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Sensitive and selective detection of *Staphylococcus aureus* via a dual-recognition colorimetric platform based on phenylboronic acid and IgY

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Abstract

Staphylococcus aureus (*S. aureus*) is one of the major food-borne pathogenic bacteria that causes epidemic and food poisoning. In this study, a colorimetric sensing platform was developed for the sensitive and selective detection of *S. aureus* based on the synergy between 3-aminophenylboronic acid-functionalized magnetic beads (APBA-MBs) and horseradish peroxidase labeled anti-protein A IgY (HRP-IgY). Target *S. aureus* bacteria can be captured on the APBA-MBs and then labelled by HRP-IgY through formation of a sandwich structure. Detection of the bacteria then involves oxidation of the colorimetric HRP substrate, thereby converting *S. aureus* concentrations into a detectable signal. The optimized method can be performed within 120 min, with a detection limit of 9.4×10^2 CFU/mL. The proposed strategy was successfully applied to detection of *S. aureus* in spring water and apple juice samples. The dual-recognition platform showed selectivity towards *S. aureus*, and other common foodborne pathogens yielded negative results. The incorporation of IgY into the proposed strategy can address the lack of selectivity observed in previously reported APBA-based methods. Our strategy has potential as a versatile platform for the sensitive detection of various bacteria using corresponding IgY.

Keywords *S. aureus*, Phenylboronic acid, Chicken anti-protein A IgY, Magnetic beads, Colorimetric detection

Introduction

Staphylococcus aureus (*S. aureus*), a prevalent Gram-positive bacterium, is a commensal organism that inhabits the nasal mucosa and skin of approximately 30% of the total population (Li et al. 2023a; Rubab et al. 2018; Tong et al. 2015). The high rate of human carriers contributes to the frequent occurrence of staphylococcal food poisoning. Owing to its robust adaptability across a wide range of temperatures, pH levels, and saline conditions, *S. aureus* is found in diverse food sources. Importantly, despite being non-spore-forming, *S. aureus* can contaminate food products during their preparation and processing (Kadariya et al. 2014; Li et al. 2024). Accordingly, *S. aureus* is a major foodborne pathogen, ranking among the top five germs that cause illness in the USA (https://

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www.cdc.gov/foodsafety/foodborne-germs.html). Inadvertent consumption of improperly processed *S. aureus*-contaminated foods can trigger an abrupt onset of nausea, vomiting, and abdominal cramping with or without diarrhea. Outbreak of *S. aureus* food poisoning have emerged as a significant public health threat in many countries, and detection of the bacterium in food is key to food safety and the lowering of risks to human health.

Because of its well characterized precision and relatively low cost, traditional "culture and colony-counting" is the gold standard method for microbial detection. However, this method takes between 24 and 72 h to yield results, and the process is tedious and laborious (JaYan et al. 2020; Kim et al. 2021). Additionally, *S. aureus* can enter a viable but nonculturable state that precludes detection (Yan et al. 2021; Yu et al. 2024). Polymerase chain reaction (PCR)-based techniques can amplify the specific nucleic acid sequences (e.g., *femA* and *arcC*) (Hassan et al. 2022) and, theoretically, detect a single bacterium. Moreover, a skilled technician is always required to perform the time-intensive processing steps, including cell lysis and DNA detection (Kasturi et al. 2017). The application of PCR-based techniques to food products is also limited by the matrix effect of food samples and the risk of false-positive results due to aerosol pollution (Leva-Bueno et al. 2020).

Achieving rapid and efficient isolation and enrichment of bacteria from complex sample matrices represents an initial important step towards sensitive bacterial detection. Recently, strategies based on magnetic separation coupled with specific recognition molecules have drawn increasing attention. Magnetic beads (MBs) have been widely applied to capture target bacteria from food matrices, owing to their ease of manipulation and fast separation speed. In previous work, we used MBs coated with either chicken IgY-(Zhang et al. 2019a) or dog IgG-(Zhang et al. 2019b) to capture *S. aureus* from food samples and thus developed highly sensitive and selective immunoassay. However, the process of coating MBs requires a relatively large amount of expensive antibodies, thereby increasing the cost of detection. Chemoselective ligands have been regarded as alternative binding agents, as they are less expensive and more resistant to both chemical and biological degradation (Wannapob et al. 2010).

Boronic acids can selectively bind with *cis*-diol moieties of glycoproteins and glycopeptides via reversible esterification (Li et al. 2018; Liu et al. 2023). Hence, the abundance of peptidoglycan and lipopolysaccharide on bacterial surfaces is compatible with boronic acid as an effective recognition agent for pathogen detection (Khanal et al. 2013; Li et al. 2023b; Wang et al. 2012). One type of amino-substituted aryl boronic acid, 3-aminophenylboronic acid (3-APBA), is a particularly suitable derivatization reagent for this purpose. Owing to the presence of amino groups in 3-APBA, it can couple with the carboxyl groups of MBs to form APBA-functionalized MBs for capturing bacteria (Geng et al. 2024; Wang et al. 2022c; Wikantyasning et al. 2023).

