., 2019). MNP is used for bacterial detection and serves as a separating agent of bacteria. The functionalization of MNPs with target molecules

such as different antibodies, antibiotics, antimicrobial peptides, bacteriophages, and aptamers will be useful in the separation and concentration of bacteria (Xu et al., 2019) (Fig. 5). MNPs conjugated with different metals enable the creation of bacterial detection techniques such as colorimetric, fluorescent, and surface-enhanced Raman detections based on surface modification. In many instances, MNPs, well known for low steric barrier, monodisperse distribution, and super-paramagnetism, are linked to biological recognition units to detect and trap the target under the external magnetic field. Immune magnet separation technology has been widely applied up until now in the isolation and concentration of foodborne bacteria for the acceleration of detection and the enhancement of sensitivity and specificity of biosensors (Guo et al., 2019). Among the sophisticated applications, MRS-based biosensors have been even more sensitive than conventional fluorescence-based assays. In this technique, MNPs with DNA intercalating fluorochromes aggregate into micro-aggregates upon complex formation with PCR-amplified DNA, resulting in alterations of the magnetic relaxation time (T_2), which can be measured precisely by using magnetic resonance methods. In variance to fluorescence-based detection methods that are prone to suffer from restriction caused by interference from the background and low sensitivity for detection in intricate matrices, MRS-based detection is based on magnetic signal alteration and can supply higher sensitivity and specificity. Detection limits as low as 0.02 femtomolar support this by representing the improved performance of MRS biosensors in ultra-sensitive pathogen detection. MRS remains less commonly applied in food diagnostics due to challenges in nanoparticles, surface modification and lack of widespread commericially. However, due to present advancements in nanoparticles synthesis which indicate broader application in food safety monitoring (Chen et al., 2015; Alcantara et al., 2016). MNPs have certain advantageous attributes that make them useful for a variety of bio-medical utilization. A notable class of nanoparticles that are susceptible to external magnetic field gradient manipulation are magnetic nanoparticles (Yang et al., 2017b; Sadanandan et al., 2023). Some factors help MNP in the detection of pathogens such as, the large surface area of MNP, it directly affects the surface/volume ratio and the efficacy of microbial adherence of particles. The other reason why MNP is best suited to detection of pathogen is that, the nano-sized particles, that offer high levels of cell wall adherence and facilitate effective separation from the aqueous solution. The last reason is due to the faster kinetics which is possible in liquid-phase reaction with the target. Target pathogens can engage in quick liquid-phase interactions with MNPs, which speeds up detection and analysis. This faster kinetics shortens the total amount of time needed for analysis by increasing the effectiveness of pathogen collection and detection. MNPs are functionalized with ligands that bind pathogens specifically to enable them to bind to the antibody. The MNPs that are still attached to their target are gathered in the direction of the magnet, which facilitates the easy removal of the undesirable material. To encourage the pathogen's release from the nanoparticles, the MNPs are subsequently rinsed in an elution buffer.

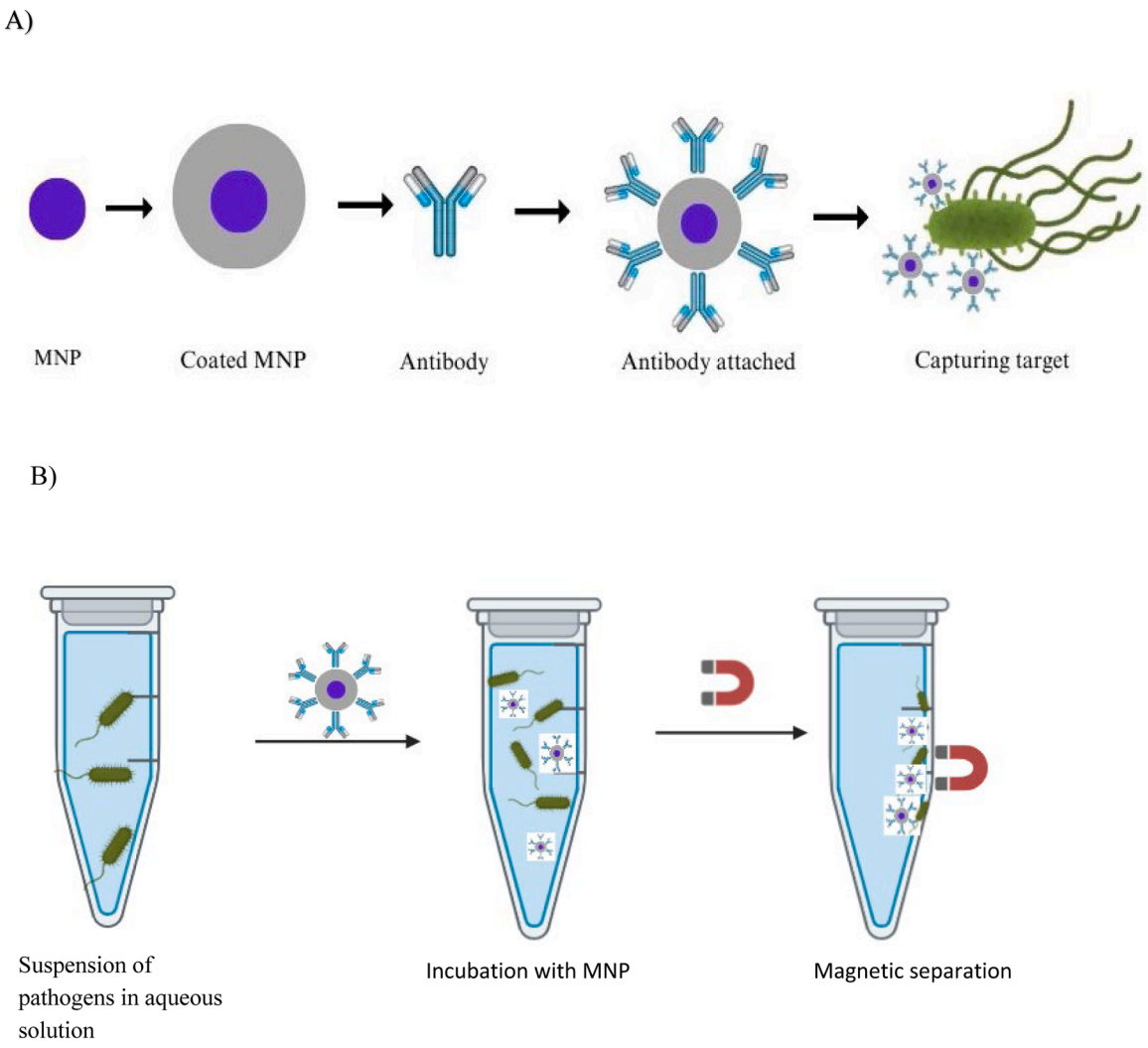

Fig. 5. Schematic representation of MNP in detection of pathogen, a) Diagrammatic depiction of a CRISPR-Cas system, b) Overview of CRISPR-based MRS.

