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ORIGINAL PAPER

Conductometric sensor for viable *Escherichia coli* and *Staphylococcus aureus* based on magnetic analyte separation via aptamer

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Abstract

A method is described to determine viable populations of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The method employs aptamer-magnetic separation combined with resistivity based detection. The bacteria were separated by means of aptamer-functionalized magnetic beads. They were then quantified by measuring their growth kinetics through time-dependent conductivity changes of culture media. The time-course of growth was logged by real-time and contactless measurements that yielded starting concentrations from the duration of lag intervals prior to the log phase of growth. In pure water samples, the linear ranges for measuring *E. coli* and *S. aureus* cells are 2.5×10^3–2.5×10^8 CFU·mL^{-1} and 4.1×10^3–4.1×10^8 CFU·mL^{-1}, respectively. In spiked tap water samples, the lower limits of detection are 2.3×10^4 CFU·mL^{-1} and 4.0×10^3 CFU·mL^{-1} for *E. coli* and *S. aureus*, with recoveries of 87.0–108.7% and 92.5–105.0%, respectively. The relative standard deviation of these measurements (10.0%) is below that of plate counting method (13.9%). The presence of micro/nanoparticles such as magnetic beads or selenium nanoparticles in the culture media does not interfere, unlike in case of automatted optical density monitoring. The *E. coli* and *S. aureus* cells captured on the aptamer-functionalized magnetic beads can be directly tested for their susceptibility to antibiotics. The process of magnetic separation and determination of load burden requires neither bulky, sophisticated equipment nor expensive reagents.

Keywords Viable bacteria · Online monitoring · Bacterial growth · Growth curve · Capacitively-coupled contactless conductivity · Non-invasive measurement · Selenium nanoparticles · Determination · Automated antibiotic susceptibility testing · Biosensor

Introduction

Escherichia coli (*E. coli*) is a common microbe of the gut of warm blooded animals and is also widely distributed in natural and man-made environments [1–3]. *Staphylococcus aureus* (*S. aureus*) is ubiquitous and can produce a wide spectrum of illnesses [4–6]. Commonly, *E. coli* and *S. aureus* are considered typical archetypes of gram-negative and gram-positive bacterium, respectively, and are frequently used as targets in bacterial experiments.

Several culture-independent methods for determination of bacteria have been developed to improve efficiency and sensitivity. They mainly involve gene or ribosome analysis (e.g. polymerase chain reaction [5, 7] and loop-mediated isothermal amplification [8]), microscopy [9], biosensor [1], bioluminescence [10], fluorescence [11], surface-enhanced Raman scattering [3], and surface plasmon resonance [12]. Although DNA/RNA analysis offers the best sensitivity with the greatest capacity for target speciation, there is frequently an over estimation of bacterial load due to the presence of non-culturable, dead cells and extracellular DNA [7]. Microscopy presents an alternative technique for directly and rapidly counting the total number of target bacteria. However, because of the requirement of observing individual cells, high magnification and small field of view, impose practical limitations on volume sampling, leading to inaccurate and imprecise enumeration

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when used with real samples [9]. Bacterial biosensors, which are based on electrochemical or optical techniques that recognize targets by antibodies, their fragments, or engineered peptides, present another pathway. Although they can be routinely performed by a trained technician, reagent stability is short-lived in the liquid state, and algorithms that directly report viable cell populations are in their infancy. As such, growth-based methods are still considered the "gold standard" for determination of viable bacteria [4, 6].