However, given that *cis*-diol groups exist on both Gram-positive and Gram-negative bacteria, 3-APBA lacks specificity for *S. aureus* detection. Fortunately, SPA on the surface of *S. aureus* represents a unique epitope for this species. The binding of the Fc subunit of pig IgG to protein A has been used in detection assays to mitigate the lack of specificity of 3-APBA (Wang et al. 2022b, c).. However, as IgG can cross-react with protein G-producing *Streptococcus* spp., which would also be recognized by 3-APBA, the use of this antibody potentially leads to false-positive results.

In the pursuit of an *S. aureus*-selective recognition agent for use in APBA-based strategies, anti-protein A IgY antibodies raised in chicken are an attractive tool, as these antibodies do not interact with protein G (Dou et al. 2022; Thirumalai et al. 2019; Zhang et al. 2017). Accordingly, we predicted that pairing chicken anti-protein A IgY with APBA would eliminate interference from protein G-producing *Streptococcus* spp. and promote the specificity of detection of *S. aureus.*

Herein, we report the development of a novel dual-recognition platform for capturing and detecting *S. aureus* using APBA-functionalized MBs and horseradish peroxidase (HRP)-conjugated chicken anti-protein A IgY (HRP-IgY). The strategy is illustrated schematically in Fig. 1. *S. aureus* cells can be enriched from a sample using APBA-functionalized MBs, and the cells can then be specifically detected by labelling them with HRP-IgY and the colorimetric HRP substrate 3,3′,5,5′-tetramethylbenzidine (TMB). In this study, we optimized detection conditions and assessed the sensitivity, specificity, and applicability of the method. To our knowledge, this is the first study to use the combination of APBA and chicken IgY for *S. aureus* detection.

Experiment

Material and reagents

APBA, casein sodium salt from bovine milk, and biotin N-hydroxysuccinimide ester (Biotin-NHS) lyophilized powder were purchased from Sigma-Aldrich (USA). Carboxylate-modified MBs (25 mg/mL), with a nominal diameter of 820 nm, were purchased from Spherotech (USA). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were both procured from Aladdin (Shanghai, China). Bovine serum albumin (BSA) was purchased from Biosharp (China). HRP-IgY and HRP

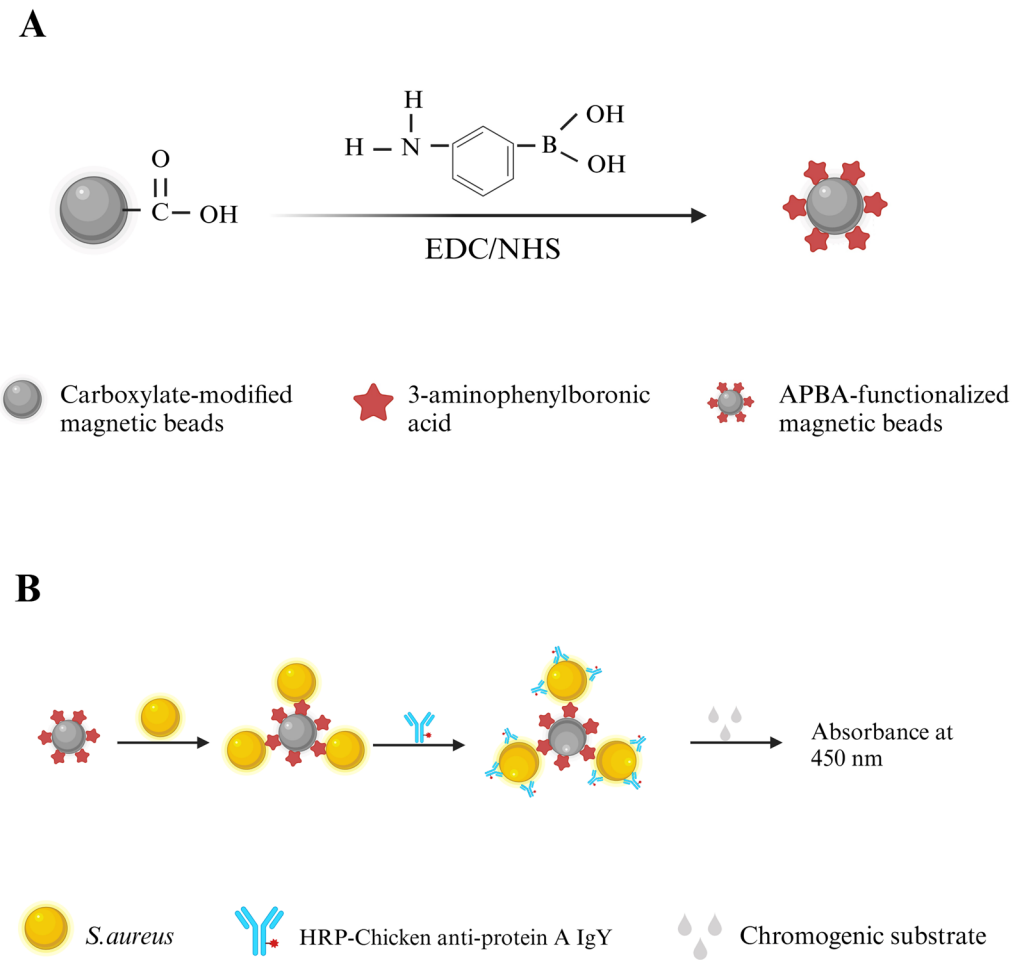

Fig. 1 Schematic diagram displaying the strategy of the proposed immunoassay. **A** The procedure for the preparation of APBA-functionalized MBs; **B** Schematic diagram (not to scale) of the colorimetric assay for *S. aureus* detection based on dual-recognition by APBA and chicken anti-protein A IgY. Created with BioRender.com

