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A novel colorimetric strategy based on canine IgM for ultrasensitive and selective detection of *Staphylococcus aureus*

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ABSTRACT

Conventional methods for *Staphylococcus aureus* (*S. aureus*) detection using mammalian immunoglobulins could yield false positive results, owing to the presence of the protein G-producing *Streptococcus*. In this study, because canine IgM can bind to protein A but not to protein G, we firstly designed a colorimetric platform based on canine IgM to identify *S. aureus*. Magnetic beads (MBs) functionalized with canine IgM acted as captors to enrich *S. aureus*, relying on the binding interaction between the Fc region of canine IgM and protein A in the bacterial cell wall, while horseradish peroxidase (HRP)-labeled rabbit anti-*S. aureus* IgG served as a tracer in the sandwich-type immunoassay, catalyzing the colorimetric reaction. Under optimal conditions, the proposed strategy achieved a satisfactory analytical performance with a broad linear range from 1.6×10^3 CFU/mL to 1.0×10^5 CFU/mL, a colorimetric minimum resolution of as low as 1.4×10^2 CFU/mL in 100 μL phosphate-buffered saline (PBS), and good selectivity in differentiating target from protein G-producing *Streptococcus*. Moreover, the proposed approach successfully identified *S. aureus* in various application scenarios (except for samples containing IgG), can be completed in less than 90 min, and was not dependent on any specialized equipment.

1. Introduction

Foodborne infections, which result from the ingestion of food infected with microorganisms or their toxins, are prevalent, albeit avoidable health burden (Hu et al., 2019). According to the World Health Organization (WHO), approximately 600 million individuals (nearly one in ten) suffer from foodborne diseases worldwide. *Staphylococcus aureus* (*S. aureus*), a common opportunistic pathogen, is among the top five foodborne pathogens responsible for a range of clinical symptoms, from gastrointestinal reaction to fatal bacteremia. Various foods, especially moist foods containing protein, are susceptible to *S. aureus* contamination since *S. aureus* can adapt to and develop in various environments, such as high or low temperatures, different pH levels, and saline environments (Shan et al., 2023). Susceptible individuals may present symptoms within 1–6 h after accidental ingestion of less than 1 μg of enterotoxin, which can be released from more than 10^5 CFU/g of *S. aureus* in contaminated food (Alarcón et al., 2006; Ding et al., 2023). Hence, the occurrence of *S. aureus* infection outbreaks and their related infectious diseases represent a substantial risk to the security of public health.

More sensitive and rapid detection methods are required for the identification of *S. aureus* at the early stage of contamination. Considerable efforts have been made to develop analytical techniques for *S. aureus* identification. The traditional "culture and colony counting" technique is the benchmark for bacterial detection owing to its high level of dependability and precision. However, the technique, including culture, identification, and confirmation steps (approximately 3–7 days), is time-consuming, which hinders point-of-care analysis (Shahrokhian & Ranjbar, 2018; Siranosian et al., 2022). Nucleic acid amplification based tests could fill this gap and shorten the time to results with high sensitivity and selectivity. DNA extraction and polymerase chain reaction (PCR) operation requires well-qualified technicians and bulky and sophisticated instruments (Cheng et al., 2023; Wen et al., 2023). Furthermore, the presence of complex food matrix

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(such as protein and lipid) and experimental conditions greatly affect the results of PCR (Brandao et al., 2015; Chen et al., 2022; Luo et al., 2018; Yin et al., 2023; Zhang et al., 2023).

Immunology-based methods depend on the selective affinity of the antibody to the corresponding antigen and are characterized by simple operation, rapid turn-around time, and cost-effectiveness (Janik-Karpinska et al., 2022). Recent IgG-based approaches for *S. aureus* detection have mainly utilized a combination of non-specific IgG and molecular recognition agents (e.g., vancomycin, daptomycin, and phenylboronic acid) to perform sandwich-like construction. Although the selectivity of these methods could be improved by utilizing the binding affinity between non-specific IgG and staphylococcal protein A (SPA), the existence of protein G producing *Streptococcus* can yield false-positive results, as it can also be recognized by the paired recognition elements. Thus, a major challenge in antibody-based methods is the search for more appropriate antibodies with high selectivity for *S. aureus*.