Next, an external magnetic field is used to separate the MNPs from the supernatant that contains the free pathogen (Farinha et al., 2021) (Fig. 5). A study conducted by Wibowo et al. (2020) showed that higher M_s (saturation magnetization) improves magnetic responsivity: larger magnetic moment particles make larger T_2 relaxation differences, increasing detection abilities. For example, 100 nm Fe_3O_4 nanoparticles possessed much larger values of M_s compared to 30 nm particles and thus produced larger signal shifts for giant magnetoresistance (GMR) biosensors (Liang et al., 2017). However, there's still a best size range, though: in magnetic particle imaging, MNPs of about 15 nm provided the strongest harmonic signals because of well-balanced Néel and Brownian relaxation dynamics (Hirt et al., 2017). Likewise, perfectly monodispersed MNPs in 20–30 nm size ranges optimize T_2 relaxivity, which optimizes detection efficiency on the basis of T_2 (Tegafaw et al., 2023; Aboushoushah, 2025). On the other hand, wide size distributions and surface defects, negatively impact sensor performance by preventing effective M_s and causing undependable relaxation behaviour, reducing signal consistency (Batlle et al., 2022; Chen et al., 2017). Highly crystalline, single-domain monodisperse MNPs provide the most stable and reproducible relaxation responses—of massive importance to reliable MRS assays in multi-component matrices.

4. Application of magnetic nanoparticles (MNP) for the detection of various pathogens in food

4.1. Detection of salmonella

Salmonella spp. are rod-shaped, facultative anaerobic, gram-negative, foodborne pathogens that belong to the *Enterobacteriaceae* family (Agregan et al., 2021). Salmonellosis is one of the most common foodborne infections. The primary reservoir of salmonella is the human and other animal intestines, but detection has also been made in insects and reptiles. The most common serotypes of Salmonella that infect humans are *Salmonella typhimurium* and *Salmonella enteritidis* (Popa and Papa, 2021).

A novel phage-based detection for Salmonella was developed from the study by Huang et al. (2023), which used a magnetic separation system, a catalyst-free trans-cyclooctene/tetrazine biorthogonal reaction for signal amplification, and an MRS sensor. This reaction improved the efficiency of covalent attachment to the phage surface of small-molecule probes. Rapid and accurate detection of Salmonella was possible with the innovation of a phage-based Magnetic Relaxation Switching (MRS) biosensor. The biorecognition components, which are bacteriophages, were employed to capture bacteria. Then, the captured bacteria are separated from other organisms using magnetism, and a signal is amplified in a complex biological system with the aid of bio-orthogonal chemistry to enhance sensitivity. Due to its high specificity and low detection limit, the biosensor was able to identify *Salmonella* successfully in foodstuffs (Table 1).

The MS (Magnetic Separation)-MRS sensor was tested to detect *Salmonella enterica* in milk, a typical real sample liable to contamination. Compared to the traditional MRS sensor and enzyme-linked immunosorbent assay (ELISA), the MS-MRS sensor had better sensitivity as it detected as few as 100 CFU/mL in spiked milk, while the traditional MRS detected only $\geq 10^4$ CFU/mL. The MS-MRS signalled 12 and the normal MRS just 2 out of 20 actual milk samples that were RT-PCR confirmed positive. Even though RT-PCR is more sensitive, it is more time-consuming and expensive. On the other hand, the MS-MRS sensor provides speedy (within 30 min), and very sensitive to pathogen detection, and thus is a potential sensor for actual practice (Chen et al., 2015).

To surpass the primary limitations of conventional MRS systems, i.e., low sensitivity and non-specific binding,

Wen et al. (2023) introduced a new MRS biosensor, UPC-MRS (uracil-DNA glycosylase (UDG) assisted V-shaped PCR driven CRISPR/Cas12a-MRS), for the highly specific and sensitive identification of *Salmonella*. The platform ensures high specificity by a quadruple signal assurance system consisting of primer specificity, CRISCR/Cas12a accuracy, MRS signal stability, and UDG decontamination by integrating a contamination-free uracil-DNA glycosylase (UDG)-aided V-shaped PCR with CRISPR/Cas12a and magnetic sensing. The sensor performs better than most existing MRS methods with a detection limit of 53 CFU/mL, which is further increased by terminal deoxynucleotidyl transferase (Tdt)-mediated signal amplification. The UPC-MRS system not only removes aerosol contamination often associated with CRISPR-based analysis but also meets real sample analysis requirements. It is an optimistic method for fast and precise foodborne pathogen recognition.

To overcome the universal problems of low instability and sensitivity in conventional MRS systems due to non-specific or inadequate cross-linking of the MNPs, the study conducted by Shen et al. (2022) described a CRISPR-Cas12a-mediated MRS biosensor for the highly sensitive detection of *Salmonella*. By taking advantage of the varying MNPs of 130 nm (MNP130) and 30 nm (MNP30) nanoparticles, the biosensor utilizes CRISPR-Cas12a's collateral cleavage activity to block the MNP130-MNP30 association in the presence of target DNA, causing more unbound MNP30, which is a T_2 signal reporter. This new CRISPR-MRS biosensor has a detection limit of 1.3×10^2 CFU/mL with better sensitivity than most of the current MRS biosensors and exhibits good performance in real food samples like spiked chicken meat, with great potential for use in food safety.

Zou et al. (2019) conducted a research where Fe_3O_4 nanoparticle clusters (Fe_3O_4 NPCs) are employed in new nuclear magnetic resonance (NMR) biosensor to rapidly detect *Salmonella* in food such as milk. The sensor detects 10^5 CFU/mL without nucleic acid extraction using a streptavidin-biotin system and nanoparticle clustering, which increase signal amplification and reduce the water proton T_2 relaxation time for detection. The technique is highly particular and fast (160 min overall) than traditional culture methods. As innovative as the work is, however, more research into signal processes, commercially accessible standard data, and verification across a range of real food matrices must be done. Overcoming these shortcomings would enhance its utility and make it a standard tool for detecting foodborne pathogens (Zou et al., 2019)

A superparamagnetic nanoparticle-based new time domain nuclear magnetic resonance (TD-NMR) biosensor was demonstrated herein for quick detection of *Salmonella* in milk within a 90-minute and detection limit of 2.3×10^3 CFU/mL. The method integrates membrane filtration, SA-functionalized super-magnetic nanoparticles (SMNs), and biotin-antibody targeting for enhanced speed and sensitivity. While this method shows great potential, especially in food matrix testing and fast detection, there is a necessity for more stringent material mechanism analysis, normalized data, and field sample verification. With development, it has great promise for commercial application and may even be used as a standard for foodborne pathogen detection (Jin et al., 2020).

A study conducted by Feng et al. (2021) introduced an electrochemical immune-sensor for the determination of *Salmonella* in milk using CoFe-MOFs-graphene nanocomposites. Gold nanomaterials that were used in this method were modified on CoFe-MOFs. It possesses a low detection limit of 1.2×10^2 CFU/mL, wide linear detection range (2.4×10^2 to 2.4×10^8 CFU/mL), and high conductivity. The combination of MOFs with graphene enhances sensitivity, and the immune-sensor shows good stability, consistency, and accuracy. Although it has lots of potential, standardization, enhanced comprehension of the materials process, and further confirmation with authentic food samples are required. These enhancements provide much commercial potential to this method and the potential to create a standard for rapid foodborne pathogen detection.