colorimetric substrate (stabilized TMB chromogen solution) were purchased from Abcam (UK) and Biodragon Immunotechnologies (Beijing, China), respectively. HRP-labeled protein A (HRP-protein A) and HRP-labeled protein G (HRP-protein G) were obtained from SouthernBiotech (USA). A recombinant protein tandem dimer tomato (tdTomato) fused to the cell wall binding domain (CBD) of plyV12 (tdTomato-CBD) was a gift from Prof. Hongping Wei (Wuhan Institute of Virology, Chinese Academy of Science, Wuhan, China). Lactose and bovine IgG were provided by Yuanye (Shanghai, China) and Solarbio (Beijing, China), respectively. Spring water and apple juice were obtained from nearby stores. All compounds and reagents used in the experiments were of analytical purity. All buffer solutions were prepared with deionized water (18.2 MΩ) that was obtained using the Ulupure UPR-II-10 T system (Chengdu, China).

Bacterial culture and counting

S. aureus (Cowan I), *Streptococcus dysgalactiae* (*S. dysgalactiae*, ATCC 35666), *Streptococcus agalactiae* (*S. agalactiae*, isolated from raw milk), *Bacillus cereus* (*B. cereus*, NC7401) and *E. coli* O157: H7 (EDL 933) were provided by Prof. Hongping Wei. *Shigella dysenteriae* (*Sh. dysenteriae*, CMCC 51252) and *Salmonella choleraesuis* (*S. choleraesuis*, ATCC 13312) were purchased from Guangdong Microbial Culture Collection Center (Guangdong, China). *S. aureus* (ATCC 12598, ATCC 2592, and ATCC 29213), *Listeria monocytogenes* (*L. monocytogenes*, ATCC 19115) were bought from the BeNa Culture Collection (Beijing, China). All the bacterial species were stored at − 80 °C in 50% glycerol until further use.

Given the diverse nutritional requirements of the various strains, *S. aureus*, *Sh. Dysenteriae*, *S. choleraesuis*, *B. cereus* and *E. coli* O157: H7 were inoculated in Luria–Bertani (LB) medium, while *S. dysgalactiae*,

S. agalactiae and *L. monocytogenes* were cultured in brain heart infusion (BHI) broth. First, bacterial stock solutions were inoculated in lines on agar plates and cultured in an incubator at 37 °C overnight. Single colonies were then picked for culturing in liquid medium with continuous oscillation at 37 °C until reaching the logarithmic phase. The samples were centrifuged (3000 r/min for 10 min) at 4 °C to collect the bacterial cells, which were subsequently washed twice with sterile phosphate-buffered saline (PBS, 0.01 mol/L, pH 7.4). After washing, the collected bacteria were re-suspended in sterile PBS (0.01 mol/L, pH 7.4) containing 20% glycerin and stored at − 80 °C until use. Bacterial concentrations were determined by plate counting.

Preparation of APBA-functionalized MBs

APBA-functionalized MBs were prepared according to previously described procedures (Wang et al. 2022c; Zhang et al. 2019a), with slight modifications (Fig. 1A). Carboxylate-modified MBs (1 mg) were activated in 1 mL of PBS (0.01 mol/L, pH 6.8) containing 0.029 mg EDC and 0.033 mg NHS. This activation step was carried out in a shaker at 37 °C and 200 r/min for 1 h. Following activation, excess EDC and NHS were removed using a magnetic separation rack (Goldmag Biotech, China). Activated MBs were mixed with 0.05 mg of 3-APBA in PBS (0.01 mol/L, pH 7.4) and coupling was performed in a shaker at 37 °C and 200 r/min for 2 h. The resulting APBA-functionalized MBs were washed thrice with PBS (0.01 mol/L, pH 7.4), dispersed in 1 mL of PBS (0.01 mol/L, pH 7.4), and stored at 4 °C for subsequent experiments.

Characterization of the binding of APBA-functionalized MBs to *S. aureus*

To investigate the capability of APBA-functionalized MBs to recognize *S. aureus*, the red fluorescent protein tdTomato-CBD was used to permit microscopic visualization of *S. aureus* cells (Fig. S1), as previous research from our lab demonstrated the ability of tdTomato-CBD to bind to *S. aureus* (Yu et al. 2016). To perform this assay, *S. aureus* cells (100 µL at 1.0×10^8 CFU/mL) were first incubated with 2 µL of tdTomato-CBD (1 mg/mL) at 37 °C and under agitation (200 r/min) for 30 min. Following the removal of unbound tdTomato-CBD, the resulting tdTomato-stained *S. aureus* cells were mixed with APBA-functionalized MBs under agitation (200 r/min) at 37 °C for 30 min. APBA-functionalized MBs@tdTomato-CBD stained *S. aureus* were imaged using a Dragonfy 202 fluorescence microscope (Andor, UK).