IgM is the primary response antibody against pathogen infection in adaptive immunity. Canine IgM is characterized by selective binding to protein A rather than protein G (Hage, 2006; Scott et al., 1997). SPA, which is highly conserved in *S. aureus*, is abundant on its bacterial cell wall, containing approximately 80,000 SPA molecules per cell (Cronin et al., 2020; Madkour et al., 2023; Zhang et al., 2019). Thus, we hypothesized that canine IgM is a potential recognition molecule for targeted identification of *S. aureus*. Herein, we investigated the potential of a novel canine IgM-affinity strategy for *S. aureus* identification. As depicted in Fig. 1, magnetic beads (MBs) coupled with canine IgM can bind to SPA when *S. aureus* is present in the sample. Variation in *S. aureus* concentration yields varying quantities of horseradish peroxidase-labeled rabbit anti-*S. aureus* IgG (HRP-IgG) that are either immobilized on the MBs or released into the supernatant, leading to differences in colorimetric intensity. This study proposes a novel and promising option for the sensitive and selective identification of *S. aureus* in diverse sample types.

2. Experimental

2.1. Regeants and materials

Carboxyl-terminated MBs, which have a diameter of 820 nm, were acquired from Spherotech (USA), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were procured from Aladdin Reagent (China). Canine IgM (1 mg/mL) and a soluble one-component 3, 3′, 5, 5′-tetramethyl benzidine (TMB) solution were provided by Biodragon Immunotechnologies (China). HRP-IgG (1 mg/mL) was bought from Abcam (ab68952) (UK). HRP-labeled protein A (HRP-protein A) and HRP-labeled protein G (HRP-protein G) were obtained from SouthernBiotech (USA). Skim milk powder and bovine serum albumin (BSA) were supplied by BD Difco (USA) and Biosharp (China), respectively. Biotin N-hydroxysuccinimide ester (Biotin-NHS) and fluorescein isothiocyanate-labeled streptavidin (FITC-SA) (0.5 mg/mL) were acquired from Sigma-Aldrich (USA) and BioLegend (USA), respectively. Jet Bio-Filtration (China) provided 96-well serological plates and enzyme-linked immunosorbent assay (ELISA) plates. Sodium chloride injection, apple juice, and milk were acquired from local pharmacies and supermarkets. All reagents utilized in this experiment were of analytical grade or better. The solutions and buffers used in this study were prepared utilizing deionized water sourced from a Pall laboratory water purification system (Pall, USA), which exhibited a resistivity of 18.2 MΩ cm at 25 °C.

2.2. Bacterial strains and culture

S. aureus (ATCC 12598), *Streptococcus dysgalactiae* (ATCC 35666), *Salmonella typhimurium* (ATCC 14028), *E. coli* O157: H7 (EDL 933), and *Listeria monocytogenes* (ATCC 19115) were purchased from Bena Culture