4.2. Detection of Pseudomonas aeruginosa

The gram-negative pathogenic bacteria *Pseudomonas aeruginosa (P. aeruginosa)* is well-known due to its several virulence characteristics, its

capacity to build biofilms, and its resistance to antibiotics (Li et al., 2023). *Pseudomonas aeruginosa* is rod-shaped, gram-negative, aerobic, endospore-negative, catalase, and oxidase-positive bacteria that belong to the *Pseudomonadaceae* family. It could be anywhere in nature and is exposed to soil, plants, water, and animals (Urgancı et al., 2022) (Table 1).

According to Jia et al. (2017), *Pseudomonas aeruginosa* causes extensive food spoilages thus, this requires to be detected with the use of MRS aptasensor. Bacteria from the species *Pseudomonas aeruginosa* attract magnetic nanoparticles which are covered with aptamers. Aptamers are molecules that have been synthesized in the laboratory with accuracy for this reason. The bacteria affect a magnetic property, the spin-spin relaxation time, by influencing the way the nanoparticles aggregate when they are trapped. This attribute can indirectly be quantified for the detection and determination of the number of germs that are present. Other than *Pseudomonas aeruginosa*, this technology, therefore does provide a possible method of detection for several food-borne infections. A fast and selective biosensor that is colourimetric for the detection of *Pseudomonas aeruginosa* has been developed. It detects the proteolytic activity of the bacteria by way of magnetic nanoparticles and a specific protease substrate. The colour change is immediate because the bacteria cleave off the substrate, thereby releasing the magnetic nanoparticles. With the simplicity of such an effectively facile system, this methodology stands promising for point-of-care diagnosis of *P. aeruginosa* infection.

4.3. Detection of Vibrio parahaemolyticus (VP)

Vibrio parahaemolyticus is the foodborne bacterium that causes the most number of outbreaks worldwide and is continually on the increase despite proper treatment. The primary causes of *V. parahaemolyticus* infection are inappropriate food handling and preparation, the bacteria's ability to survive in a human's gut and to develop virulence, the bacterium's resistance to antibiotics, and the inability of regulatory authorities to protect the quality of food (Letchumanan et al., 2019). *Vibrio parahaemolyticus*, also known as *V. parahaemolyticus*, is a Gram-negative bacterium most commonly found in foods, food products, and any source of water. The bacterium has been known to cause gastroenteritis. Additionally, it elaborates toxins resulting in diarrheal illness (Beshiru and Igbinosa, 2023). The marine and estuarine bacteria *Vibrio parahaemolyticus* pose a serious global hazard to food safety and well-being. It causes foodborne outbreaks that harm the aquatic industry (Li et al., 2020) (Table 1).

Recently, Chen et al., 2023 published a report on a novel MRS biosensor capable of the rapid detection of the pathogenic bacterium *Vibrio parahaemolyticus* (VP) in seafood, based on both low-field nuclear magnetic resonance (LF NMR) detection and click chemistry-mediated sol-gel technology; a target-responsive aptamer is embedded within hollow mesoporous silica microspheres (HMSMs) in this sensor. This leads to the binding of aptamer to the bacterium in the presence of VP, hence a sol-gel transition. This is brought about by the relaxation behaviour of the water molecule. The transition allows the use of LF NMR to detect VP. The advantages of this technique make it suitable for pathogen onsite testing: samples can be prepared in an easy, straightforward procedure, and the detection takes place in one step in 30 min, the sensitivity being about 5 CFU/mL.

The MRS biosensor identifies *Vibrio parahaemolyticus* (VP) by sensing variations in the magnetic relaxation time (T_2) of a test sample. In this platform, signal units made up of LAP-MXene@AuNPs-ssDNA are released upon the attachment of aptamers to VP in a pipette tip-based reaction system. These signal units are subsequently appended to a solution of a disulfide-crosslinked gel, which is cleaved by the LAP under UV light to start a gel-to-sol phase transition. This phase transition disperses magnetic materials, changing the T_2 relaxation time, which is measured with low-field NMR. Increasing T_2 signals the presence of VP, providing this strategy label-free, extremely sensitive, and efficient for the detection of pathogens (Chen et al., 2025).

4.4. Detection of Listeria monocytogenes

Listeria is an intrinsic pathogen that is gram-positive, microaerophilic, rod-shaped, non-spore-forming, and positive for catalase. *Listeria monocytogenes* is the pathogen that causes listeriosis, a serious illness that can afflict both humans and animals. Listeriosis can be either invasive or non-invasive (Zamuz et al., 2021). *L. monocytogenes* can adapt and even multiply within various environmental stress conditions, such as temperature changes, pH levels, high salt concentrations, UV light exposure, and the presence of biocides and heavy metals. Additionally, in food production contexts, these bacteria might build biofilm structures on various surfaces, making it difficult to remove them and stay there for long. This leads to an extremely heightened risk of food production facilities and the food being contaminated (Osek et al., 2022) (Table 1).

For sensitive and quick detection of *Listeria monocytogenes* a new DNA biosensor has been constructed using Au@Pt nanozyme-mediated MRS. The Au@Pt nanoparticles with elevated stability and high peroxidase-like activity cause the hydrogen peroxide decomposition. With magnetic separation and DNA hybridization, a sandwich structure is formed (MNP180-probe1-target DNA-probe2-Au@Pt), which controls the Mn(VII)/Mn(II) redox conversion, changing the transverse relaxation time. The Au@Pt content is directly proportional to the *L. monocytogenes* concentration. This redox-mediated signal amplification and nanozyme catalysis-based system has a 30 CFU/mL detection limit, which is 33 times that of ELISA, a highly sensitive method, hence it is a potentially powerful tool for the detection of foodborne pathogens in complex food matrices (Wu et al., 2022).

MRS biosensor based on DNA is introduced for the rapid and precise detection of *Listeria monocytogenes* in ready-to-eat foods. To form a sandwich nanocomplex upon target DNA binding, specific probes were tagged onto 30 nm and 250 nm magnetic nanoparticles through a nucleic acid hybridization method. The unbound 30 nm magnetic nanoparticles (MNP30-probe2) served as the T_2 magnetic signal readout following magnetic separation, which was measured through monitoring changes in transverse relaxation time. The assay recovered to a high rate of 92.6 % in spiked ham sausage samples and could detect 50 CFU/mL with no prior DNA amplification requirement within 2 h. Therefore, for pathogen detection in complex food matrices, the MRS mechanism provided a sensitive and precise platform (Qi et al., 2021).

4.5. Vibrio alginolyticus

Vibrio alginolyticus, a salt-loving bacterium in oceans and estuaries, which leads to cholera-like soft tissue infections, sepsis, and other extra-intestinal infections. Individuals are exposed to the bacteria via water or through ingestion of infected seafood (Slifka et al., 2017).

For the detection of *V. alginolyticus* a sensitive and quick MRS sensor was created with aptamer-functionalized magnetic nanoparticles (Fe_3O_4@SiO_2-AptVA). The sensor was based on the dispersion or aggregation of these nanoparticles upon target binding, influencing the transverse relaxation signal (ΔT_2w), which had the best correlation with bacterial concentration. The optimized system had a detection limit of 26 CFU/mL, with 91–113 % recovery, good specificity, and finished detection in 15 min which is considered as fast compare to traditional methods. The method provides a simple, quick, and precise tool for the detection of foodborne pathogens (Wang et al., 2021).