Colorimetric detection of *S. aureus*

The entire process leading to the detection of *S. aureus* is shown in Fig. 1B. A suspension of *S. aureus* (200 µL) was incubated with APBA-functionalized MBs with agitation (200 r/min) at 37 °C for 30 min. The supernatant was removed by magnetic separation, while the precipitate containing *S. aureus*-loaded APBA-functionalized MBs was washed with PBS (pH 7.4) containing 0.05% Tween 20 (PBST) to remove uncaptured bacteria and food matrix components. Then, the cells were incubated with BSA (100 µL at 1 mg/mL) for 30 min to block nonspecific absorption sites. The sample was then re-suspended in 100 µL of HRP-IgY. After incubation at 37 °C for 30 min, excessive HRP-IgY was removed by washing with PBST. A TMB chromogen solution (100 µL) was added, and the mixture was allowed to incubate for 10 min at at 37 °C in the dark. Sulfuric acid (2 mol/L, 100 µL) was added to terminate the reaction. Colorimetric intensity was measured at 450 nm using a Multiskan MK3 Microplate reader (Thermo Scientific, USA).

Results and discussions

Interaction of APBA-functionalized MBs to *S. aureus*

The proposed detection method depends on the effective attachment of MBs to *S. aureus*. MB composites decorated with boronic acid moieties, such as 3-APBA, have been shown to adhere to bacteria via covalent bonding with the *cis*-diol moieties of saccharides located on the bacterial cell wall. The binding of APBA-functionalized MBs was evaluated by incubating them with *S. aureus* cells fluorescently labelled with tdTomato-CBD and then visualizing the cells with bright-field and fluorescence microscopy. The APBA-functionalized MBs@tdTomato-CBD stained *S. aureus* cells were observed as bubble-like structures under bright-field microscopy (Fig. 2A). The precise matching of these structures with the red fluorescence of the known *S. aureus* indicator tdTomato-CBD (Fig. 2B). To validate the specificity of the fluorescence signals observed, a negative control (MBs only without *S. aureus*) was processed to confirm that MB autofluorescence does not contribute to the observed signals (Fig. 2C, D), thereby demonstrating the ability of APBA-functionalized MBs to capture *S. aureus*.

Optimization of the dual-recognition assay

Following capture of the bacteria, the proposed assay involves specific interactions between SPA on *S. aureus* and IgY conjugated to HRP. Detection is achieved by incubation with a chromogenic HRP substrate. In order to optimize detection, the assay was performed while adjusting several parameters, including the amount of APBA-functionalized MBs added, the incubation time,