In order to address the problems that manual methods face, several automated approaches have been developed in which growth is automatically monitored in real time [13]. Among them, optics-based and electrochemistry-based methods are the most popular. The image pattern analysis, based on time-lapse microscopy, is capable of determining the morphology of *E. coli* down to single-cell resolution [14]. However, the number of individual cells that can be continuously monitored by microscopy is limited [15]. Another pattern based on turbidity/absorbance can achieve fast, continuous, non-invasive, high-throughput, and real-time measurement in an indirect manner. Consequently, several commercial instruments have been successfully adapted for numerous application requirements [16]. However, several concerns with optic-based methods merit further consideration including: the requirement of sample transparency, elimination of optical and electrical noise, adhesion of co-segregated materials, light scattering by cell debris, and interference from gas bubbles or other micro- and nanoparticles [16, 17]. Electrochemistry-based methods are easier to miniaturize and usually more cost-effective compared to optical methods. In particular, they can provide label-free pattern detection capability [18]. Nevertheless, electrode deterioration and non-specific capture are difficult to eliminate completely because normally working electrodes must be in galvanic contact with the arbitrary medium or solution. The erratic noise that results decreases the accuracy of measurement. To address these problems, we developed a conductometric method for monitoring bacterial growth in real time. A capacitively coupled contactless conductivity detectors was used to measure and record the conductivity changes of the culture medium in a non-invasive manner as bacterial populations multiplied [16]. It incorporates some of the advantages of both classical electrochemistry- and turbidity/absorbance-centered approaches.

Some rapid approaches, other than selective culture, have been developed to facilitate identification and separation of target bacteria in food and environmental samples prior to a final quantification step. A number of antigen/antibody [3], bacteriophage [6, 11] and aptamer [4, 9–12] technologies are capable of specifically recognizing targeted bacteria species. They have led to widespread exploration and have found applications in medicine, food protection, and environmental safety [2, 19]. Compared to antigen/antibody and bacteriophage selection, aptamers have several advantages, such as good stability over a vast range of pH, temperature, and ionic environments. In addition, they are low cost, non-toxic, easy to synthesize, highly resistant to denaturation, possess long shelf-life, and are amenable to well-controlled modifications. Therefore, it is rather attractive to use aptamers for identifying *E. coli* [20, 21] and *S. aureus* [22, 23]. When used in conjunction with magnetic beads, aptamers can be instrumental in identifying as well as separating target from debris contained within the same sample source [24]. However, we know of no reports based on automated growth methods for directly determining bacterial load captured on magnetic beads.

In this paper, we demonstrate that viable *E. coli* and *S. aureus* populations can be simply and efficiently determined by magnetic analyte separation via aptamer selection in combination with a multichannel conductometric sensor (MAS@CS). For the first time, automated quantification of viable target species has been achieved by directly culturing cells captured on the magnetic beads. The limit of detection (LOD) is determined for each species. The distinguishing feature of this method is the absence of interferencefrom micro/nanoparticles that are present in the assay medium. We also show using this method that susceptibility and resistance of *E. coli* and *S. aureus* strains to antibiotics can be directly tested in the presence of the capturing particles.

Materials and methods

Materials and reagents

Standard bacterial strains of *E. coli* (ATCC35150), *S. aureus* (ATCC25923), *L. monocytogenes* (ATCC19116), *S. typhimurium* (ATCC14028), *P. aeruginosa* (PAO1, ATCC15692) and *S. flexneri* (CGMCC11868) were purchased from BIOBW Biotechnology Co., Ltd. (Beijing, China, http://biobw.org/). Chloramphenicol, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (USA, https://www.sigmaaldrich.com/). Reduced glutathione (GSH), bovine serum albumin (BSA) and sodium selenite (98.0%, Na_2SeO_3) were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China, http://www.aladdin-e.com/). Other common chemicals were all purchased from Shanghai Chemical Reagent Co. (Shanghai, China, https://www.sinoreagent.com/index/index.jsp) and were of analytical grade. Unless otherwise indicated, solutions were prepared with ultrapure water (Resistivity: 18.2 MΩ·cm at 25 °C) from Poseidon-R70 water purification system (Research Scientific Instruments Co. Ltd., Xiamen, China, http://www.ro-di.com/cn/index.html).