4.6. Aspergillus flavus

Aspergillus flavus is a saprophytic fungus that occupies soil naturally as a facultative parasite, infecting various significant food crops pre- and post-harvest. *Aspergillus flavus* produces the carcinogenic and mutagenic polyketide secondary metabolite aflatoxin. Aflatoxin contamination is

an international food safety issue for the marketability and safety of many food crops (Abd El-Hack et al., 2023).

Lu et al. (2023) developed a very sensitive and simplified MRS biosensor for the detection of Aflatoxin B1 (AFB1) based on a new approach by combining one millimeter-sized polystyrene sphere (PSmm) as a micro-reactor and 150 nm magnetic nanoparticles (MNP150). The Single Polystyrene Sphere MRS (SMRS) sensor increases signal concentration on the PSmm surface by an immune-competitive reaction without signal dilution and without cumbersome washing steps. It had a detection range of 0.02–200 ng/mL and a limit of detection of 14.3 pg/mL, and was perfectly validated in maize and wheat samples, providing a simple, enzyme-free, and very sensitive approach applicable to trace detection of small molecules.

4.7. Noro-virus

Norovirus (NoV) is responsible for nearly one-fifth of all foodborne disease cases and ranks as one of the leading causes of domestically acquired foodborne acute gastroenteritis and outbreaks. NoV illness frequently is linked with eating contaminated fresh and ready-to-eat produce, fresh and frozen berries, raw or undercooked bivalve mollusks and products that become contaminated through handling (Guix et al., 2019).

A dual-mode biosensor consisting of Fe_3O_4@Au nanoparticles was fabricated for the ultrasensitive and specific detection of norovirus (NV) via magnetic relaxation and fluorescence signals. The antibody-FAM-labelled aptamer-functionalized probe reacts to NV through nanoparticle aggregation (changing magnetic relaxation) and aptamer release (revising fluorescence), and the detection limits reached as low as 0.38 and 3.45 pg/mL. It demonstrated excellent accuracy in NV detection from spiked food materials such as oysters and strawberries, and was comparable to ELISA performance. This dual-signal, dual-recognition strategy improves sensitivity, decreases false positives, and is highly promising for effective food safety monitoring of viruses (Wang et al., 2023).

5. Limitations of traditional MRS biosensors and their improvements

MNPs are used to detect bio-macromolecules but struggle with tiny components like antibiotics due to low sensitivity. Xianyu et al. (2020) introduced a new magnetic probe combining 150 nm MNPs and Gd^{3+}-chelates to boost magnetic relaxation and sensitivity in biosensing applications (eMRS).

To overcome the drawbacks of MRSs which as low sensitivity, researchers have reported a straightforward-to-use, fast, robust, and sensitive MRS-based sensing method for bacteria and viruses based on magnetic separation (MS) as well as protein, nucleic acid, and enzymes. In this new approach, superparamagnetic iron-oxide nanoparticles are used, which self-assemble differently in the presence of target analytes. This then affects the spin-spin relaxation time (T_2) of the surrounding water molecules to allow for sensitive detection of a wide range of biomolecules. Results from MRS sensors show that it has high sensitivity and selectivity in detecting varying objects. Reports have documented that it can reach detection limits as low as 0.5 nM in lysozyme and 0.2 pM in telomerase activity (Gui-Ying et al., 2018).

Traditional techniques for detecting pathogens are time-consuming and expensive equipment. Thus, the work performed by Wei et al. (2021) proposed a rapid and accurate method of detecting foodborne pathogens based on a new biosensing platform. This exploits the direct T_2 biosensor detecting specific alterations in water proton relaxation time due to pathogen-antibody interaction coupled with an ALP-mediated hydrogenation process.

Research by Chen et al. (2017) noted the importance of the timely sensitive and accurate detection of pathogenic bacteria in seafood. Even with the currently available detection methods, such as molecular biology, immunoassays, and microbial enumeration, they are sensitive and precise; however, background interference might be imminent. Therefore, a novel MRS biosensor based on a redox reaction for the detection of *Vibrio parahaemolyticus* (VP) in seafood was developed using LF NMR detection combined with click chemistry-mediated sol-gel technology. Upon binding with VP, the signal unit releases sodium ascorbate, which triggers a sol-gel transition that changes the water state and reduces T_2 values.

Huang et al., 2021 reported a new MRS biosensor that detects amplified BPA (bisphenol A) by the self-assembly of magnetic nanoparticles and polystyrene microspheres. Unlike traditional MRS, this process provides greater sensitivity and accuracy wherein it can identify a smaller amount of BPA. However, the process contains so many steps that it may become difficult to apply.

A novel biosensor, uracil-DNA glycosylase (UDG) assisted V-shaped PCR-driven CRISPR/Cas12a (UPC)-MRS, was developed for rapid sensitive detection of foodborne pathogenic bacteria by integrating CRISPR/Cas12a with deoxynucleotidyl transferase (Tdt)-mediated signal amplification and UDG-assisted V-shaped PCR. Compared to traditional MRS methods, UPC-MRS improves sensitivity and specificity, preventing disease outbreaks and potential economic losses (Wen et al., 2023) (Table 2).

To overcome the limitations of MRS, Shen et al. (2022) integrated the CRISPR-Cas12a system into an MRS biosensor. It makes use of the collateral cleavage activity of Cas12a to control the binding of two different sizes MNPs (MNP130 and MNP30), thus detecting Salmonella sensitively. With the distinct magnetic properties of bound and free MNPs, it provides a clear readout without any complex signal transduction. Two substrates developed for cleavage have been used: MNP130-S1 for qualitative detection, and biotin-S1, which is used for quantitative detection and is more sensitive.

According to Dong et al. (2022), the traditional MRS biosensors hold a low sensitivity and possible instability. A novel approach was developed for improving the MRS biosensors for detecting biomolecules. It employed polydopamine, as a precursor material biocompatible that can capture copper ions. The concentration of captured ions triggered a copper-catalysed click reaction resulting in the agglomeration of magnetic nanoparticles. This agglomeration caused a large effect on the magnetic signal and changed this detection process into a more sensitive and stable one.

Dong et al. (2024) conducted a study which revealed Cu^{2+}-induced MRET (magnetic resonance tuning), and Fe_3O_4@PDA (polydopamine)-assisted signal transduction to form a new immunosensor, the Cu-M-MRS, that allowed the selective and sensitive detection of foodborne pathogens. Because it avoided the "prozone effect" and exploited a new MRET strategy-based magnetic signal detection technology, this new method surpassed the shortcomings of traditional MRS. This was a prospective tool for food safety since it could perform better than the existing biosensors in operation.

Hou et al. (2023) developed a new method for the analysis of pyrethroids (PYR) in environmental and dietary samples. Advanced detection methods are needed because PYRs are pesticides that might impact human health and the environment. The technique utilized an MRS sensor. The novelty approach provided by the modern MRS sensors is sensitive to use, mainly because of the employment of nanoparticles with gold, and a unique antibody that improves the overall accuracy of detection. At very low levels, it could detect different PYRs in complex material compositions such as milk or water (Table 2).

6. A new method based on MRS

CRISPR/Cas systems use a guide RNA to target specific DNA or RNA regions, enabling accurate recognition and cleavage (Lei et al., 2024). CRISPR-based nucleic acid detection has emerged as a popular study issue in the field of food fast detection due to its benefits of high sensitivity, high specificity, modularization, programmability, convenience,

Table 2
The key improvement over the traditional biosensors.

