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Rapid and highly sensitive detection of *Salmonella* using specific aptamers and nucleic acid enrichment technology

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Abstract

Salmonella is a significant pathogen that is responsible for foodborne diseases, and the rapid detection of this pathogen is crucial for ensuring food safety. Traditional detection methods are often time-consuming due to the requirement of bacterial enrichment. To address these challenges, this study developed a novel *Salmonella* enrichment technique utilizing carboxylated magnetic beads and ultrafiltration membranes, and designed and synthesized a specific nucleic acid aptamer targeting the *invA* gene of *Salmonella*. By integrating nucleic acid enrichment technology with a specific aptamer, we achieved quantitative detection of low-concentration *Salmonella* using fluorescence spectrometry without the need for conventional enrichment steps. This method demonstrated a detection limit as low as 1 colony-forming units (CFU)/mL, a linear range from 10^0 CFU/mL to 10^3 CFU/mL, and a correlation coefficient of $R^2 = 0.9864$. Compared with conventional methods, this approach exhibited 2.4-fold greater sensitivity accompanied by significant signal amplification. In terms of stability, when the concentration of unknown *Salmonella* was measured via plate counting methods, a value of 5.76×10^2 CFU/mL was obtained, comparing well with our method, which yielded a concentration of 5.49×10^2 CFU/mL, indicating good performance. Additionally, this method exhibited high specificity, which allowed for the accurate detection of *Salmonella* even in the presence of multiple interfering bacteria, including *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes*, and *Shigella* spp. This novel technique overcomes the limitations of traditional enrichment-based methods, thereby offering an efficient and rapid approach for *Salmonella* detection in food, with broad potential for practical applications.

Keywords: Salmonella; aptamer; Salmonella detection; InvA gene; food safety

Introduction

Salmonella is a leading global cause of bacterial foodborne illnesses and ranks among the four pathogens responsible for diarrhea diseases (Besser, 2018; Billah & Rahman, 2024). It can also cause severe conditions, including enteric fever, septicemia, and gastroenteritis characterized by symptoms like diarrhea, abdominal cramps, and fever (Zha *et al.*, 2019). *Salmonella* causes approximately 95 million infections worldwide, resulting in approximately 150,000 deaths (Cuypers *et al.*, 2023). Moreover, it primarily resides in the intestines and feces of animals and is transmitted to humans through contaminated food (Bai *et al.*, 2023;

Gonçalves-Tenório *et al.*, 2018; Pui *et al.*, 2011). The public health risks associated with *Salmonella* necessitate the urgent development of rapid and reliable detection methods for food safety monitoring.

Currently, bacterial culture is considered the gold standard for detecting *Salmonella*. This method requires preenrichment, culture, and isolation, followed by biochemical and serological confirmation. These techniques are time-consuming and labor-intensive, which hinders detection speed and potentially causes significant delays in *Salmonella* detection (Ranjbar *et al.*, 2022; Wang *et al.*, 2021). Polymerase chain reaction (PCR) is an alternative conventional technique for detecting *Salmonella* (He *et al.*, 2023; Zhang *et al.*, 2022); although a recommended method, the reliability can be compromised by the typically low concentrations of *Salmonella* in contaminated food, thus leading to false-negative results (Su *et al.*, 2021; Wei *et al.*, 2022). In contrast, enzyme-linked immunosorbent assays (ELISAs) have been used for *Salmonella* detection and exhibit a low incidence of false-positive results; however, preenrichment is still required to detect low concentrations of *Salmonella* (Bai *et al.*, 2023). Raman spectroscopy (RS) and immunomagnetic separation (IMS) have been increasingly utilized for the detection of pathogenic microorganisms via spectroscopic analyses. IMS can increase detection sensitivity and reduce the duration of the enrichment cycle (Qiu *et al.*, 2023). However, these techniques are susceptible to interference from electrochemical reactions occurring on the electrode surface, complex experimental conditions, and concerns regarding electrode stability (Buehler *et al.*, 2019; Bülte & Jakob, 1995; Tas *et al.*, 2025). These detection methods are time-consuming and require preenrichment steps, which limits their implementation in rapid detection technologies and onsite applications (Lin *et al.*, 2020; Liu *et al.*, 2022; Qiao *et al.*, 2023). Therefore, there is an urgent need to investigate and develop innovative new technologies that rapidly detect *Salmonella* without relying on preenrichment processes.

Hence, this study employs specific aptamers and nucleic acid enrichment techniques to develop a method for detecting low levels of *Salmonella*. A nucleic acid enrichment device was created using carboxylated magnetic beads and ultrafiltration membranes to concentrate *Salmonella*, thereby bypassing traditional preenrichment steps. Specific aptamers targeting the *invA* gene, including a 5'-aminated capture aptamer linked to magnetic beads and a 3'-TAMRA (carboxytetramethylrhodamine)-labeled detection aptamer, were synthesized. The resulting magnetic aptamer-targeted nucleic acid detection aptamer complex enables the rapid, sensitive, and precise quantification of low concentrations of *Salmonella*. This method combines specific aptamers with nucleic acid enrichment technology to amplify the signal and enhance detection sensitivity, while circumventing traditional enrichment processes, thus shortening the detection time. This approach provides an innovative solution for onsite food safety testing, addressing urgent public health needs.