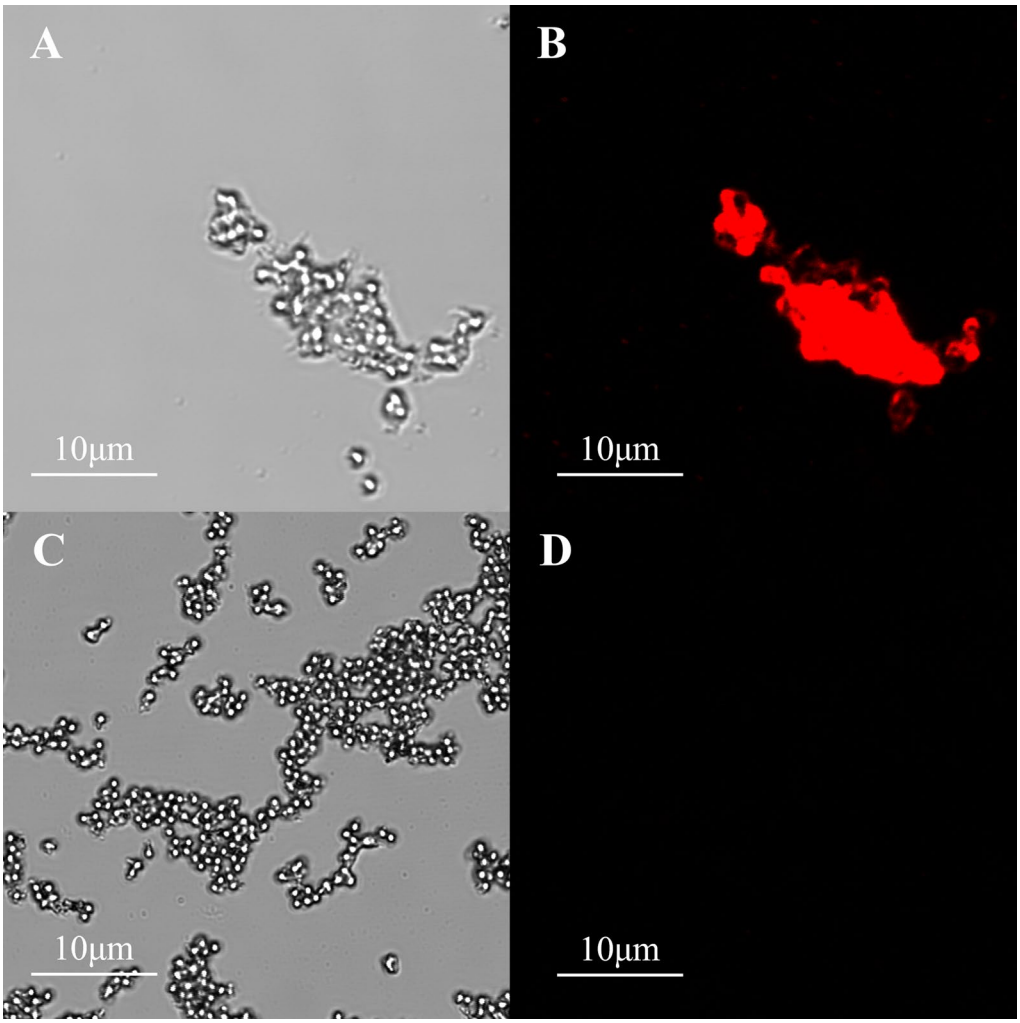

Fig. 2 Fluorescence micrographs of APBA-functionalized MBs@tdTomato-CBD stained *S. aureus* (cell bind domain from plyV12, (Yu et al. 2016)) and control MBs. **A** Bright-field image of APBA-functionalized MBs@tdTomato-CBD stained *S. aureus* (cell bind domain from plyV12; **B** Corresponding red fluorescence image; **C** and **D**: Bright-field and fluorescence images of MBs (negative control), respectively (Scale bar: 10 μm)

and the concentration of HRP-IgY. The absorbance intensities measured at 450 nm for the experimental group, which comprised 5.0×10^4 CFU/mL of *S. aureus* suspended in PBS, and the blank group, consisting solely of PBS, were designated as A and A_0, respectively.

The performance of the assay is governed by the specific recognition of *S. aureus* (quantified as A) and non-specific adsorption of HRP-IgY onto the MBs (quantified as A_0). As shown in Fig. 3A, B and Table S1, both A and A_0 increased as the amount of added APBA-functionalized MBs increased, and the A/A_0 ratio was maximized at 20 μg. At lower values, the specific binding that was observed was lower, but at higher values, the non-specific binding increased. In a similar manner, both A and A/A_0 increased over time, ultimately reaching a plateau at 30 min (Fig. 3C, D and Table S2). Statistical analysis (Table S3) confirmed significant differences ($^{***}P<0.001$) between 30 min and shorter durations. Thus, the amount of APBA-functionalized MBs used for subsequent experiments was 20 μg and the time of incubation was 30 min.

Concentrations of IgY-HRP that are too low would be expected to lead to decreased signal, while excessive concentrations could lead to non-specific adsorption. Thus, we performed the assay with different concentrations of HRP-IgY over the range of 0.25 μg/mL to 2 μg/mL. Accordingly, as shown in Fig. 3E, F and Table S4, both A and A_0 increased with increasing HRP-IgY concentrations. Because the A/A_0 value was maximized at 0.5 μg/mL HRP-IgY, this concentration was used in subsequent assays.

Therefore, the optimized conditions for *S. aureus* detection were as follows: an addition of 20 μg of APBA-functionalized MBs, an incubation time of 30 min, and a concentration of 0.5 μg/mL of HRP-IgY.