Limitations	Description	Improvement	Method	Reference
Low sensitivity	Researchers create a highly sensitive magnetic sensor using gadolinium-loaded nanoparticles for detecting ractopamine at trace levels in complex samples.	Improved sensitivity using 150 nm MNPs and Gd^{3+} bases chelates	Gd^{3+}-nanoparticle-mediated MRS biosensor	Xianyu et al., 2020
Low sensitivity	Use of superparamagnetic nanoparticle-based MRS for quick and precise detection in the sectors of medicine and food safety	Magnetic Relaxation Switch (MRS) biosensors are being improved with an emphasis on integrating cutting-edge techniques including aptamer technology, optimizing detection settings to increase sensitivity, and improving nanoparticle modification for improved stability and biocompatibility. Furthermore, MRS can test quickly and non- destructively, which makes it appropriate for real-time monitoring of complicated samples. All of these developments improve the functionality and adaptability of MRS biosensors across a range of industries.	SPIO(super magnetic nanoparticle) based MRS	Gui-Ying et al., 2018
Limited sensitivity	Researchers created a new foodborne pathogen sensor that detects bacteria by monitoring changes in water molecule movement instead of relying on magnetic fields.	Direct T_2 biosensing using alginate hydrogenation mediated by ALP	Electrochemical biosensor, MRS biosensor	Wei et al., 2021
Limited detection time	It describes a one-step magnetic biosensor using click chemistry for rapid detection of *Vibrio parahaemolyticus* bacteria at very low concentrations.	Quick detection using LF NMR and sol-gel technology	Sol-gel technology	Chen et al., 2023
Complexity	A new type of MRS biosensor was created to detect BPA (bisphenol A) with high sensitivity. The magnetic nanoparticles and polystyrene microspheres used in the sensor self-assemble when BPA is present, changing the magnetic relaxation time. When compared to conventional MRS approaches, this method offers better sensitivity and accuracy, making it possible to detect BPA in a variety of samples, such as water and materials used in food packaging.	In comparison to traditional MRS techniques, the enhancement resides in the use of magnetic nanoparticles and polystyrene microspheres to increase the magnetic relaxation signal, allowing for more accurate and sensitive BPA detection.	Polystyrene microspheres-MRS	Huang et al., 2021
Laborious PCR method	A novel biosensor (UPC-MRS) that combines DNA amplification and CRISPR for highly sensitive and specific *Salmonella* detection to overcome limitations in the magnetic detection of pathogenic germs	Novel uracil-DNA glycosylase-assisted V-shaped PCR-driven CRISPR/Cas12a-MRS (UPC-MRS) biosensor	CRISPR-MRS	Wen et al., 2023
Less sensitive and low stability	Traditional MRS have low sensitivity due to the non-specific binding of MNP therefore CRISPR-Ca 12 method is used for the detection of *Salmonella*	Controlling MNP binding precisely with the CRISPR-Cas12a system. Certain sequences of *Salmonella* DNA are recognized by CRISPR-Cas12a.Cas12a's collateral cleavage activity intensifies the signal. The overall stability is improved by less non-specific binding.	CRISPR-MRS biosensor	Shen et al., 2022
Unsatisfactory sensitivity	Traditional MRS biosensors' MNP probes can aggregate due to complex sample matrices, which can produce inaccurate readings.	A new approach enhances magnetic relaxation switching (MRS) biosensors for biomolecule detection. It integrates polydopamine (PDA) and copper-catalyzed click chemistry (CuCC). PDA selectively captures copper ions, and their concentration controls a CuCC reaction that triggers magnetic nanoparticle clumping. This controlled clumping results in a stronger, more stable magnetic signal allowing for detection of tiny biomolecule amounts, overcoming limitations of traditional MRS sensors in sensitivity and stability.	PDA-MRS	Dong et al., 2022
Low sensitivity	Traditional biosensor suffers from a narrow liner detection range due to the prozone effect and also suffer in detecting analytes that have low concentration	The method tackles the issue by using Cu^{2+} ions to quench the signal and MNPs to enhance it. The sensitivity and accuracy are increased by a factor of 77 and the new method becomes a highly adaptable platform for detecting food safety.	Cu-M-MRS	Dong et al., 2024
Limited sensitivity	PYRs are insecticides that are harmful to human health. Traditional MRS have various limitations for the detection of trace amounts of PYRs so there's a need for a good detection method.	The new sensor detects several PYRs at once by using a broad-spectrum antibody (CL-CN/1D2). This new sensor uses gold nanoparticles which enhance the effectiveness of capture, resulting in enhanced detection limits (down to 2.72 μg/L for cypermethrin).	Gold-functionalized MRS sensor	Hou et al., 2023

low cost, and less time consumption (Sun et al., 2022). It is well known that CRISPR targets DNA. CRISPR technology was initially used for gene editing. A Cas12a protein is attached to a target sequence upon recognition by a specific crRNA molecule (Dai et al., 2019). Recent studies conducted by Gama-Brambila et al. (2021); Shi et al. (2024) indicate two orthogonal strategies to engineer Cas proteins' stability problems in practical applications. One strategy is to design Cas proteins with a π-clamp Phe-Cys-Pro-Phe (FCPF) tag, which enables chemical, precise control over their activity and stability through PROTAC (Proteolysis--targeting chimeras)-FCPF molecules. The targeted degradation of Cas proteins is facilitates by the ubiquitin-proteasome system, increasing biosensing safety and reducing off-target effects. Simultaneously, another important area concerns the application of thermostable Cas variants, such as Brevibacillus-derived Cas12b, that are still functionally stable at higher temperatures. These enzymes enable one-pot detection systems such as SHERLOCK-LAMP, (Specific High-sensitivity Enzymatic Reporter unlocking-loop-mediated isothermal amplification) simplifying complexity and enzyme degradation during point-of-care diagnostics. When utilized with portable microfluidic or paper-based biosensors, both chemical and thermostability approaches increases the reliability, accuracy, and convenience of CRISPR-based diagnostics in on-site and point-of-care applications.