Carboxyl-functionalized core–shell type magnetic beads (0.1–1.0 μm) were produced by Tianjin BaseLine Chromatography Technology Development Center (Tianjin,

China, http://www.qiuhuan.com/). The sequence of DNA aptamer specific to *E. coli* was 5′- GCAATGGTACGGTACTTCCCCATGAGTGTTGTGAAATGTTGGGACACTAGGTGGCATAGAGCCGCAAAAGTGCACGCTACTTTGCTAA-3′ (called aptamer E). The aptamer specific to *S. aureus* was 5′-GCAATGGTACGGTACTTCCTCGGCACGTTCTCAGTAGCGCTCGCTGGTCATCCCACAGCTACGTCAAAAGTGCACGCTACTTTGCTAA-3′ (called aptamer S) [23]. All of the 5′- amino modified aptamers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China, https://www.sangon.com/). Their specificity was tested using *L. monocytogenes*, *S. typhimurium*, *P. aeruginosa* and *S. flexneri* as negative controls (see Electronic Supplementary Material).

Culture and plate counting bacteria

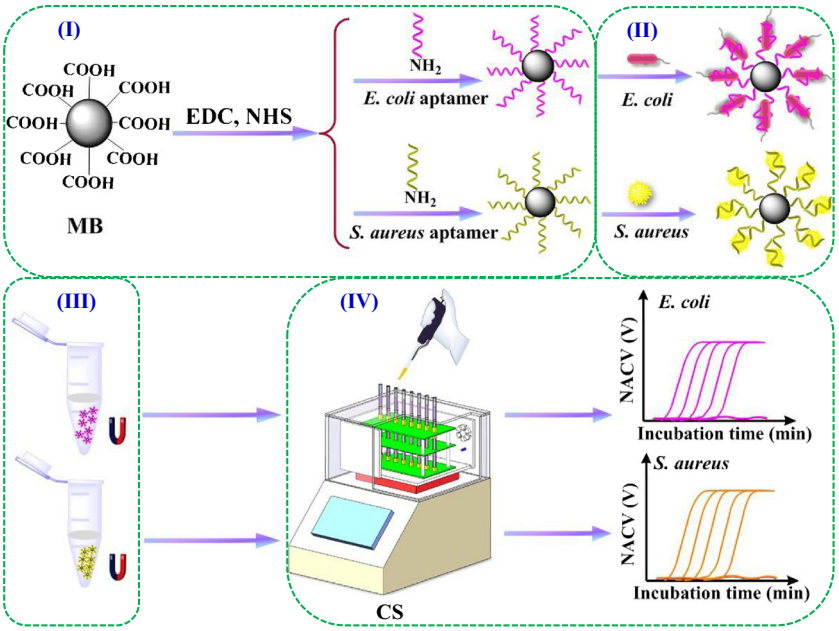
E. coli, *S. aureus*, *L. monocytogenes*, *S. typhimurium*, *P. aeruginosa* and *S. flexneri* were all cultured in LB medium. The culture and propagation followed previously described protocols [25] with minor modifications. Briefly, strains were taken from −80 °C storage, seeded in media, and (pre-grown) cultured overnight in the recommended media with constant shaking at 37 °C. Active strains were then selected and further transferred to new culture media. After a second incubation for~10 h to achieve mid-exponential phase, cell numbers were determined using the colony counting method that we have used previously [16]. The cultures were then immediately diluted to achieve desired cell concentrations for further use, or otherwise centrifuged to collect cells.

Determination of bacteria by magnetic analyte separation in combination with a multichannel conductometric sensing (MAS@CS)

As shown in Scheme 1, there are four steps for determining target bacteria by the MAS@CS method. These include the preparation of aptamer-functionalized magnetic beads, identification/capture of bacterial cells from aqueous sources, separation of bacterial cells captured on the magnetic beads from the source and transporting them to the assay vessel, and quantifying viable bacteria by monitoring the binary fission process using the CS (details are shown in Electronic Supplementary Material). In order to obtain sensitive and stable measurements, we sought to determine the optimal operational parameters. Detailed steps and results are shown in the Electronic Supplementary Material. Unless otherwise indicated, experiments were conducted under optimal conditions.