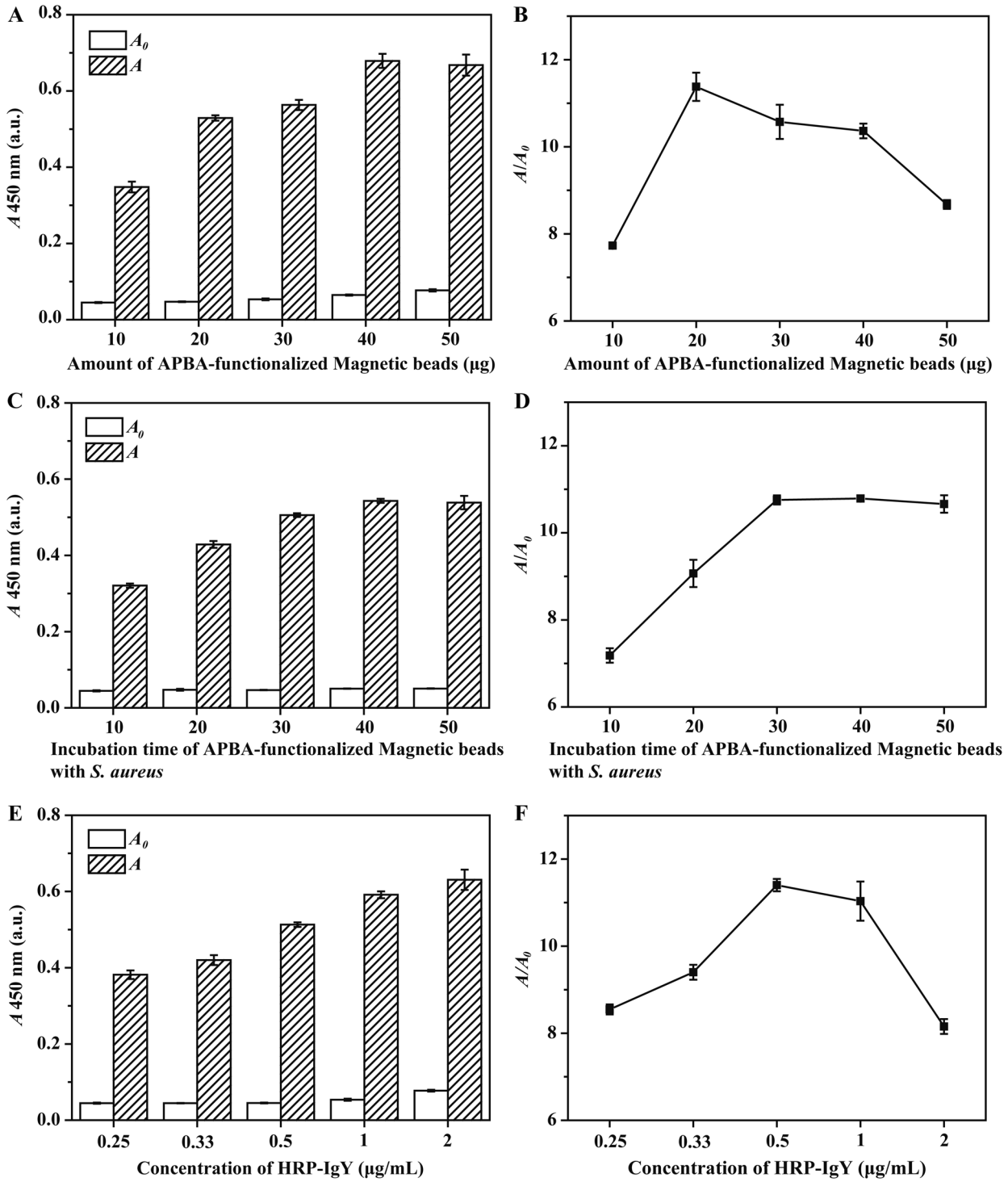

Fig. 3 Condition optimization for the APBA-IgY based dual-recognition method. The assay was performed under the noted conditions in the absence (A_0) or presence (A) of 5.0×10^4 CFU/mL *S. aureus*. After addition of a chromogenic HRP substrate, the signal was recorded as A_{450}. (**A**, **B**) The amount of APBA-functionalized MBs was varied, with an incubation time of 30 min and 0.5 μg/mL HRP-IgY; (**C**, **D**) Incubation time of APBA-functionalized MBs with *S. aureus* was varied, with 20 μg MBs and 0.5 μg/mL HRP-IgY; (**E**, **F**) The concentration of HRP-IgY was varied, with an incubation time of 30 min and 20 μg MBs. The bacterial density was 5.0×10^4 CFU/mL. (Error bars: standard deviation (SD), n = 3)

Detection of *S. aureus* in PBS

The responsiveness of the assay to the quantity of *S. aureus* present in PBS was determined, over a concentration range of 1.6×10^3 CFU/mL to 2.0×10^5 CFU/mL. As shown in Fig. 4, the colorimetric intensity at 450 nm was linearly correlated ($R^2=0.9988$) with the *S. aureus* concentration. In addition, as shown in Table S5, the relative SD values of the measurements (n = 3) were less than 5%. The linear equation describing the relationship between the Y and the bacterial concentration (X) was $Y=(8.8366\times10^{-6})\times X+0.0497$. According to the IUPAC guidelines, the limit of detection (LOD) is defined as 3σ, where σ is the SD of blank PBS measurements (n = 10) processed under the same conditions as test samples. The LOD was calculated using the IUPAC-recommended 3σ/S method, where σ is the SD of blank measurements and S is the slope of the calibration curve. According to the data provided in Table S6, the limit of detection for the proposed method was calculated to be 9.4×10^2 CFU/mL.

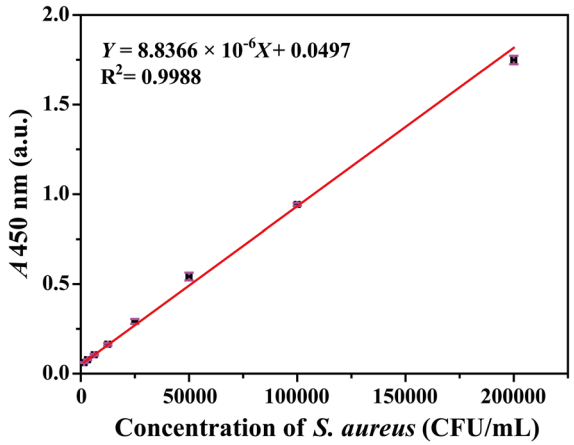

Fig. 4 Dependence of signal on the concentration of *S. aureus*. The detection of *S. aureus* samples in PBS (CFU/mL) was performed under optimized conditions, and the signal was obtained as absorbance intensity at 450 nm. (Error bars: SD, n = 3)

The analytical sensitivity of the developed method was comparable to that of previously reported colorimetric methods that combine magnetic separation and molecular recognition strategies (Table 1). The ability of MBs to enrich and concentrate target bacteria from large-volume samples allowed us to enhance the detection sensitivity so that even extremely low concentrations of bacteria could be detected by increasing the detection volume of sample ten-fold or more.