Materials and Methods

Chemicals and materials

Reagents and materials

HindIII and EcoRI (haemophilus influenzae d strain third enzyme isolated and *Escherichia coli* R strain first enzyme isolated) restriction enzymes were purchased from Bioneer Corporation (Shanghai, China). A 0.22 μm pore size membrane filter, proteinase K, and bovine serum albumin (BSA) were obtained from Takara Biotech Co. Ltd. (Dalian, China). Tris-EDTA (tromethamine-ethylenediaminetetraacetic acid) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Sodium chloride (NaCl) was obtained from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). Tris, NaCl, ethanol, and isopropanol were of analytical grade. The capture probe aptamer, detection probe aptamer, and validation probe aptamer were diluted to a concentration of 100 mM in ultrapure water. Beef extract peptone medium (BPM) was prepared in-house.

Lysis buffer

The lysis buffer consisted of 1.5% (w/v [weight/volume]) sodium dodecyl sulfate (SDS), 50 mM Tris-HCl (pH 8.0), 100 mM EDTA, and 200 mM NaCl. SDS was purchased from Shanghai Solarbio Bioscience & Technology Co., Ltd. (Shanghai, China). Tris-HCl was purchased from NOVON Scientific Co., Ltd. (Beijing, China).

Main instruments and equipment

Scanning electron microscopy (SEM) images were acquired with a JEM-2100 scanning electron microscope from EOL Japan Electronics Co., Ltd. (Tokyo, Japan). UV-Vis (ultraviolet-visible) spectra were obtained via a UV-1800 spectrophotometer supplied by Shimadzu Corporation (Kyoto, Japan). Laser scanning confocal microscopy employed an Olympus BX53 system. The fluorescence spectrometer FS5 used for fluorescence spectrum analysis was provided by Edinburgh Instruments (Edinburgh, UK).

Pathogens

Salmonella enterica subspecies *enterica* serovar Typhimurium (strain no. 21482), *Staphylococcus aureus* (strain no. 10001), *Escherichia coli O157:H7* (strain no. 21530), *Listeria monocytogenes* (strain no. 21639), and *Shigella* spp. (strain no. 10865) were obtained from the China Center for Industrial Culture Collection (CICC).

Experimental methods

Design and synthesis of nucleic acid aptamers

The sequence of the *invA* gene of *Salmonella* was retrieved from the National Center for Biotechnology Information (NCBI) database. On sequence alignment, a capture probe modified with NH_2 at the 5' end and a detection probe modified with TAMRA-N at the 3' end were designed. The sequence of the capture probe aptamer was 5'-NH_2-TTTTTTTGGTTGTTACGGCTATTC-3', the sequence of the detection probe aptamer was 5'-ATGCTGTTATCGCTC ACGTTTTTTT-TAMRA-N-3', and the sequence of the validation probe aptamer was 5'-NH_2-TTTTTTTGGTTG TTACGGCTATTC-Cy5-3'. The nucleic acid aptamers were synthesized by Sangon Biotech Co., Ltd. for this study.

Nucleic acid enrichment

Pathogenic bacteria in the detection samples were enriched using a specially designed enrichment device. The cells were subsequently lysed to release deoxyribonucleic acid (DNA), which was subsequently fragmented into nucleic acid fragments that included the *invA* gene. This process amplified the signal, enabling the detection of low-concentration pathogens. The principle of the detection method is illustrated in Figure 1.

Material preparation

Fe_3O_4 nanoclusters were synthesized via the solvothermal method and $Fe_3O_4@SiO_2$ magnetic nanoparticles (MNPs) were prepared via the sol-gel method. Carboxylated magnetic beads ($Fe_3O_4@SiO_2@COOH$, 10 mg/mL) were obtained via chemical carboxylation of the $Fe_3O_4@SiO_2$ surfaces (Fuentes-García *et al.*, 2018; Khalid *et al.*, 2023; Wei *et al.*, 2012). Both materials were characterized using transmission electron microscopy (TEM).

To prepare the magnetic capture probes, carboxylated magnetic beads were washed and resuspended in deionized water (10 mg/mL), after which capture and validation probe aptamers (50 μL each) were added to 50 μL of magnetic beads and incubated at room temperature for 60 minutes. To block nonspecific binding, 10 μL of 1% BSA was added, and the mixture was allowed to react for 5 minutes. After magnetic separation and removal of the supernatant, three washing cycles were performed to eliminate unbound probes. Probe conjugation was confirmed via fluorescence microscopy.

Correlation between Salmonella absorbance and bacterial count

Salmonella cultures (12-hour incubation) were centrifuged in 1 mL aliquots at 4000 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended in 1 mL of 0.9% sterile saline. Serial dilutions were prepared by transferring 500 μL, 400 μL, 300 μL, 200 μL, and 100 μL aliquots of the suspension, with each aliquot diluted to a total volume of 1 mL with sterile saline.

Absorbance measurements at 600 nm were obtained in triplicate using a spectrophotometer and parallel