Preparation of aptamer-functionalized magnetic beads

Prior to the conjugation of aptamers, 200 μL magnetic beads (5 $g \cdot L^{-1}$) were pretreated with freshly prepared EDC and NHS as described previously [23] to activate the carboxylic acid groups [19, 26]. This was followed by washing with 3× exchanges of PBS buffer (pH 7.4). Next, 5 $g \cdot L^{-1}$ of magnetic beads in water was mixed with an equal volume of amino modified aptamer at the desired concentration (10^{-7} M, unless otherwise indicated) in TE buffer (pH 7.4). This was followed by an incubation at 37 °C for 16 h to allow the conjugation reaction to run to completion. Finally, non-attached aptamers were washed away from the magnetic beads. After the final



Scheme 1 Schematic of the working steps of the MAS@CS method, including the preparation of aptamer-functionalized magnetic beads (I), selective capture (II) and separation (III) of bacterial cells, and determination of viable bacteria by the CS (IV)

rinse, the aptamer-functionalized magnetic beads were re-suspended in 200 μL water, and stored at room temperature prior to further use within 2 h. In some experiments, aptamer E and aptamer S at equal concentrations were combined so that they were simultaneously conjugated to the same batch of magnetic beads.

Identification/capture and separation

Identification/capture and separation experiments were carried out according to the method reported by Feng et al [27] with minor modifications. In brief, 100 μL of capture sample (containing *E. coli*, *S. aureus*, or both of them combined) was mixed with 25 μL of aptamer-functionalized magnetic beads (5 $g{\cdot}L^{-1}$). The mixtures were incubated for 60 min at room temperature with gentle rotation. The unpurified bead-cell complexes were collected as-found from solution using the magnetic force gradient method by cementing the particles on an Affimag-2 magnetic particle concentrator (Tianjin BaseLine Chromatography Technology Development Center, Tianjin, China, http://www.qiuhuan.com/). This was followed by resuspension and washing with 3× water to remove non-specifically bound cells and debris. Finally, the bead-cell complexes were re-suspended in 100 μL water. Unless otherwise indicated, the beads were directly transferred into disposable glassy tubes immediately for further analysis in the CS. Samples undergoing examination by scanning electron microscopy (SEM; JSM-6700F, Japan, www.jeol.co.jp) were dried and sputter Au coated to avoid charging in the electron beam.

Determination of viable *E. coli* and *S. aureus*

The determination of viable *E. coli* and *S. aureus* that were captured on the magnetic beads was carried out with the method we reported previously [16]. In brief, several (up to eight) capped glass tubes, in which LB culture medium and bead-cell complexes were loaded (1.3 mL in total), were inserted into the CS simultaneously for continuous monitoring at 37 °C. To monitor the conductivity of culture media, we set the CS operating parameters at an excitation frequency of 2.0 MHz, the excitation amplitude of 16 V, and the recording rate at 1 point per 60 s. Bacterial growth curves were generated by plotting the normalized apparent conductivity values (NACV, showed in voltage) against incubation time. For simplicity, we defined the time needed for the NACV to reach 50 mV as a detectable time (D_t). The D_t values obtained from the growth curves were plotted against the logarithmic values of viable cell concentrations originally in the capture samples to form a calibration plot for each species.

Determining viable bacteria in the presence of nanoparticles

Selenium nanoparticles (nano-Se, an experimental antibiotic) were used to demonstrate that nanomaterials in the assay vessel did not interfere with the determination of viable bacteria by the CS method. Nano-Se was prepared according to the method reported by Kong et al [28] with minor modifications, and was characterized by transmission electron microscopy (TEM) and UV − VIS absorption spectroscopy (see Electronic Supplementary Material). To ascertain the effect of this nanomaterial on the bacterial growth, we added different amounts of the nano-Se to form desired concentrations in the culture medium before monitoring bacterial growth by the CS.

Direct antimicrobial susceptibility testing of bacteria captured on magnetic beads

Chloramphenicol was chosen as a model antibiotic for direct testing of bacterial cell susceptibility to antimicrobials after targeted species are captured on magnetic beads. For validation of killing, 10^8 $CFU{\cdot}mL^{-1}$ of *E. coli* or *S. aureus* were used as capture samples. After capture, bead-cell complexes of *E. coli* were transferred immediately into glass tubes, which contained 0, 0.001, 0.005, 0.010, 0.050, 0.100 and 0.500 $\mu g{\cdot}mL^{-1}$ chloramphenicol. For *S. aureus*, the concentration of chloramphenicol was 0, 0.005, 0.010, 0.050, 0.100, 0.500 and 0.750 $\mu g{\cdot}mL^{-1}$. The growths of bacterial cells were monitored by the CS.