When compared with PCR-based, ELISA-based and SPR-based assays, the proposed method stands due to its unique combination of sensitivity and simplicity. While the detection of a single CFU is theoretically feasible using PCR, the practical efficiencies of DNA extraction and amplification, especially due to the heterogeneous distribution of bacteria in a sample, mean that such sensitivity remains a formidable challenge. In addition, given the small template volumes (less that 5 μL) that are required for PCR-based methods, the identification of a single bacterium in 1 mL of sample without pre-enrichment is rather unlikely. Our method achieved a sensitivity of 9.4×10^2 CFU/mL within 120 min, costing around $ 0.07 per test with basic equipment (magnetic rack and plate reader). In contrast, PCR offers higher sensitivity (1 CFU/mL–10 CFU/mL) but requires 250 min (Ndraha et al. 2023), specialized training, and costs $ 1-$ 5 per test. The main challenges in the implementation of ELISA-based methods involve identifying an appropriate antibody pair and ensuring sufficient sensitivity to detect the low concentrations of bacteria in food. IgG antibodies can be used to capture *S. aureus* by binding to SPA, but the secondary antibody might compete for binding to SPA, potentially causing the release of the captured bacteria and decreasing the sensitivity of the assay (Yu et al. 2016).While loop-mediated isothermal amplification

Table 1 Comparison of various colorimetric methods for detection of *S. aureus* that combine magnetic separation with molecular recognition

Recognition agent	LOD (CFU/mL)	Time (min)	References
Chicken IgY-rabbit IgG	1.1×10^2	90	Zhang et al. (2019a)
Dog IgG-Chicken IgY	1.0×10^3	90	Zhang et al. (2019b)
APBA-pig IgG	3.0×10^2	105	Wang et al. (2022b)
Mouse IgG-CBD	4.0×10^3	90	Yu et al. (2016)
Vancomycin-IgG	3.3×10^3	210	Wang et al. (2022a)
Mouse monoclonal anti-*S. aureus* antibody	1.5×10^3	40	Sung et al. (2013)
Wheat germ agglutinin-chicken IgY	3.9×10^2	90	Zhang et al. (2024)
Bacteriophage-goat IgG	2.5×10^3	90	Yan et al. (2017)
Phage-derived bacterial-binding proteins	2.7×10^4	145	Hong et al. (2024)
This study	9.4×10^2	120	

(LAMP) and SPR balance speed and sensitivity but remain more expensive and equipment-dependent. Though less sensitive than PCR theoretically, these results clearly confirm the potential of the APBA-IgY based dual-sensing platform as a promising alternative to conventional nucleic acid- and antibody-based systems for resource-limited laboratories.

Selectivity of the APBA-IgY based method

Selectivity is a key parameter in a detection method, as it influences both the risk of false positive signals and the risk of missing the target signal due to interference by off-target bacteria. To assess potential cross-reactivity, the APBA-IgY based dual-recognition method was applied to the detection of the potentially interfering pathogenic bacteria *Sh. dysenteriae*, *S. choleraesuis*, *S. dysgalactiae*, *S. agalactiae*, *L. monocytogenes*, *E. coli* O157: H7 and *B. cereus* at a concentration of 5.0×10^5 CFU/mL. As shown in Fig. 5, the colorimetric signals resulting from the analysis of the off-target bacteria were almost negligible close to the background signal. In addition, the signal obtained for the the detection of *S. aureus* (at a concentration of 5.0×10^4 CFU/mL) in the presence of all seven of these bacteria was similar to the signal obtained with *S. aureus* (at a concentration of 5.0×10^4 CFU/mL) alone (Fig. 5 and Table S7). Based on these results, we conclude that while some types of interfering bacteria might be captured by APBA-functionalized MBs, the selectivity of the IgY for SPA, which is exclusive to strains of *S. aureus*, makes the detection method highly specific.

Previous studies have indeed reported the use of APBA for capturing *S. aureus* (Wang et al. 2022b, c), and these methods utilized pig IgG to address the lack of capture specificity. However, pig IgG also lacks specificity, as it can bind to both protein A and protein G, which is produced by *Streptococcus* spp. Here, we performed an ELISA assay to test the binding capacity of antibodies for protein A and protein G (as depicted in Fig. S2). As shown in Fig. S3, the absorbance intensities from HRP-protein A and protein G in the pig IgG group were nearly identical, whereas in Chicken anti-protein A IgY group, the absorbance from HRP-protein A was significantly higher than that from HRP-protein G ($^{***}P<0.001$, statistical analysis results are shown in Table S8). Pig IgG can bind to both protein A and protein G, while the chicken anti-protein A IgY antibody used in our method showed exclusive binding to protein A, with no interaction observed for protein G. These results are consistent with other reported findings (Butler et al. 2009; Zhang et al. 2024). Thus, the use of chicken anti-protein A IgY facilitates accurate identification of *S. aureus* and reduces the risk of false positive outcomes. These findings confirm the superior ability of the recognition pair "APBA-IgY" in distinguishing *S. aureus* from other pathogenic bacteria, highlighting its promising potential for enhancing detection accuracy.