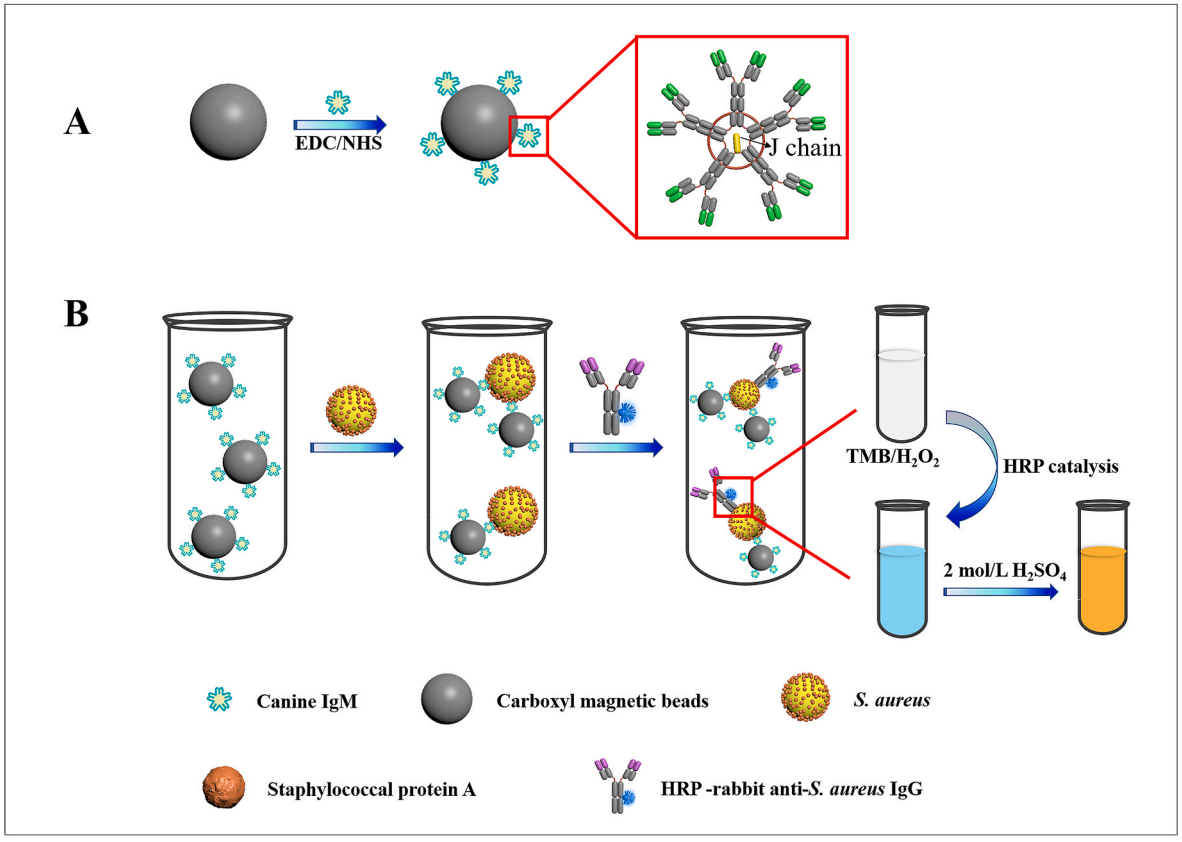

Fig. 1. Schematic diagram of the proposed canine IgM-based strategy for *S. aureus* detection.

Collection (China). Prof. Hongping Wei provided the *Streptococcus agalactiae* (extracted from raw milk; Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China). Luria–Bertani medium was used to culture *S. aureus*, *E. coli* O157: H7, and *Salmonella typhimurium*, and the brain–heart infusion broth for *Streptococcus agalactiae*, *Streptococcus dysgalactiae,* and *Listeria monocytogenes* at 37 °C with constant shaking overnight. Bacterial cells were collected by centrifugation for 10 min at 3000 r/min, washed twice with PBS at 0.01 mol/L and pH 7.4, and resuspended in sterile PBS at pH 7.4 and 0.01 mol/L with 20% glycerol. The concentration of each bacterial strain was determined using the plate count technique, which involved counting the number of colony-forming units (CFU). The pure bacterial cultures were stored at −80 °C until further use.

2.3. Apparatus

Absorbance was measured by Multiskan MK3 microplate reader (Thermo Scientific, USA). Magnetic separation was performed using magnetic separation racks (Goldmag Biotech, China). MBs were resuspended using an Oscillator Vortex-Genie 2 (Scientific Industries, USA) to ensure uniform particle distribution. Bacterial incubation and antibody response to antigen were carried out using a ZWY-100H incubator shaker (Labwit Scientific, China). An Eclipse Ti–S fluorescence microscope (Nikon, Japan) was used to capture fluorescence micrographs.

2.4. Characterization of the selective interaction of canine IgM towards S. aureus

An ingenious ELISA was designed to validate the selective binding of canine IgM to protein A rather than protein G (Fig. S1). The ELISA plates were prepared by coating 10 ng of canine IgM per well in carbonate-coating buffer at 0.05 mol/L and pH 9.6, followed by overnight incubation at 4 °C. Wells coated with BSA were used as controls for non-specific binding. Then, wells were blocked with 5% BSA at 37 °C for 2 h. A two-fold serial dilution of HRP-protein A and HRP-protein G (100 μL), ranging from 1:4000 to 1:16000, was added into the wells, which were incubated at 37 °C for 30 min and washed five times with PBST (0.01 mol/L, pH 7.4). Finally, the reaction was initiated and quenched by adding 100 μL of the chromogenic substrate TMB and 100 μL of 2 mol/L sulfuric acid, respectively.

The proposed strategy was based on the affinity between canine IgM and *S. aureus*. Biotinylated canine IgM (biotin-canine IgM) was prepared as previously described with minor modifications (Yu et al., 2016; Zhang et al., 2017) (The detailed protocol is uploaded in Supplementary Material). Briefly, 50 μL of biotin-canine IgM (1.4 mg/mL) was incubated with 1 mL of *S. aureus* (1.0×10^8 CFU/mL) for 30 min at 37 °C in an incubator shaker at 180 r/min. Immediately after, unbounded antibodies were removed by repeated washing with PBS containing Tween 20 (PBST; PBS at pH 7.4 with 0.05% Tween-20). The *S. aureus* cells labeled with biotin-canine IgM were stained with FITC-SA at 37 °C for 30 min. Finally, *S. aureus* cells were visualized by fluorescence microscopy.