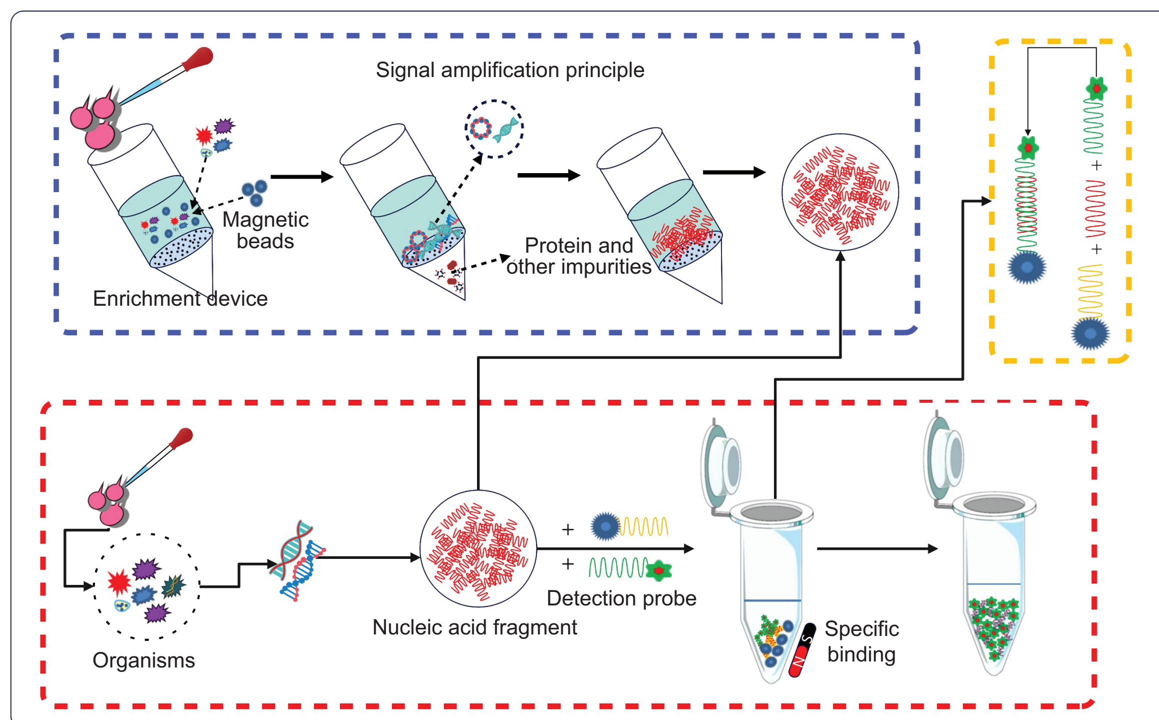

Figure 1. Principle of nucleic acid enrichment technology.

plating of 40 μL aliquots from each dilution was conducted (n = 3) to determine its actual concentration. A standard curve correlating the absorbance at optical densities (OD600) with the actual concentrations of *Salmonella* (CFU) was established using the obtained OD600 values as the dependent variable and the corresponding actual concentrations of the dilutions as the independent variable.

Quantitative detection of Salmonella

To quantify *Salmonella*, bacterial suspensions were prepared on national standard limits (ISO 6579-1:2017 and GB 29921-2021) and the actual sample concentrations. The bacterial suspension was adjusted to 10^3 CFU/mL using an established bacterial absorbance count standard curve. Aliquots (750 μL, 500 μL, 250 μL, 100 μL, 10 μL, and 1 μL) were collected from this suspension and diluted to a final volume of 1 mL using sterile 0.9% saline, resulting in six distinct concentrations.

Excess impurities were removed from samples of varying concentrations using an enrichment device. Subsequently, 500 μL of lysis buffer and 10 μL of proteinase K were added, mixed thoroughly, and incubated at 65°C for 20 minutes. Subsequently, 10 μL each of *HindIII* and *EcoRI* were added to each mixture and allowed to react at room temperature for 3 minutes. Magnetic separation was performed, and the supernatant was discarded. Finally, the beads were washed twice with 500 μL of 75% ethanol, followed by magnetic separation and removal of the supernatant.

Afterward, the enrichment device was supplemented with 30 μL of the magnetic capture probe and 30 μL of the detection probe. After mixing and incubation for 30 minutes, unbound detection probes were removed via magnetic separation. The remaining beads were resuspended in 100 μL of deionized water. Fluorescence intensities were subsequently measured at 580 nm via a fluorescence spectrophotometer. A standard curve was established using the concentrations of bacterial suspensions and fluorescence intensity at 580 nm to evaluate the linear relationship and determine the limit of detection.

Feasibility study of enrichment technology

An unknown concentration of the *Salmonella* suspension was prepared by plating and counting, according to the established bacterial count standard curve. This suspension was then added to 100 mL of commercially purchased milk, followed by a 10-fold dilution. Nucleic acid fragments were subsequently extracted using both nucleic acid enrichment technology and conventional extraction methods. Fluorescence intensity measurements were conducted to evaluate the signal amplification effect of nucleic acid enrichment.

The conventional method involved a 10-fold dilution of 10 mL of bacterial culture, centrifugation at 12,000 rpm for 5 minutes, and discarding removal of the supernatant. Lysis buffer and 10 μL of proteinase K were added, mixed, and incubated in a 65°C water bath for 20 minutes. The mixture was then centrifuged at 12,000 rpm for 10 minutes, and the supernatant was transferred to a new centrifuge tube. Afterward, 30 μL of a magnetic capture probe and 30 μL of a detection probe were added to the tube, mixed, and allowed to stand for 30 minutes. Unbound detection probes were magnetically separated and discarded, and the remaining beads were resuspended in 100 μL of deionized water (Chavan *et al*., 2022).

To further validate the method, 10 mL of bacterial culture was diluted 10-fold, and the concentration of *Salmonella* was determined using the method described in section 2.2.5. Concurrently, 40 μL of the bacterial culture was plated and counted (n = 3) to obtain the actual concentration of *Salmonella*. This value was compared with the detection results from this study to assess the feasibility of using nucleic acid enrichment technology and specific aptamer detection for *Salmonella*.

Anti-interference capability

To evaluate the anti-interference ability of the detection system, the target pathogen *Salmonella* was sequentially replaced with other potential pathogens likely to exist in actual samples. These pathogens included *Staphylococcus aureus, Escherichia coli O157:H7, Listeria monocytogenes*, and *Shigella* spp., which were cultured in LB liquid media at 37°C for 24 hours, resulting in OD600 between 0.6 and 0.8.

Results

Preparation of carboxylated magnetic beads and capture probes

In this study, carboxylated magnetic beads and microporous membranes were utilized to construct a nucleic acid enrichment device for rapid and convenient enrichment of *Salmonella* DNA. The principle of nucleic acid enrichment is illustrated in Figure 1.