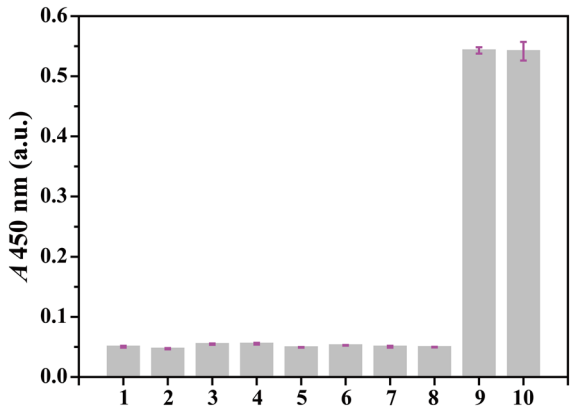

Fig. 5 Selectivity of the proposed method for identifying *S. aureus*. The signal output was measured as A_{450} upon applying the APBA-IgY based dual-recognition strategy to samples containing equal concentrations of various pathogenic bacteria. 1: blank, 2: *Sh. dysenteriae*, 3: *S. choleraesuis*, 4: *S. dysgalactiae*, 5: *S. agalactiae*, 6: *L. monocytogenes*, 7: *E. coli* O157: H7, 8: *B. cereus*, 9: *S. aureus* and 10: *S. aureus* with all 7 off-target bacteria. (Error bars: SD, n = 3)

Detection of *S. aureus* in real samples

To evaluate the application potential of our proposed system, we employed the tool to detect *S. aureus* in representative fluids (spring water and apple juice). The accuracy of the proposed method was tested using the standard addition method, in which different known amounts of *S. aureus* was added to the samples prior to detection. As shown in Figs. S4 and S5, the two calibration curves that were obtained had R^2 values of approximately 0.99, indicating linearity (The detailed data are shown in Tables S9 and S10). The detection limits (3σ) in undiluted spring water and apple juice were 9.3×10^2 CFU/mL and 9.5×10^2 CFU/mL, respectively.

As expected, the detection limit of this method in PBS was within the same order of magnitude as those in the actual samples, indicative of robust stability and effective resistance to interference by the sample matrix. However, a major limitation of the proposed strategy is that it cannot be applied to milk samples,which are rich in nutrients and have a neutral pH and thus are frequently contaminated by *S. aureus*. To investigate the cause of this failure, competitive inhibition assays were performed by spiking *S. aureus* suspensions with milk's major constituents-casein (28 mg/mL), BSA (0.63 mg/mL), lactose (53 mg/mL) and bovine IgG (0.63 mg/mL)-at physiological concentrations (Costa et al. 2019; Vincent et al. 2016). Expected outcomes (Fig. S6) include: 1) high concentrations of non-glycoprotein-mediated interference: severely

diminished absorbance signals in the casein and BSA groups indicated their ability to interfere with the capture of *S. aureus* by APBA-MBs, and 2) *cis*-diol-mediated interference: reduced absorbance signals in the IgG and lactose groups confirmed their competitive binding to APBA-MBs via *cis*-diol structures, which hindered *S. aureus* capture.

To demonstrate broad applicability, the method was tested against multiple *S. aureus* strains. While *S. aureus* Cowan I was initially utilized for assay optimization, additional strains (ATCC 12598, ATCC 25923, and ATCC 29213) were evaluated to address strain diversity. As shown in Supplementary Fig. S7, detection signals varied across strains, confirming cross-strain applicability. In some cases, the proposed method can be used to detect *S. aureus* qualitatively.

Conclusion

We describe here a user-friendly and sensitive detection technique for *S. aureus* that takes 120 min (including washing steps and operations) and achieves a low detection limit of 9.4×10^2 CFU/mL. The APBA-IgY based dual-recognition platform has several advantages. First, the capture efficiency of APBA-functionalized MBs is optimized by the abundant *cis*-diol groups in the cell wall of *S. aureus*. Second, the sensitivity of the method is further enhanced by the abundance of protein A molecules in each *S. aureus* cell, and the selectivity of the method is supported by the use of chicken IgY antibodies, which do not interact with protein G, as opposed to mammalian IgG. Third, owing to its low price and small molecular weight, the use of 3-APBA greatly reduces the cost of detection. Fourth, this proof-of-principle study can serve as a versatile platform for developing additional methods to be applied to the sensitive detection of other bacteria using appropriate IgY. Lastly, the proposed method can be automated by integrating the MBs with a simple magnetic particle-handling machine. Benefiting from these advantages, the novel dual-recognition assay stands as a refined complement of other phenylboronic acid-based systems and holds great potential for screening *S. aureus*-contaminated food samples.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-025-00501-4.

Supplementary file1.

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Author contributions
YZ: Writing-review and editing, Supervision, Project administration. CH: Conceptualization, Investigation. XY: Methodology. YC: Formal analysis. YH: Methodology. QG: Resources, Funding acquisition. QW: Formal analysis, Funding acquisition. MW: Data curation, Validation. YZ: Writing-original draft, Methodology.

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Availability of data and materials
Almost all details of experimental data are presented in the article or additional file.

Declarations

Competing interests
The authors declare that they have no competing interests.

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