2.5. Preparation of canine IgM-modified MBs

Carboxyl-terminal MBs coated with canine IgM were prepared using the EDC/NHS coupling system as previously reported (Zhang et al., 2019). MBs (1 mg) were washed twice with PBS (0.01 mol/L, pH 6.8) to eliminate the storage buffer. Then, 200 μL of PBS (0.01 mol/L, pH 6.8) containing 2 mg of EDC and 1 mg of NHS, were introduced to activate the carboxyl groups of MBs. After 30 min of shaking at 180 r/min and 37 °C, the activated MBs were rinsed thrice with PBS (0.01 mol/L, pH 7.4), and 100 μL of canine IgM (1 mg/mL) was added. After 4 h of incubation under constant stirring (180 r/min), coupling was achieved by the formation of an amide bond between IgM and MBs. Subsequently, the MBs–canine IgM bioconjugates were separated with a magnet and washed thrice with PBST (0.01 mol/L, pH 7.4) to eliminate unbound IgM.

To reduce non-specific binding, 0.05 g of BSA and 0.15 g of skim milk powder were mixed in 1 mL of PBST (0.01 mol/L, pH 7.4) with the aforementioned MBs-canine IgM bioconjugates for 2 h. Lastly, the prepared immunomagnetic beads (IMBs) were stored in 1 mL of PBS (0.01 mol/L, pH 7.4) containing 0.1% BSA at 4 °C for further analysis.

2.6. S. aureus detection

Briefly, canine IgM-modified MBs (10 μg) were incubated with 100 μL of *S. aureus* solution for 40 min with shaking at 37 °C to enrich *S. aureus* from samples, as canine IgM could bind with SPA. The magnetic separation rack adsorbed the complex of MB–*S. aureus* onto the inner side of the tube wall. After discarding the supernatant, the complex was washed five times with PBST (0.01 mol/L, pH 7.4) and added with 100 μL of HRP-IgG (2 μg/mL) to obtain a sandwich-type binding complex. After 30 min of incubation on a shaker at 37 °C and 180 r/min, unbound HRP-IgG molecules were removed by washing five times with PBST (0.01 mol/L, pH 7.4). The sandwiched complex comprising MBs–*S. aureus*–HRP–IgG was isolated using a magnet to facilitate the catalytic chromogenic reaction with the TMB substrate.

Chromogenic reaction was conducted as follows. First, the "MBs–*S. aureus*–HRP–IgG" complex was dispersed in 100 μL of the soluble one-component TMB. Then, the mixture was incubated at 37 °C away from light. After 10 min, the reaction was terminated by adding 100 μL of sulfuric acid (2 mol/L). Lastly, magnetic separation was performed to remove MBs, and the supernatant was transferred into the 96-well serological microplate using a pipette. Absorbance was measured at 450 nm.

3. Results and discussion

3.1. Characterization of the binding capability of canine IgM towards S. aureus

As illustrated in Fig. S2, the canine IgM-coated wells showed significant signals for HRP-protein A, whereas no signals were observed for HRP-protein G (***$P < 0.001$, data are listed in Table S1). Our results confirm that canine IgM is selective for protein A, which is consistent with the results of previous studies (Hage, 2006; Scott et al., 1997).

We further verified the feasibility of canine IgM as a recognition agent for *S. aureus* identification. As illustrated in Fig. 2A and **B**, the morphology of FITC-stained *S. aureus* was consistently round, accompanied by a prominent distribution of bright green fluorescence on the surface of the cell membrane. This observation supports that canine IgM is located on *S. aureus* cell membrane.

3.2. Optimization of experimental conditions

The analytical performance of the proposed method was subject to several factors, such as the quantity of added IMB, HRP-IgG concentration, and incubation time. These experimental conditions were optimized to obtain the ideal detection results. The detection signals were defined as A and A_0, where A and A_0 represent the absorbance intensity at 450 nm obtained from experimental (containing 5×10^4 CFU/mL *S. aureus* in PBS) and blank groups (only PBS), respectively.

The amount of IMB added was first optimized under 0.5 μg/mL of HRP-IgG and 30-min incubation time for canine IgM-modified MBs recognizing *S. aureus*. From Fig. 3A, *An* increased significantly with increasing amounts of added MBs (3–10 μg), reaching the maximum when 10 μg of IMBs was added. After this, there was no increase in signal intensity, indicating saturated IMBs. Meanwhile, the corresponding A_0 was slightly elevated as more IMBs were added, indicating higher non-specific adsorption of HRP-IgG towards IMBs. Therefore, 10 μg of IMBs was chosen owing to its superior A/A_0 value (Fig. 3B).