Spiked experiments

Prior to the preparation of spiked samples, tap water was filtered using 0.22 μm Sterivex filters to remove potential bacterial contaminants. *E. coli* or *S. aureus* cells were obtained from fresh culture medium by centrifugation (9168×g for 15 min) and washed with 3× changes of water. The pelleted biomass was then re-suspended in water to constitute a bacterial suspension, whose concentration was determined by the colony counting method. Using this suspension, we prepared spiked tap water samples at desired concentration. Unless otherwise indicated, all samples were used within 20 min to avoid loss of viable cells.

Results and discussion

SEM characterization of the identification/capture beads

Figure 1a shows the SEM image of carboxyl-functionalized core–shell type magnetic beads, whose diameters are approximately 1 μm, as specified by the commercial supplier.

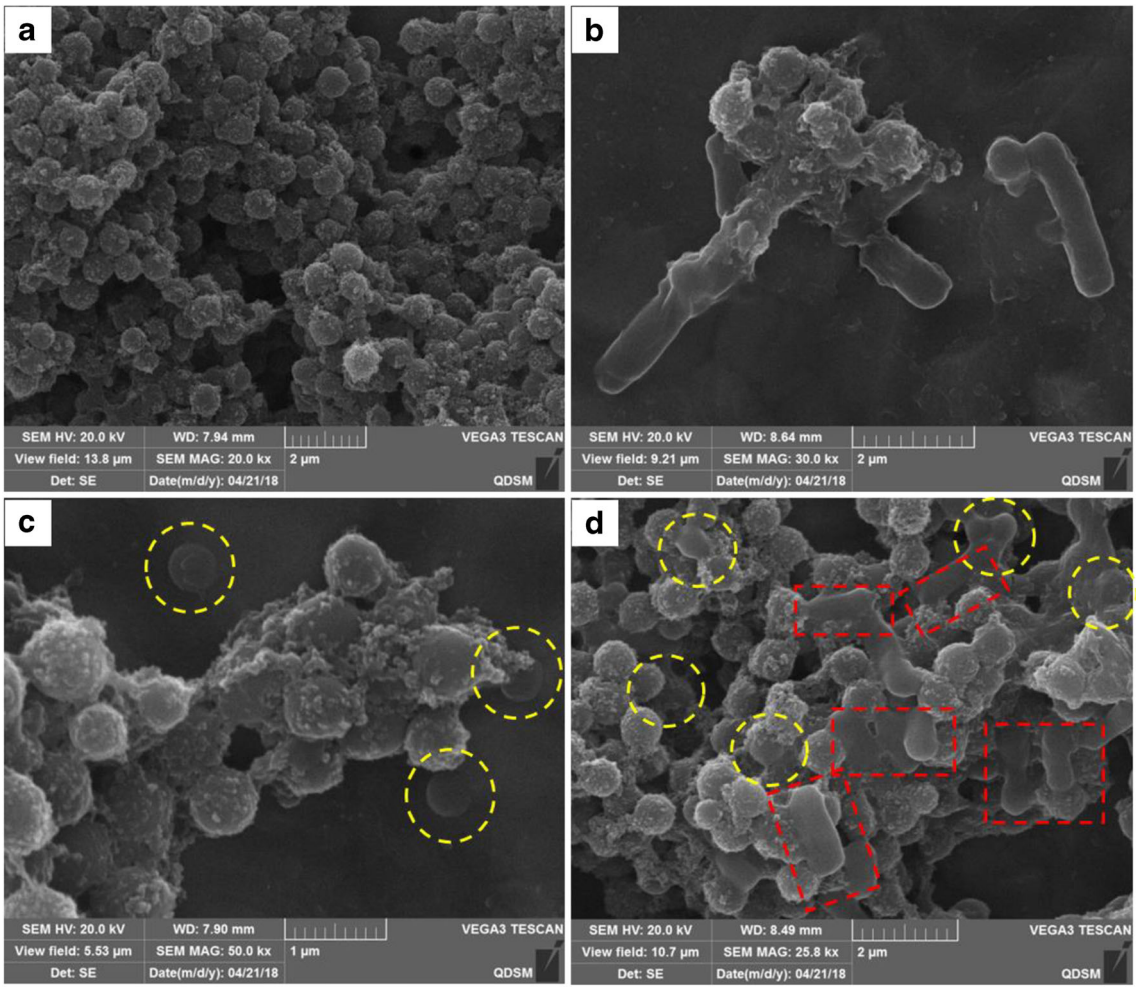


Fig. 1 SEM images of the naked magnetic beads (**a**), *E. coli* cells captured by the aptamer E-functionalized magnetic beads (**b**), *S. aureus* cells captured by the aptamer S-functionalized magnetic beads (**c**) and *E. coli* and *S. aureus* cells simultaneously captured by the aptamer E + aptamer S-functionalized magnetic beads (**d**)

Figure 1b and c show *E. coli* and *S. aureus*, respectively as captured by aptamer E and aptamer S on the magnetic beads. The morphology and size of *E. coli* and *S. aureus* are in accord with those reported previously [29]. For samples in which magnetic beads were functionalized with both aptamer E and aptamer S to capture the two bacteria species simultaneously, we find both *E. coli* and *S. aureus* cells