The small size and large specific surface area of the carboxylated magnetic beads facilitate efficient enrichment of low-concentration nucleic acids. TEM images of the prepared carboxylated magnetic beads are shown in Figure 2; Figure 2A depicts $Fe_3O_4@SiO_2$ and Figure 2B depicts $Fe_3O_4@SiO_2@COOH$. The prepared beads exhibited a uniform size distribution and good dispersion. Furthermore, observation of a smooth surface with a visible halo (as shown in Figure 2B) indicated the presence of carboxyl groups on the magnetic bead surface.

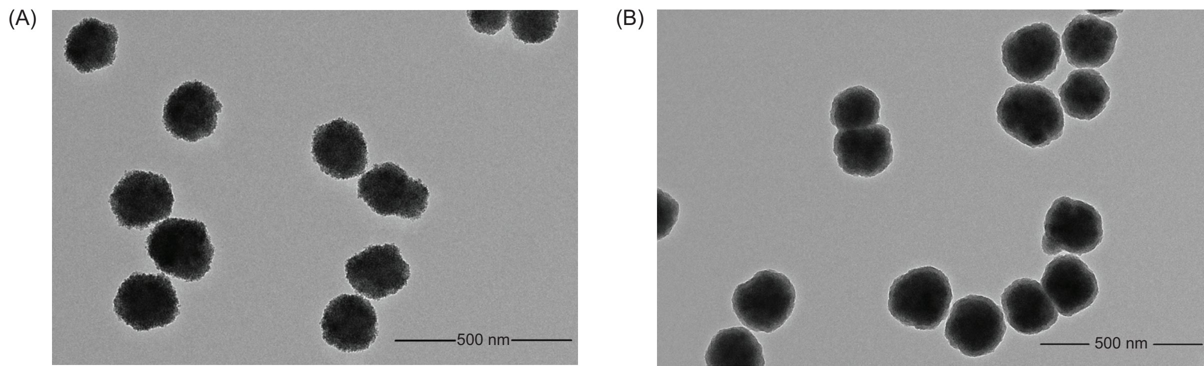

Figure 2. Magnetic nanoparticle TEM.

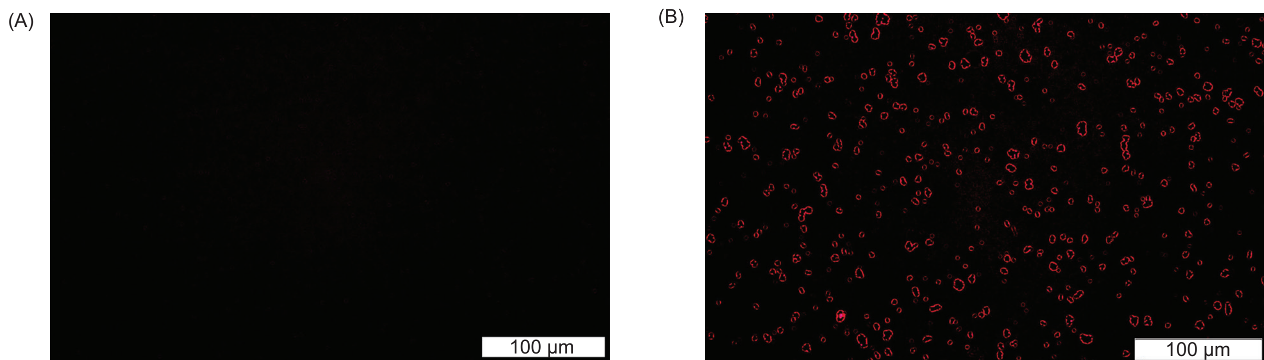

Figure 3. Magnetic probe validation.

Fluorescence microscopy images of the magnetic capture probes are shown in Figure 3; Figure 3A depicts the magnetic capture probe, whereas Figure 3B displays the magnetic detection probe. The distinct fluorescent spots observed in Figure 3B confirm successful conjugation, which corresponds to the detection probe labeled with Cy5 at the 3' end. These results further indicate that the capture probes were effectively bound to the carboxylated magnetic beads (Figure 3A), confirming successful preparation.

MNPs have gained significant attention for their excellent biocompatibility and magnetic properties, making them widely applicable in molecular biology and medical fields (Chavan *et al.*, 2022; Materón *et al.*, 2021). Previous studies have shown that MNPs effectively enrich target nucleic acids (He *et al.*, 2019; Zhao *et al.*, 2024). Fluorescent materials, known for their high fluorescence intensity, good stability, and biocompatibility, are commonly employed in bacterial detection and medical diagnostics (Pgi & Rathnayaka, 2018; Zhang *et al.*, 2023). Therefore, the use of Cy5-labeled aptamers in conjunction with magnetic materials enable rapid and highly sensitive detection of *Salmonella*. Additionally, fluorescently labeled aptamers can be used to validate effective conjugation with carboxylated magnetic beads.

Correlation between *Salmonella* absorbance and bacterial count

To establish the correlation between *Salmonella* absorbance and bacterial count, the OD600 values of various concentrations of *Salmonella* were measured and plotted as a standard curve (Figure 4). The resulting linear equation was y = 0.71953x-0.1393, with a correlation coefficient of R^2 = 0.99325, indicating a strong linear relationship between *Salmonella* concentration and absorbance. A plate count image of *Salmonella* is shown in Figure 5.

Optical density measurements at a wavelength of 600 nm (OD600) is the most common method for estimating the number of cells in liquid suspensions (Montesinos-Cruz & Somerville, 2024; Myers *et al.*, 2013). Establishing a linear relationship between the OD and *Salmonella* concentration within a limited range enables the determination of *Salmonella* concentration during the study (Stevenson *et al.*, 2016).