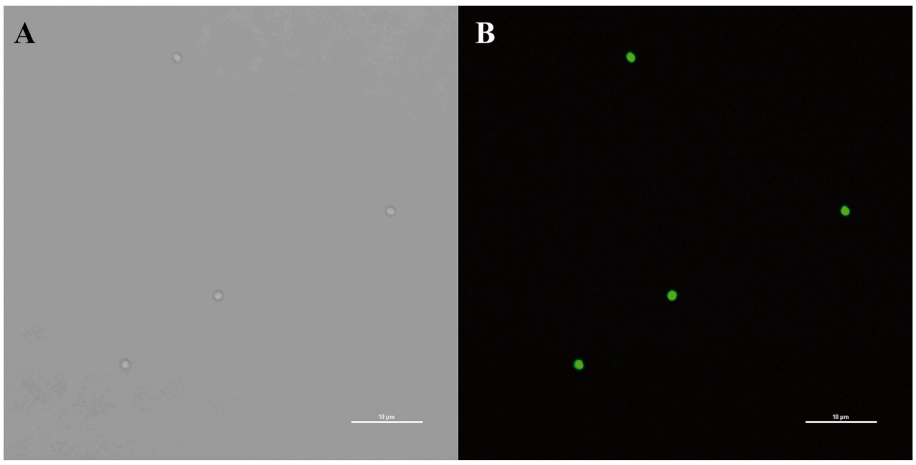

Fig. 2. Fluorescence microscopic images of *S. aureus* stained with tagged FITC. (A) Bright field image and (B) corresponding green fluorescence image (Bar: 10 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Then, the effects of HRP-IgG concentration were monitored for 30 min for canine IgM-modified MBs recognizing *S. aureus*. As illustrated in Fig. 3C, increases in A and A_0 were observed with increasing HRP-IgG concentration (0.5–8 μg/mL). When the concentration was <2 μg/mL, the increase in A was higher than that in A_0. This is because a low HRP-IgG concentration cannot saturate the binding sites on *S. aureus* surface. By contrast, when there was >2 μg/mL of HRP-IgG, there was a higher increase in A_0 than in A, as higher HRP-IgG concentration corresponds to higher non-specific adsorption. According to Fig. 3D, the largest A/A_0 was obtained when the concentration of HRP-IgG was 2 μg/mL. Moreover, using too much antibody increases the experimental cost. Thus, 2 μg/mL of HRP-IgG was selected as the optimum concentration for the subsequent trials.

Lastly, the effect of the incubation time of canine IgM-modified MBs and *S. aureus* was investigated. As shown in Fig. 3E and **F**, A and A/A_0 increased with increasing incubation time and stabilized at 40 min, indicating that the binding between canine IgM and *S. aureus* nearly reached saturation. At this point, A_0 remained almost unchanged. Therefore, 40 min was determined the optimum incubation period.

3.3. Performance of the proposed strategy

To assess the sensitivity of the proposed canine IgM-based method, various *S. aureus* concentrations were mixed with canine IgM-modified MBs and detected by HRP-IgG to produce colorimetric signals. Three replicates were performed for each concentration. From Fig. 4, the absorbance of the chromogenic reaction solution at 450 nm increased as the concentration of *S. aureus* was increased. The standard calibration curve showed a significant linear relationship between the intensity of absorbance at 450 nm and the concentration of *S. aureus,* ranging from 1.6×10^3 CFU/mL to 1.0×10^5 CFU/mL. The linear equation is $Y = 2.2817 \times 10^{-5} X + 0.0495$ ($R^2 = 0.9902$), where Y is the 450 nm absorbance intensity and X is the *S. aureus* concentration. The detection limit (3σ, where σ is the standard deviation of PBS processed similarly as the *S. aureus* samples, n = 10, data are listed in Table S2) was calculated as approximately 1.4×10^2 CFU/mL (Wijesinghe et al., 2023).

Immunomagnetic bead enrichment can concentrate targets from samples, that is, the detection limit can be further lowered and theoretically reach 1 CFU by increasing the volume of the analyzed sample. General real-time PCR obtained a good detection limit of 10^2 CFU/mL in a minimal sample volume (1 μL) (Law et al., 2014; Wang et al., 2020). However, in reality, bacteria, unlike protein or salt, are not uniformly dispersed in the liquid; thus, the pre-concentration step is necessary for specimens with low target concentration (Nguyen & Gu, 2023), implying that the proposed method offers unique advantages over PCR in terms of sensitivity and simplicity. This satisfactory detection performance was attributed to two reasons. First, there are over 80,000 SPA molecules in one *S. aureus* cell, offering sufficient binding sites for the efficient capture of the target bacteria by canine-modified MBs. Second, HRP-IgG, a type of polyclonal immunoglobulin, can bind with multiple epitopes of *S. aureus* and SPA, which possesses five homologous IgG-binding regions. Consequently, this interaction facilitated a substantial loading of HRP-IgG onto the captured *S. aureus*, allowing the colorimetric signal to be highly and efficiently amplified. Concurrently, we conducted a comparative analysis between our method and several other molecular recognition based colorimetric methods. The results (as listed in Table 1) indicated that our proposed method boasted a comparable detection limit while offering advantages in terms of simplicity of operation, reagent commercialization, and superior specificity. Moreover, the reproducibility of the canine IgM-based platform is crucial for its practical use. In this study, 5.0×10^4 CFU/mL *S. aureus* cells were measured for eleven independent samples (data are listed in Table S3). Results revealed excellent reproducibility and reliability with a relative standard deviation (RSD) of 4.2%, confirming the feasibility of *S. aureus* detection using this colorimetric strategy.