in the SEM images (Fig. 1d). In control experiments, on the naked magnetic beads (i.e. functionalized with neither aptamer E nor aptamer S) we observe neither *E. coli* nor *S. aureus* (image not shown), suggesting that capture is specific to the targeted species due to the high binding affinity of the aptamers, rather than resulting from non-specific adsorption.

Determination of viable *E. coli* and *S. aureus* loads by the MAS@CS method

The capture sample, 2.5×10^8 CFU·mL^{-1} *E. coli* cells in water, was mixed with 1 g·L^{-1} aptamer E-functionalized magnetic beads. The as-obtained bead-cell complexes were directly transferred to a glassy tube for culture in LB media. After a short lag phase, bacterial division transforms previously un-ionized or weakly ionized metabolites in the media (e.g. yeast, peptone, and sugar) into highly charged end products, such as amino acids, aldehydes, ketones, and other charged metabolites, resulting in a progressive increase in the ionic strength of the culture medium. This transformation is reflected in an increasing conductivity [30] that is continuously monitored in real time by the capacitively coupled contactless conductivity detector of the CS. As shown in Fig. 2a, a typical S-shaped growth curve is found, that is similar to that is obtained by the optical density method [17]. A lag phase of approximately 220 min is followed by an acceleration phase during which the growth rate increases. An exponential phase lasts for approximately 80 min. Subsequently, the growth rate begins to decline during the deceleration phase. Eventually, the growth ceases, coming to a stationary phase. As in most examples where quantitative