Quantitative detection of *Salmonella*

On the basis of the nucleic acid enrichment method and the established standard curve correlating *Salmonella*

absorbance with bacterial count, a *Salmonella* suspension with an initial concentration of 10^3 CFU/mL was serially diluted within the range of 10^0–10^3 CFU/mL. Under optimized experimental conditions, the fluorescence intensities of different concentrations of *Salmonella* were measured (Figure 6). The linear range of the method was evaluated, and from 10^0 to 10^3 CFU/mL, the fluorescence ratio demonstrated a linear correlation with concentration ($y = 0.5911 + 0.0022x$, $R^2 = 0.9864$). The detection limit was determined to be 1 CFU/mL.

Fluorescence spectroscopy exhibits exceptional sensitivity, specificity, and accuracy, and has been widely applied in bacterial detection because of the linear relationship between fluorescence spectrum and bacterial concentration (Nakar *et al.*, 2020). For example, Simões and Dong (2018) developed an optofluidic sensor for detecting pathogens in drinking water that achieved an accuracy of up to 10^6 CFU/mL. Du *et al.* (2022) utilized laser-induced fluorescence (LIF) technology to investigate the fluorescence emission spectra of *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*, with detection limits below ~10^2 cells/mL. These findings demonstrate that fluorescence emission spectroscopy is a sensitive, rapid, and stable method for bacterial detection.

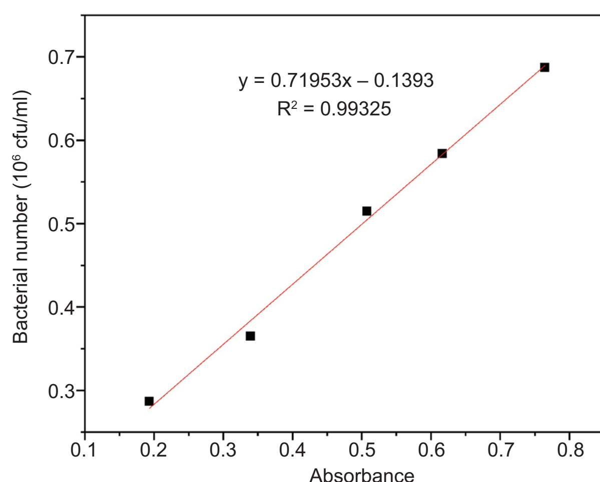

Figure 4. ***Salmonella*** **absorbance and bacterial count standard curve.**

Feasibility study

The feasibility of the proposed method was evaluated by comparing it with conventional extraction methods for detecting *Salmonella*. Figure 7 shows the fluorescence intensity spectra obtained from this study. Curve 1 represents the fluorescence spectrum of *Salmonella* detected using the nucleic acid enrichment method and Curve 2 corresponds to the fluorescence spectrum obtained using the conventional nucleic acid extraction method. Fluorescence intensities obtained with the nucleic acid enrichment method were significantly greater than those obtained with the conventional method, demonstrating a 2.4-fold increase in sensitivity. These findings confirm that the nucleic acid enrichment method effectively amplified the detection signal for *Salmonella*. To ensure consistent concentrations of the detection samples, quantification was performed via the plate counting method, as shown in Figure 1A. This method yielded a *Salmonella* concentration of 5.76×10^2 CFU/mL, while the concentration determined using the method proposed in this study was 5.49×10^2 CFU/mL.

Anti-interference capability

The anti-interference ability of the nucleic acid enrichment method was evaluated, and the results are presented in Figures 8 and 2A. The fluorescence response values of the detection system in the presence of interfering pathogens were significantly lower than those observed for *Salmonella*. These findings indicate that the nucleic acid enrichment method has high specificity and selectivity for *Salmonella*, even in the presence of other pathogens (such as *Staphylococcus aureus, Escherichia coli O157:H7, Listeria monocytogenes*, and *Shigella* spp).

Research has demonstrated that aromatic amino acids can serve as indicators for detecting bacterial

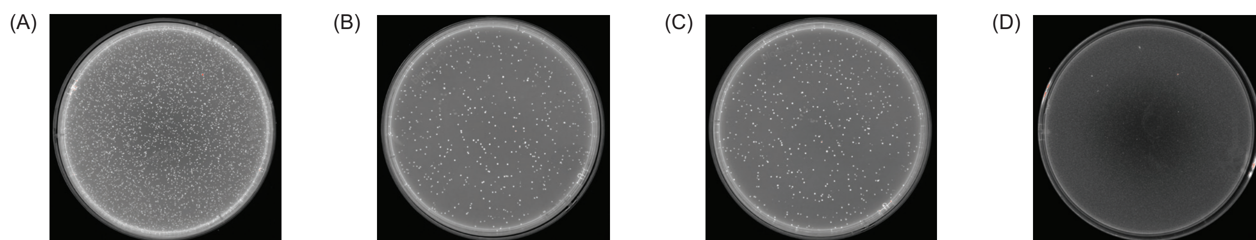

Figure 5. ***Salmonella*** **colonies on agar plates. (A–C) correspond to plate count images with concentrations ranging from high to low; (D) represents the negative control.**

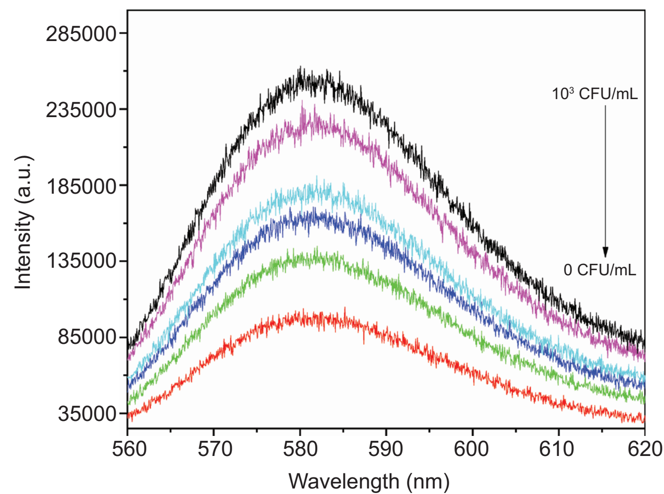

Figure 6. Fluorescence spectra of different concentrations of *Salmonella*.