The accuracy of our method for *S. aureus* identification for real samples was assessed using the typical addition technique. The typical calibration curves of the three matrix samples (sodium chloride injection, milk without any dilution and apple juice with a 10-fold dilution) exhibited minor discrepancies in comparison to those produced in PBS buffer, mostly attributed to the existence of the sample matrix (Figs. S3, S4, and S5). The three calibration curves revealed a good linear relationship ($R^2 > 0.99$) and detection limits (3σ, where σ is the standard deviation of real samples processed similarly as the *S. aureus* samples, n = 10; data are shown in Tables S4, S5, and S6) of 1.8×10^2 CFU/mL, 1.7×10^2 CFU/mL, and 2.1×10^2 CFU/mL, respectively. The detection limits in real samples were slightly higher than those in PBS, indicating favorable stability and anti-interference ability in the sample matrix. Hence, the proposed canine IgM-based assay could be robust enough to detect *S. aureus* in the real world. However, the proposed method is not suitable for the detection of *S.aureus* in serum and plasma samples that contain IgG. Specifically, the IgG present in serum or plasma samples has the potential to bind with SPA on the surface of *S. aureus*, thereby hindering recognition by canine IgM-modified MBs and HRP-IgG and ultimately affecting the detection results.

3.4. Selectivity detection of S. aureus

The attribute of selectivity holds significant importance in analytical detection, as it necessitates the ability of the technique to identify the