A new approach in combining the CRISPR/Cas system with MNPs and biosensor techniques has been designed to identify targets and read signals for CRISPR-based MRS biosensors (Hu et al., 2024; Shen et al., 2022). In CRISPR-based MRS biosensors, a specific nucleic acid sequence is identified and bound by the CRISPR-Cas system. Release or aggregation of MNPs occurs as a result of the activation of collateral cleavage activity of Cas proteins when it comes into contact with this target. The change in state of the MNP (aggregation or dispersion) is sensed through a variation in the magnetic relaxation time, T_2, and this can produce a measurable signal that correlates to the concentration and presence of the target analyte. CRISPR-based MRS is an effective method for detecting pathogens. This biosensor makes use of two crucial elements: Accurate manipulation of magnetic nanoparticles (MNPs) and Cas12a's target-specific cleavage activity. Adding nanomaterials such as MNP improves the signal's sensitivity, stability, and amplification (Wei et al., 2023). Biosensing has gained strength with the combination of magnetic particles and CRISPR/Cas systems. Cas proteins, which can cleave or trans-cleave targets, allow for precise target recognition and cleavage in these systems. In the case of target recognition, these proteins cleave not only the target nucleic acid but also nearby single-stranded DNA or RNA molecules, allowing signal amplification. Because of these advantageous characteristics, adding magnetic particles improves biosensor performance (Wang et al., 2024). During pathogen detection, a specific target RNA sequence is conjugated to the target pathogen bounded by CRISPR/Cas12a. Cas12a becomes activated when it binds and excises non-specifically cleaving the MNP30-MNP1000-HCR hybridization chain reaction complex. The complex releases MNP30 cleaved from the reaction. The discrimination between the smaller MNP30 and the complex of the bigger MNP1000-HCR would be achievable using magnetic fields. MNP30 released follows the inverse relation of its concentration with T_2 relaxation time used for quantification. This technique offers great sensitivity and specificity for the detection of pathogens in food samples (Wei et al., 2023; Shen et al., 2022). The two main components of the CRISPR/Cas9 gene-editing technique are Cas9 (CRISPR-associated protein 9), an endonuclease that breaks double strands of DNA to allow alterations to the genome, and a (gRNA)guide RNA that matches a particular target gene (Koonin and Makarova, 2019). A certain sequence in the bacterial DNA is recognized by the system when bacteria is infected by a virus. A section of the bacterial DNA is subsequently cleaved by the Cas enzyme and integrated into the CRISPR locus. This spacer sequence that was acquired acts as an infection memory (Hu et al., 2023). There are multiple crucial phases in the CRISPR/Cas9 genome editing mechanism. The target gene must first be identified before a guide RNA (gRNA) that matches its complementary sequence can be created. After this, a combination consisting of the gRNA and the Cas9 enzyme is added to the cell. When Cas12a is added to a sample it scans the genetic material. If the target pathogen gene is present, the crRNA binds to it and this activates Cas12a. Once activated Cas12a cuts the target gene (Fig. 6). The Cas12 enzyme is directed to cut both strands of the DNA molecule at the desired site by the gRNA, which binds to the target DNA sequence. A double-strand break is caused by this exact cleavage, which can be repaired by cellular machinery and may result in desired genetic changes (Mc Ginn & Marraffini, 2019; Chakraborty et al., 2022) (Fig. 6). Firstly, it accurately cuts target DNA sequences. Secondly, after cleaving the target DNA, Cas12a cleaves neighbouring single-stranded DNA (ssDNA) molecules without showing preference (Shen et al., 2022). The high sensitivity and specificity of CRISPR-based biosensors arise from their ability to precisely recognize the target. This makes it feasible to detect scarce targets. Another reason for their extensive versatility is the wide range of biomolecules with which they can be used-directly with proteins, DNA, RNA, as well as extremely small molecules. The swift measurement of signals facilitates real-time or near-real-time detection and allows point-of-care applications that require minimal sample preparation (He et al., 2023). However, CRISPR-based MRS is still in its initial stage (Wen et al., 2023). A brief comparative examination of new biosensing technologies signifies significant variation in sensitivity, specificity, detection range, and complexity. ELISA is highly sensitive but has moderate specificity, whereas nanopore electrochemical sensors yield real-time, label-free detection with broad detection ranges. Isothermal amplification methods such as LAMP exhibit high sensitivity and specificity with low detection thresholds. MRS biosensors uses magnetic relaxation for ultrasensitive detection but have no data on standardized specificity. CRISPR-based sensors exhibit high genetic specificity with low detection thresholds (Table 3). Even though MRS and CRISPR-based biosensors are well-documented to be effective,

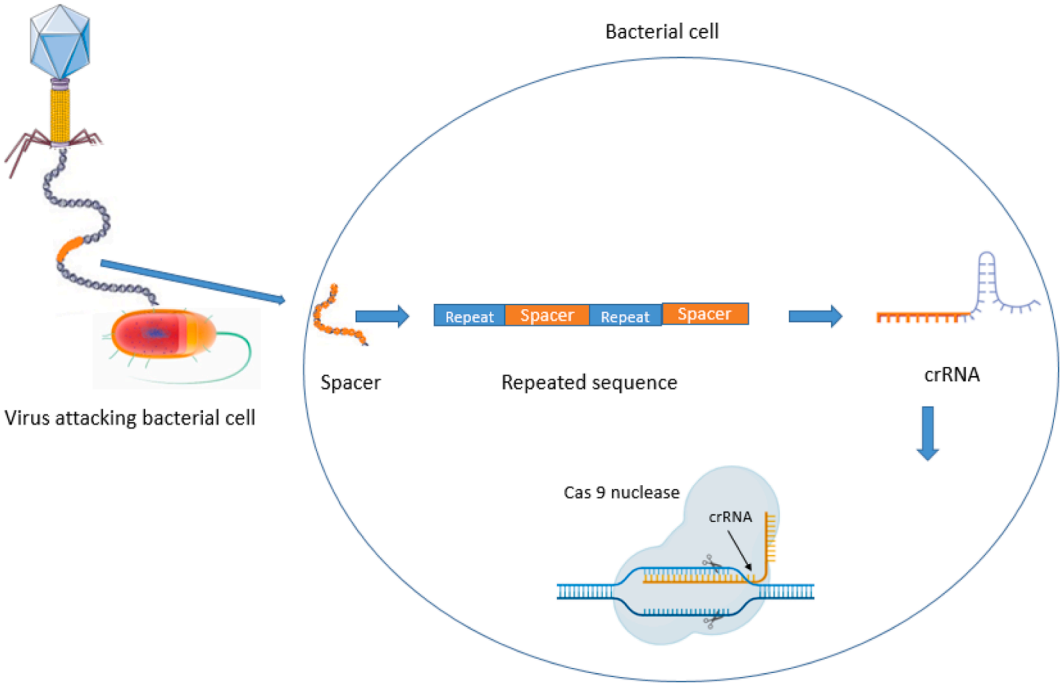

Fig. 6. a) Diagrammatic depiction of a CRISPR-Cas system.

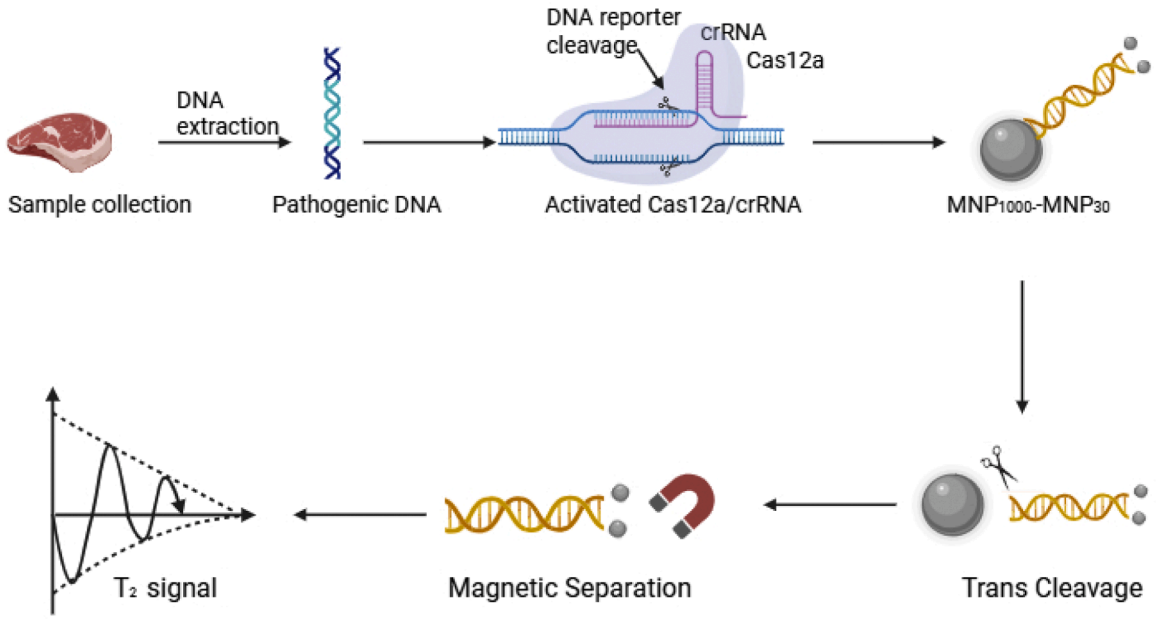

Fig. 6. b) Overview of CRISPR-based MRS.