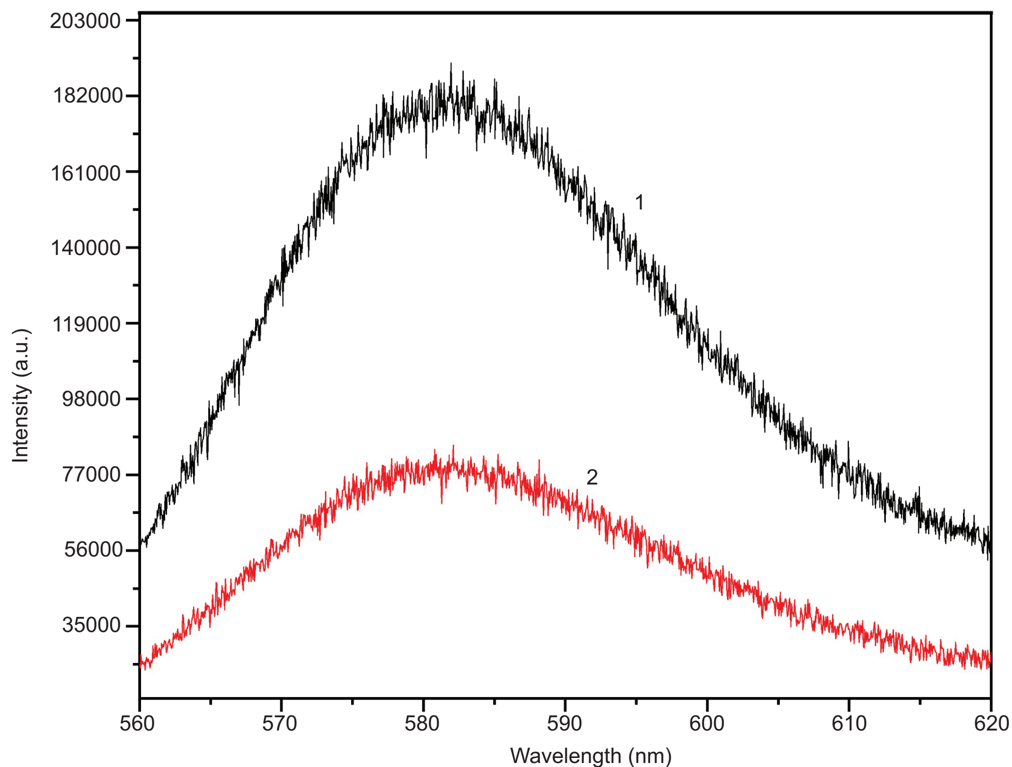

Figure 7. Feasibility study of the nucleic acid enrichment method. Curve 1 represents the fluorescence intensities obtained using the nucleic acid enrichment method and Curve 2 represents the fluorescence intensities achieved using the conventional extraction method.

distribution, concentration, and species (Pan, 2015). Tryptophan-like fluorescence (TLF) has also been widely and effectively utilized to monitor bacterial contamination in water (Baker *et al.*, 2015). Furthermore, some studies have proposed that coenzymes are the primary sources of most endogenous fluorescence in cells (Aubin, 1979). These natural fluorescent substances exhibit robust characteristic fluorescence intensities at various laser wavelengths. In this study, an innovative approach was employed that utilized nucleic acid enrichment techniques to release *invA* gene fragments for the detection of *Salmonella*, effectively circumventing these