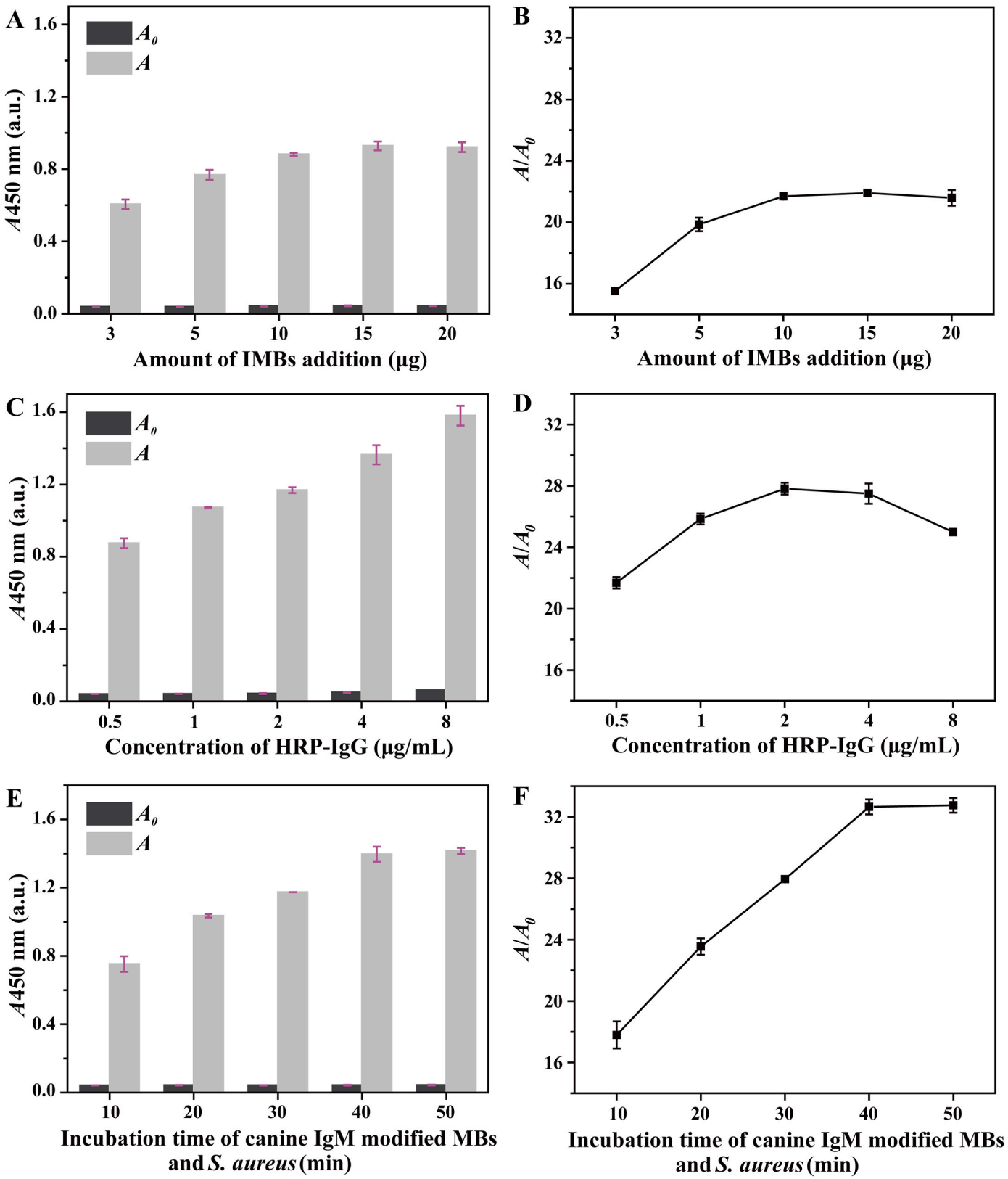

Fig. 3. Optimization of experimental conditions and performance of the canine IgM-based assay. (A) Effect of amount of IMBs added on A and A_0 at 450 nm, (B) effect of the amount of IMBs added on A/A_0, (C) effect of HRP-IgG concentration on A and A_0 at 450 nm, (D) effect of HRP-IgG concentration on A/A_0, (E) effect of incubation time of canine IgM-modified MBs and *S. aureus* on A and A_0 at 450 nm, (F) effect of incubation time of canine IgM modified MBs and *S. aureus* on A/A_0. The bacterial density was 5.0×10^4 CFU/mL. Error bars represent the standard deviation (SD) of three independent measures.

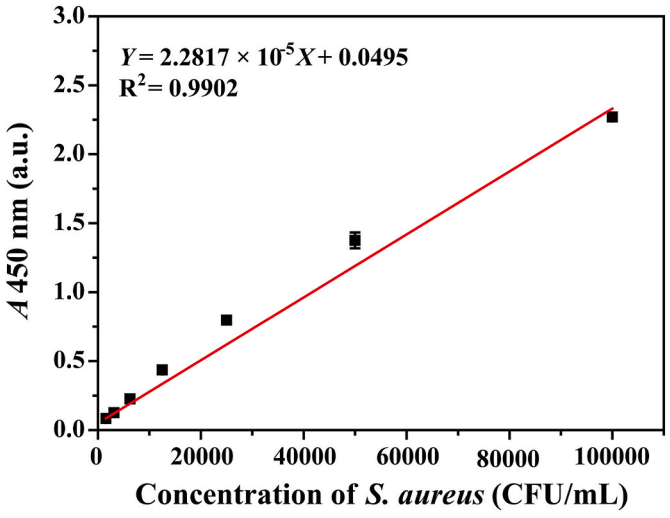

Fig. 4. Concentration of *S. aureus* in PBS plotted against absorbance intensity at 450 nm. Three distinct measures were obtained from three separate preparations for identification. Error bars represent the SD of triplicate measurements.

Table 1
Comparison of molecular recognition based colorimetric methods for *S. aureus* detection.

Recognition agents	Detection limit (CFU/mL)	Time (min)	Reference
Vancomycin	6.7×10^3	140 min	Gao et al. (2015)
Phenylboronic acid	3.0×10^2	100 min	Wang et al. (2022)
Bacteriophage	2.5×10^3	90 min	Yan et al. (2017)
Cell wall binding domain of phage endolysin	4.0×10^3	90 min	Yu et al. (2016)
Aptamer	81	330 min	Yu et al. (2020)
Chicken IgY	1.1×10^2	90 min	Zhang et al. (2019)
Canine IgM	1.4×10^2	90 min	This work

intended target(s) amidst the presence of other interfering targets. To access the selectivity of our assay for *S. aureus* identification, several prevalent foodborne pathogenic bacteria were selected. These interfering bacteria, including *E. coli* O157: H7, *Salmonella*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Listeria monocytogenes*, were used at a concentration of 10^6 CFU/mL. As illustrated in Fig. 5, the absorbance intensities from non-target bacteria were similar to that from the control ($P > 0.05$, statistical analysis results are shown in Table S7) and were significantly lower than that from *S. aureus* ($^{***}P < 0.001$, statistical analysis results are shown in Table S8). In contrast, the inclusion of *S. aureus* led to a significant increase in absorbance intensity ($^{***}P < 0.001$, statistical analysis results are shown in Table S9). These findings suggest that the established biosensor possessed an ideal selectivity for *S. aureus* identification.