Table 3
Comparative analysis of emerging bio-sensing technologies.

Method	Description	Sensitivity	Specificity	Detection range	Linearity	References
ELISA	Uses antibody to detect harmful substances	100 %	81 %	1×10^4CFU/mL	0.15–34.5 ng/ml	Pang et al., 2018; Di Febo et al., 2019
Nanopore electrochemical sensors i	Identify pathogens through the monitoring of ionic current or electrochemical signals alterations while single pathogen molecules traverse or bind with a nanometer-sized pore, providing real-time, label-free, and ultra-sensitive detection.	1–10^8 CFU/mL	1.9 × 10^3CFU/mL to 1.9 × 10^6 CFU/mL	10^1 CFU/mL to 10^{10} CFU/mL	–	Bobrinetskiy et al., 2021
Isothermal amplification	LAMP works as amplifying specific DNA producing fluorescent signal	96.6 %	97.6 %	LOD-2CFU/mL	–	Sadeghi et al., 2021
MRS Biosensor	Measures changes in the spin relaxation time of surrounding water proton which is due to MNP aggregate or disperse upon binding pathogen	0.1ng/mL	–	LOD-37/pg/mL	4.4×10^4–4.4 × 10^5(pmol/L)	Huang et al., 2021
CRISPR	Guide RNA s used to recognize specific genetic sequence of pathogen and upon binding the Cas get activated that cleave the one that produce signal	40CFU/mL	–	LOD-4.0 × 10^1CFUm/L	–	Sun et al., 2020

real-world food samples are highly challenging and underrepresented in the review. Foods have complex matrices—proteins, fats, sugars, particulates—that can suppress amplification reactions or prevent CRISPR enzyme activity, resulting in false negatives or compromised sensitivity (Nayak and Dutta, 2023). The research conducted by Xing et al. (2023) adequately remedies the insufficient multiplex detection approaches by proposing a finger-actuated microfluidic biosensor (FA-MB) that allows the simultaneous detection of seven foodborne pathogens, including Bacillus cereus. Through the combination of recombinase aided amplification (RAA) and CRISPR/Cas12a within a one-pot system featuring isolated wells and one-way valves, it reduces interference and cross-contamination from complicated food matrices. This handheld, low-cost, and highly sensitive (LOD < 500 CFU/mL) biosensor provides the results in one hour and shows good performance in real food samples, providing a potential solution for on-site multiplex food safety testing in resource-limited environments.

For pathogen detection, only a few organisms can be detected by using the CRISPR-Cas, (Table 4). CRISPR has worked on *E. coli* (Sun et al., 2020), *E.coli* (Wang et al., 2021), *Zymomonas mobilis* (Banta et al., 2020), *Listeria monocytogenes* (Li et al., 2021), *Listeria monocytogenes* (Li et al., 2021), *Listeria monocytogenes* (Xiao et al., 2023), Methicillin resistance *Staphylococcus aureus* (Suea-Ngam et al., 2021), *Salmonella* (Wang et al., 2022), *Salmonella* (Liu et al., 2024), *Salmonella* (An et al., 2021), *Campylobacter jejuni* (Li et al., 2022), *Campylobacter jejuni* (Huang et al., 2021), *Campylobacter jejuni* (Zhang et al., 2024), *Campylobacter jejuni* (Xiao et al., 2023) *Shigella flexneri* (Shi et al., 2022).

7. Conclusion and future perspectives

The field of pathogen identification has significantly improved with the integration of modern biosensor technologies, particularly those that use MRS. The risk of foodborne illnesses brought on by microbial contamination has grown in importance as global trade expands. However, MRS also faces various challenges during pathogen detection. One of the main challenges is their dependence on heavy, temperature-sensitive low-field NMR equipment, which constrains portability to point-of-care applications. The next challenge is that MNPs tend to be unstable because of their physicochemical nature and sensitivity to nonspecific biochemical interaction. Also the true multiplex identification is challenging to attain because the majority of MRS systems do not have discrete magnetic signals with which to identify multiple targets in one step. Therefore, the CRISPR-MRS biosensor is a breakthrough for

Table 4
CRISPR/Cas systems for pathogen detection.

Pathogens	System used	Target nucleic acid	Principle	Cas used	Target gene	Sensitivity	Time	Detection	Advantage	Ref
E.coli O157:H7	CRISPR	DNA	CRISPR-Cas9 targets specific genes of E.*coli*	Cas9	hylA gene	4.0 × 10^1CFU mL−1	2 hr	Fluorescence detection	This CRISPR-Cas9 based method offers specific, sensitive, and easy detection of *E. coli* O157:H7 with a wide range	Sun et al., 2020
E.coli O157:H7	CRISPR/Cas12a	DNA	This method uses CRISPR-Cas12a to target the rfbE gene in *E. coli* O157:H7 DNA for specific and sensitive identification.	Cas12a	rfbE gene	9 μg/mL and 6.5 × 10^8 CFU/mL)	4 hr	Fluorescence detection	Compared to traditional method this approach offers rapid detection with high sensitivity.	Wang et al., 2021
Z. mobilis	CRISPRi	RNA	CRISPRi system to precisely repress gene expression in *Z. mobilis*, including essential genes, for improved biofuel production.	dCas9	rpIL, pdc	–	–	Fluorescence detection	This enables comprehensive gene function discovery and rational design of high-yield biofuel strains.	Banta et al., 2020
Listeria monocytogenes	RAA(recombinase assisted amplification) based E-CRISPR	DNA	RAA-based E-CRISPR amplifies target *Listeria* DNA and leverages CRISPR for specific detection	Cas12a	–	0.68× 10^{-17} M and 9.4 × 10^2	45mins	Fluorescence detection	This method offers high-sensitivity and simplicity for detecting	Li et al., 2021
Listeria monocytogenes	CRISPR	DNA	Cas12aFDet integrates PCR/RAA amplification with Cas12a cleavage in a sealed tube for rapid (15 min) and contamination-free detection of amplified nucleic acids.	PCR-Cas12aFDet and RAA (recombinase-aided amplification)-Cas12aFDet	–	3.37 × 10^1 CFU/mL and 1.35 × 10^2 CFU/mL	15mins	Fluorescence detection	Cas12aFDet offers significant time savings (15 min vs. 2 h) and avoids amplicon contamination risk for precise and accurate detection of pathogens like Listeria.	Li et al., 2021
Listeria monocytogenes	CRISPR	DNA	By using a targeted molecule (crRNA) to target specific *Listeria* DNA, CRISPR-Cas12a activates a protein (Cas12a) that cuts a reporter molecule and produces a signal.	Cas12a-MA (micro-amplification)	hyla, prfa, Lm8	4.4CFU/g	25mins	Fluorescence detection	Compared to conventional approaches, this approach provides faster and less expensive detection of *Listeria*, and its visual readout may be useful in environments with limited resources.	Xiao et al., 2023
Methicillin resistance Staphylococcus aureus	E-Si-CRISPR	DNA	E-Si-CRISPR is an amplification-free biosensor using CRISPR/Cas12a with silver metallization to directly detect target genes	Cas12a	mecA gene	3.5 and 10 fM	1.5 hr	Electrochemical	E-Si-CRISPR offers ultrasensitive, specific, and amplification-free MRSA detection with simple gRNA modification for various targets.	Suea-Ngam et al., 2021
Salmonella	Cas9-LFS(lateral flow strips)	DNA	Using a particular FITC label, Cas9-LFS amplifies *Salmonella* DNA. It then targets the amplicons with Cas9/sgRNA and finds them using gold nanoparticles on a lateral flow strip for visual readout.	Cas9	fimA gene	10^2CFU/mL	–	–	When compared to conventional LFS, Cas9-LFS provides a more precise and sensitive approach for detecting *Salmonella* with fewer false positive results.	Wang et al., 2022
Salmonella	RPA-CRISPR	DNA	This method combines RPA for rapid target DNA amplification with CRISPR-Cas12a for specific detection of *Salmonella*, making it suitable for on-site applications.	Cas12a	fimY gene	1 × 10^{-4} ng/μL	45mins	Isothermal amplification methods	RPA-CRISPR/Cas12a offers a simple, rapid, and sensitive method for on-site detection of *Salmonella* in food samples without requiring complex equipment.	Liu et al., 2022
Salmonella spp	RPA(recombinase polymerase amplification)-CRISPR	RNA	This method combines RPA for target DNA amplification with Cas13a-based detection to achieve	Cas13a	invA gene	10^2 copies	One step RPA-Cas13a-20 mins and two steps	RPA	This approach offers a one-tube (simple) or two-step (more sensitive) option for *Salmonella* detection with a great degree of	An et al., 2021