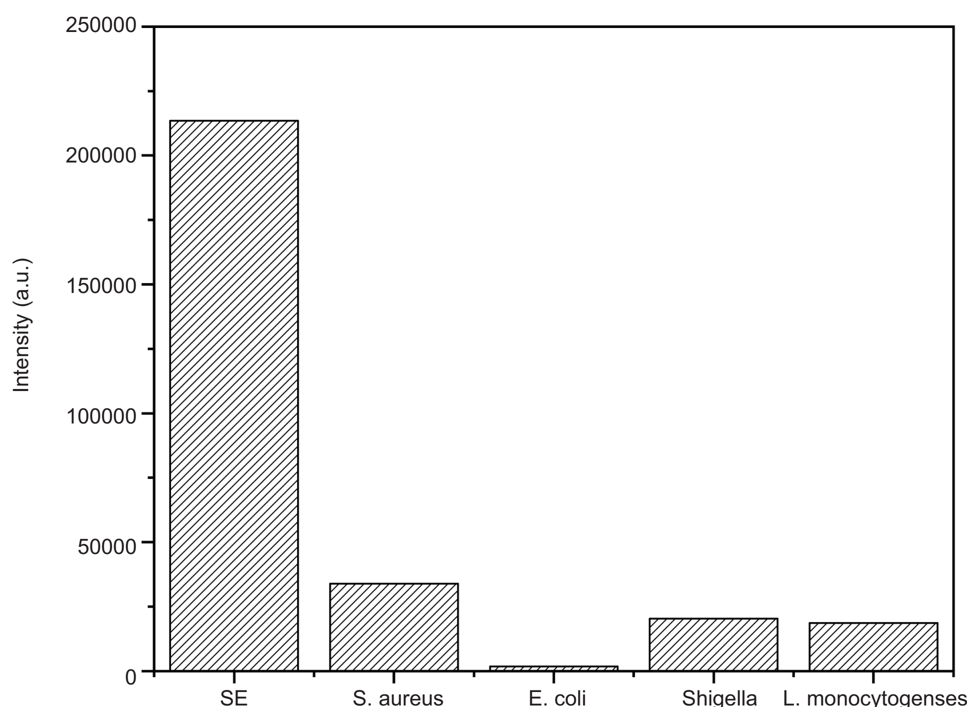

Figure 8. Specificity of *Salmonella* detection methods.

substances and minimizing interference signals. This strategy is advantageous for lowering detection limits and enhancing resistance to interference.

Discussion

The development of nucleic acid detection technologies, particularly aptamer-based methods for detecting pathogens such as *Salmonella*, enhances onsite rapid testing strategies for foodborne pathogens. The development of rapid, sensitive, and specific detection methods for *Salmonella* is critical to ensure food safety and prevent outbreaks of foodborne illnesses. Our method exhibited a 2.4-fold increase in sensitivity compared with conventional nucleic acid extraction techniques (Figure 7), primarily due to the effective enrichment of low-concentration nucleic acids using carboxylated magnetic beads. This result aligns with recent studies demonstrating the use of MNPs to enhance detection sensitivity. Previous studies have demonstrated that due to their magnetic properties and bioaffinity, MNPs can adsorb nucleic acids (Liu *et al.*, 2023). Furthermore, MNPs can be surface-functionalized to specifically capture target nucleic acid molecules and achieve rapid separation via an external magnetic field (Khamlamoon *et al.*, 2023; Li *et al.*, 2021; Yue *et al.*, 2020). For example, MNPs functionalized with carboxyl groups can increase the efficiency of nucleic acid capture via electrostatic interactions and large surface-to-volume ratios (Gautam, 2022; Li *et al.*, 2025). Additionally, the dual-probe design (utilizing both capture and detection aptamers targeting the *invA* gene) ensured high specificity, as evidenced by minimal cross-reactivity with *Staphylococcus aureus, Escherichia coli*, and other pathogens (Figure 8). Therefore, this study demonstrates that the enrichment of target nucleic acids using MNPs and the dual recognition strategy of connecting nucleic acid aptamers to their surfaces can improve the efficiency of target capture.

Traditional methods, such as plate counting and PCR-based detection, are time-consuming and require specialized equipment. This is particularly important, given the technical limitations in detecting low concentrations of pathogens in actual samples. For example, a recombinase-aided amplification (RAA) method combined with DNA-specific nucleases has a detection limit of 10 CFU/mL; however, this method requires isothermal amplification equipment (Zhou *et al.*, 2024). Moreover, Xie *et al.* (2022) have validated the use of digital microfluidic chip detection technology for foodborne pathogens using spiked milk and simultaneously detected all four pathogenic bacteria at concentrations as low as 10^3 CFU/mL within 50 minutes. Our approach relies on fluorescence spectroscopy detection, which provides a solution that is more suitable for field deployment. Moreover, its linear detection range of 10^0–10^3 CFU/mL (Figure 6) encompasses both regulatory thresholds (including ISO

6579-1:2017 and GB 29921-2021) and trace contamination levels, making it suitable for routine monitoring and outbreak investigations.

Zhao *et al*. (2023) combined concanavalin A with aptamers for target recognition of *Salmonella* Typhimurium, thereby achieving a detection limit of 23 CFU/mL in complex food matrices (Zhao *et al*., 2023). Although their method utilized visual detection, our fluorescence-based approach demonstrated higher sensitivity (1 CFU/mL), highlighting the advantages of the nucleic acid enrichment method employed in this study with regard to signal amplification. Moreover, although a newly developed colorimetric biosensor can rapidly detect *Salmonella* at concentrations as low as 168 CFU/mL within 25 minutes via color changes (Jin *et al*., 2023), this method requires improvement with regard to detection sensitivity. In addition, though Shin *et al*. (2018) developed an aptamer-based sandwich assay to detect *Salmonella enterica* ser. Typhimurium with a detection range of 2×10^1 to 2×10^5 CFU/mL, our method eliminates the preenrichment step, allowing for rapid completion of tests that meet the time control principles required in food safety. Furthermore, our method's robust anti-interference ability in milk samples (Section 3.5) highlights its applicability to complex food matrices.

In summary, our nucleic acid enrichment-based method significantly reduces the detection time and improves the sensitivity of *Salmonella* detection. Nonetheless, challenges remain regarding the use of this method in the field. Our approach leverages MNPs and dual aptamer probes to address critical limitations of traditional techniques. Moreover, it aligns with the growing demand for rapid, onsite pathogen detection in the food industry. Future refinements that focus on portability and multiplexing will further solidify the role of this method in global food safety surveillance.