Recently, some approaches with high sensitivity have been reported, relying on IgG-SPA recognition to identify *S. aureus* (He et al., 2018; Wang et al., 2019; Wang et al., 2022; Wang et al., 2022; Yu et al., 2016; Zhang et al., 2017). However, the selectivity of these IgG-based *S. aureus* detection methods is affected by the presence of protein G producing *Streptococcus*. Owing to the affinity between canine IgM and *S. aureus*, our proposed method exhibits superior performance in terms of selectivity and could identify *S. aureus* even in the presence of *Streptococcus*. Despite its novelty, the proposed method inherits a major limitation from these SPA-recognition based techniques, specifically the variability

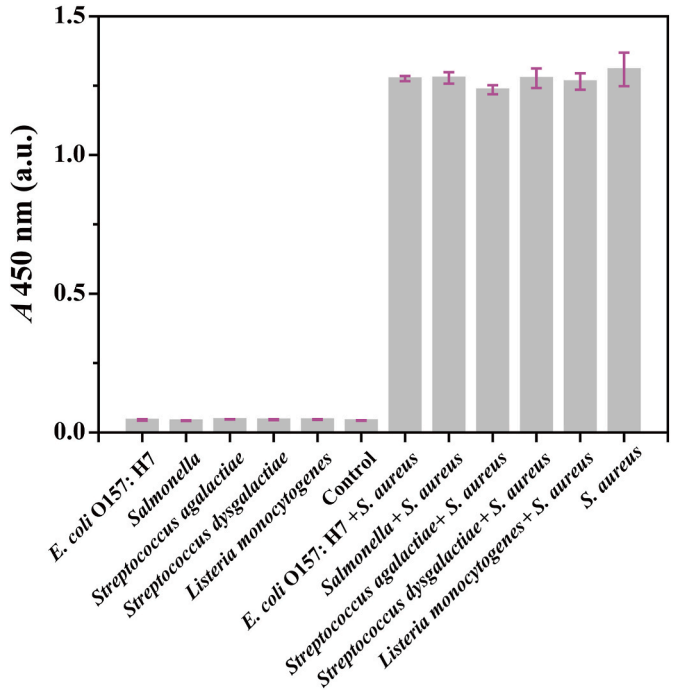

Fig. 5. Absorbance intensity at 450 nm using the canine IgM-based strategy for identifying *S. aureus* and interfering bacteria. 1. *E. coli* O157: H7, 2. *Salmonella*, 3. *Streptococcus agalactiae*, 4. *Streptococcus dysgalactiae*, 5. *Listeria monocytogenes*, 6. Control, 7. Mixture of *E. coli* O157: H7 and *S. aureus*, 8. Mixture of *Salmonella* and *S. aureus*, 9. Mixture of *Streptococcus agalactiae* and *S. aureus*, 10. Mixture of *Streptococcus dysgalactiae* and *S. aureus*, 11. Mixture of *Listeria monocytogenes* and *S. aureus*, and 12. *S. aureus*. Error bars represent the SD of three replicates.

in SPA genes expression levels noted among different strain types (Zhang et al., 2019), thus leading to the detection signal not so strong in some cases.

4. Conclusion

We resolved the interference of protein G-producing *Streptococcus* in traditional immunoassays for *S. aureus*. In our proposed strategy, canine IgM modified MBs were utilized for *S. aureus* separation and enrichment from samples and HRP-IgG for signal output and amplification. Owing to its good enrichment efficiency, plenty of binding sites, and high catalytic activity, the analysis have a short turnaround time (sample-to-answer in less than 90 min), wide detection range (1.6×10^3 CFU/mL to 1.0×10^5 CFU/mL), and an ultralow detection limit (1.4×10^2 CFU/mL). This performance was comparable to those of the most commonly used PCR methods, but its operation is much simpler. Our proposed colorimetric detection approach serves as a visible method for the rapid detection of *S. aureus*. This approach obviates the need for lengthy sample preparation protocols and intricate instrumentation, making it suitable for implementation in the fields of environmental and food safety management.

CRediT authorship contribution statement

Yun Zhang: Writing – original draft, Methodology, Investigation, Conceptualization. **Yibing Zhang:** Methodology, Investigation. **Ruihua Lun:** Investigation. **Qingshan Fu:** Validation, Conceptualization. **Yuqiao Chang:** Formal analysis. **Jiansen Du:** Methodology, Investigation. **Yi Zhang:** Validation, Software, Data curation. **Junping Yu:** Writing – review & editing, Supervision, Resources, Project administration.

Declaration of competing interest

The authors have declared no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116525.

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