(*continued on next page*)

Table 4 (*continued*)

Pathogens	System used	Target nucleic acid	Principle	Cas used	Target gene	Sensitivity	Time	Detection	Advantage	Ref
			rapid and specific detection of *Salmonella* spp				RPA-Cas13a −45mins		accuracy and comparable sensitivity to real-time PCR.	
Campylobacter jejuni	ICB-LAMP-CRISPR	DNA	The ICB-LAMP-CRISPR/Cas12a method first enriches the bacteria using magnetic beads, amplifies a particular DNA sequence using LAMP, and then uses CRISPR-Cas12a to cleave the amplified DNA and a reporter molecule, producing detectable fluorescence.	Cas12b	hipO gene	8 CFU/mL	70mins	Digital fluorescence detection	It can be used in resource-constrained environments since it is quick, easy to use (one-tube reaction), sensitive (detects down to, specific (CRISPR-Cas12a targeting), and visually appealing.	Li et al., 2022
Campylobacter jejuni	CRISPR	DNA	This technique targets a particular and conserved 20-base sequence in the DNA of *C. jejuni* by using CRISPR-Cas12b. Cas12b cleaves a reporter molecule to produce visible fluorescence if *C. jejuni* is present.	Cas12b	mapA gene	~10CFU/g	~40 mins	Green Fluorescence was observed	Rpid detection and high sensitivity compared to traditional method	Huang et al., 2021
Campylobacter jejuni	RAA-CRISPR	DNA	This technique combines CRISPR-Cas12a for detection with RAA to amplify a distinct *C. jejuni* gene (yclQ). Cas12a cleaves a reporter molecule to produce visible fluorescence if *C. jejuni* is present.	Cas12a	qclQ gene	1.8×10^1CFU/ mL	50 mins	Fluorescent illumination and test strips for nucleic acids	Rapid detection with a 100 % accuracy rate	Zhang et al., 2024
Clostridium perfringens	RAA-CRISPR/ Cas12a-FL (fluorescence signal) and RAA-CRISPR/ Cas12a-LFS(lateral flow strips)	DNA	This technique combines CRISPR/ Cas12a for specific detection with recombinase-aided amplification (RAA) for rapid DNA amplification. In the event that *C. perfringens* DNA is present, RAA amplifies the target gene, and Cas12a cleaves a reporter molecule, producing a visible band on a lateral flow strip or a fluorescent signal.	Cas12a	cpa gene	1×10^3CFU	Within 1 hr	Fluorescent signal and LFS	The technique provides excellent sensitivity (detects as few as two copies of DNA), specificity (no cross-reactivity with other bacteria), and fast detection (within an hour). For a variety of uses, there are formats for both fluorescence and lateral flow strips.	Xiao et al., 2023
Shigella flexneri	CRISPR	DNA	This technique identifies the bacterium by activating Cas12a in response to target DNA and then cleaving a reporter molecule to produce fluorescence. It combines LAMP for *Shigella* DNA amplification with CRISPR-Cas12a guided cleavage.	Cas12a-E-LAMP		$4 \times 10^{\circ}$ copies/ μL	40 mins	Fluorescence detection	This approach provides a visual readout through fluorescence and simplifies detection by combining LAMP and CRISPR-Cas12a in a single tube. Because it does not require complicated equipment, it is perfect for environments with minimal resources.	Shi et al., 2022

food safety and detection of pathogen. It serves as a valuable tool in solving important challenges in those fields, such as high sensitivity, specificity, and usability. This review discusses the development of CRISPR-Cas12a-assisted MRS biosensors to increase detection sensitivity for pathogens towards improved food safety.

Future generations of CRISPR-MRS biosensors are anticipated to leverage the upcoming technologies in artificial intelligence (AI), AI based nano-biosensor technologies and machine learning, especially for autonomous signal processing and pattern recognition, which can effectively improve real-time detection efficiency and decision-making. Moreover, the integration of these technologies can create environmentally friendly, biodegradable nanomaterials which can enhance the environmental sustainability and biocompatibility of biosensors. Integration with mobile phone-based platforms and handheld diagnostic devices may facilitate rapid *in-situ* testing even in resource-poor environments. Overcoming the existing impediments, including resilience in difficult food matrices and multiplexed detection capacity, should be one of the high-priority areas of research. With ongoing interdisciplinary advancements, these biosensors have the enormous potential to revolutionize pathogen surveillance, minimize foodborne disease outbreaks, and maximize global food safety and security.

Ethical statement -studies in humans and animals

This article is a review and does not contain any studies involving human participants or animals performed by any of the authors. Therefore, ethical approval and informed consent were not required

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Hiya Borah: Writing – original draft, Visualization, Validation, Conceptualization. **Omar Bashir:** Writing – original draft, Visualization, Supervision. **Prashant Anil Pawase:** Writing – review & editing, Investigation. **Imdad Ul Hoque Mondal:** Writing – review & editing, Visualization, Validation. **Szilvia Várallyay:** Writing – review & editing, Visualization, Methodology. **Diána Ungai:** Writing – review & editing, Resources, Investigation, Formal analysis. **Béla Kovács:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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