Conclusions

This study developed a novel *Salmonella* detection method that employs a nucleic acid enrichment technique utilizing carboxylated magnetic beads and ultrafiltration membranes, combined with *Salmonella*-specific capture probes. This method circumvents traditional enrichment processes to capture *Salmonella*, enabling the rapid detection of low concentrations of this pathogen. Moreover, our method amplifies the signals detected, resulting in a 2.4-fold increase in sensitivity compared with conventional approaches. We also achieved a linear detection range of 10^1–10^3 CFU/mL (R^2 = 0.9864) and a detection limit of 1 CFU/mL, thereby meeting practical requirements. Hence, this new detection technology provides a viable alternative to traditional pathogen testing, in that timely and reliable detection is crucial to ensuring food safety (Paniel & Noguer, 2019). Despite our advancements, challenges remain in achieving a balance between accuracy and efficiency in foodborne pathogen detection. Semiquantitative methods often fail to deliver the sensitivity required for low-concentration targets, whereas quantitative methods can be hindered by time-consuming preenrichment steps. For example, lateral flow immunoassays that employ gold nanoparticles provide rapid visual estimates but lack quantitative precision (Gong *et al*., 2023). Our proposed method addresses these limitations by eliminating traditional enrichment steps, thereby streamlining the detection workflow and enabling more rapid responses to potential contamination events.

Future research will focus on constructing nucleic acid aptamer-based biosensors utilizing the proposed detection method. Utilizing the ability of biosensors to convert biological recognition signals into electrical signals, we aim to develop a simple and rapid onsite detection platform by leveraging the stability and cost-effectiveness of aptamers in conjunction with nucleic acid enrichment techniques to eliminate the enrichment step (Ansari *et al*., 2017; Duan *et al*., 2012). Research has shown that, because of their simplicity, methods employing nanotechnology and biosensors for DNA detection are excellent alternatives to historical methods for pathogen detection in food (Duan *et al*., 2020; Lee & Hwang, 2020). Our rapid detection platform is designed to facilitate onsite testing across a broader range of sample types and conditions.

Author Contributions

All authors contributed equally to this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary

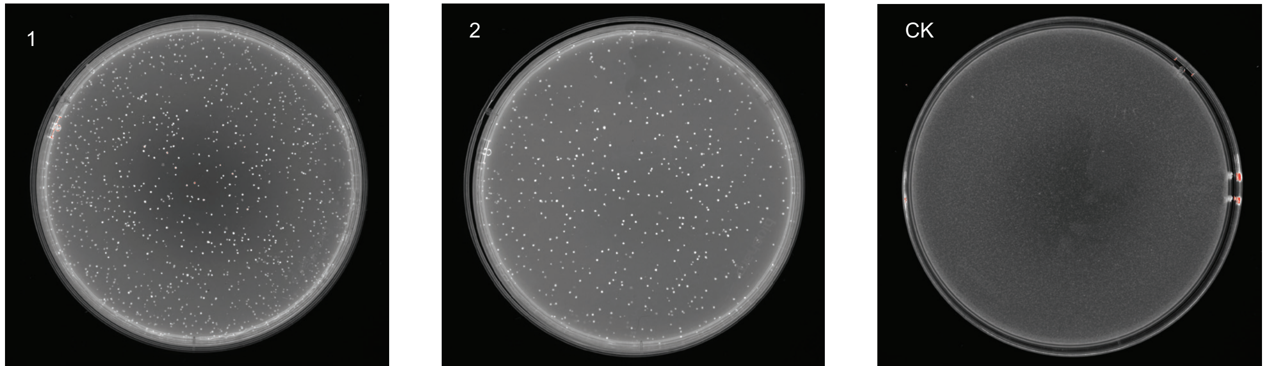

Figure S1. **Photos of *Salmonella* colonies of unknown concentrations on agar plates.**

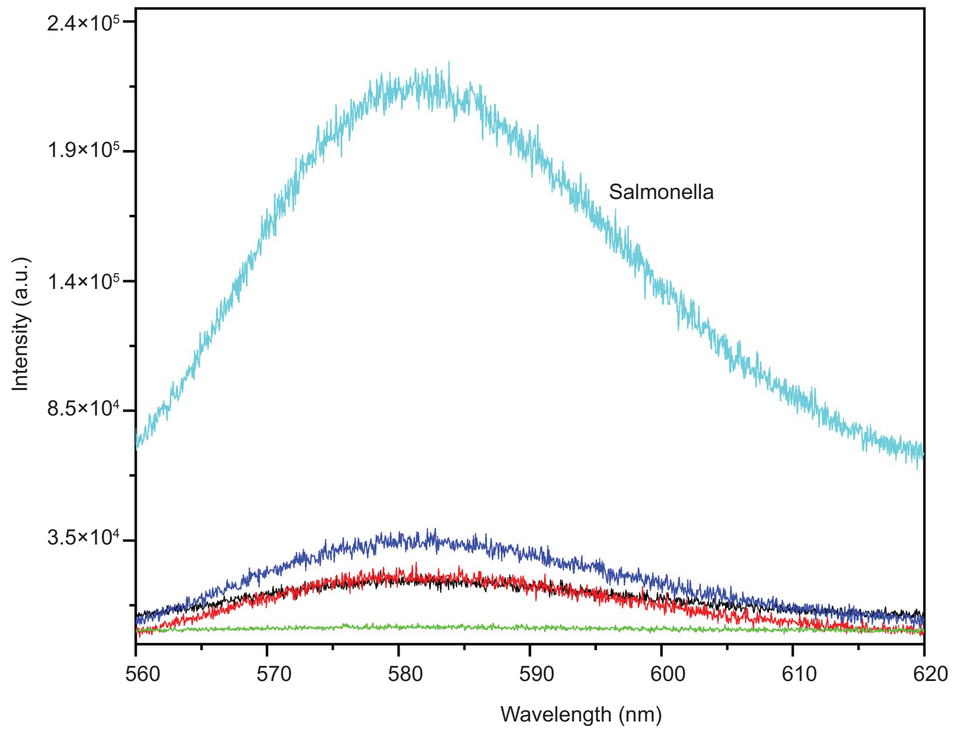

Figure S2. **Specificity of *Salmonella* detection methods.**