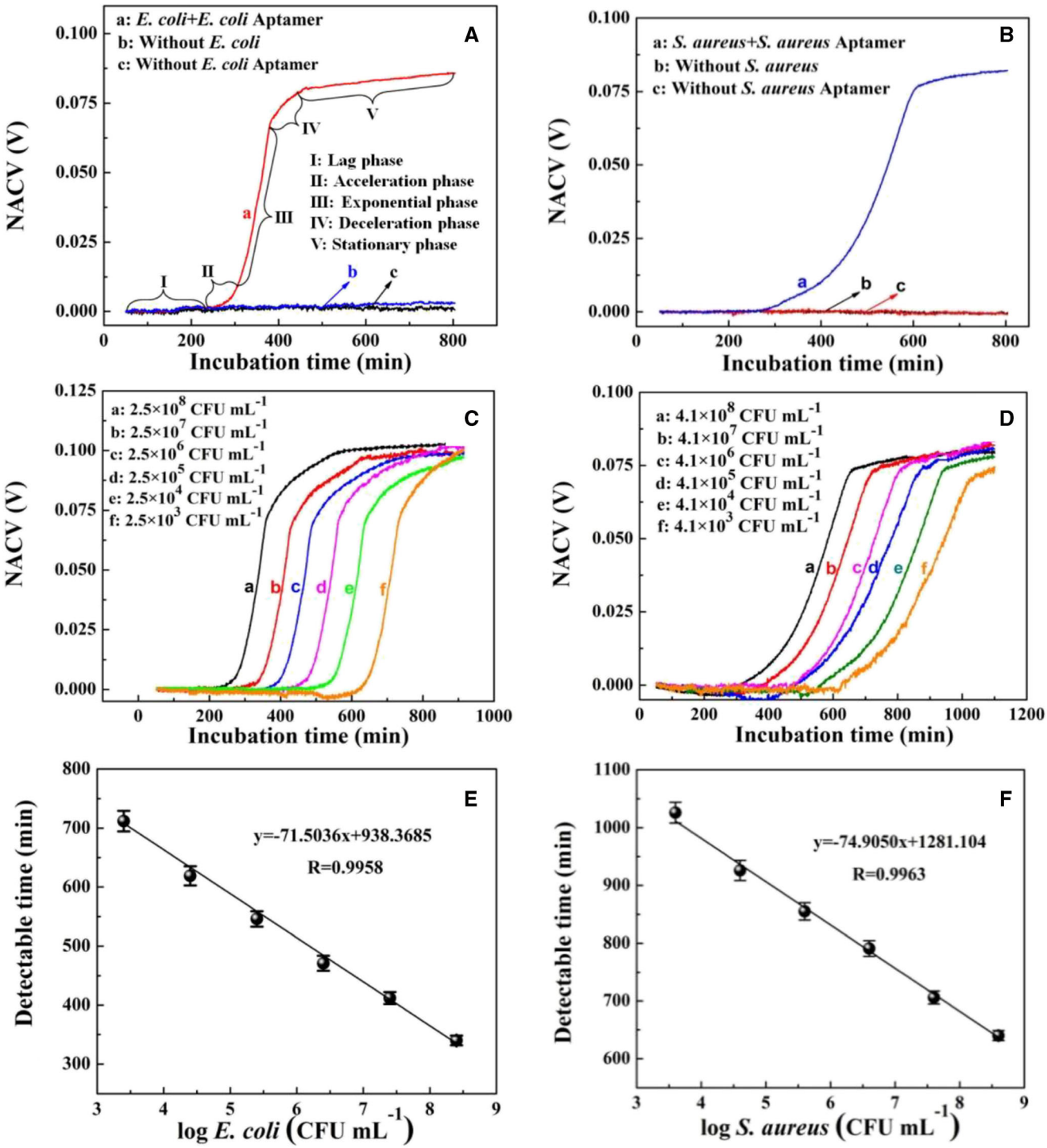


Fig. 2 Typical growth curve (NACV vs. incubation time, S-shaped curve) of viable *E. coli* cells (**a**) and viable *S. aureus* cells (**b**) captured by aptamer-functionalized magnetic beads, obtained by the CS. Horizontal lines show the results of negative control experiments. Growth curves of viable *E. coli* cells (**c**) and viable *S. aureus* cells (**d**) captured by aptamer-functionalized magnetic beads from a series of capture samples containing *E. coli* or *S. aureus* at different cell concentrations. The linear relationships between the logarithm of *E. coli* (**e**) and *S. aureus* (**f**) cell concentrations in the capture samples and D_ts

bacteriology methods are used, the duration of the lag phase is one with the greatest usefulness [25]; it can be obtained directly from the curve. Of significance, the existence of the growth curve also confirms that viable bacterial cells captured by aptamers on magnetic beads can still directly proliferate in culture [31]. We obtain no growth curves from the negative

control samples, confirming that no viable bacterial cells are captured in the absence of the species-specific aptamer [23]. These data support the results obtained from the SEM images.

To determine the sensitivity and quantitative accuracy of the MAS@CS method for *E. coli* capture, we ran a series of tests in which the *E. coli* concentration varied by six orders of magnitude from 2.5×10^3 to 2.5×10^8 CFU·mL^{-1}. Identical amounts of aptamer E-functionalized magnetic beads were used to capture *E. coli* cells in each sample. Figure 2c shows the typical growth curves of this series of *E. coli* concentrations superimposed and synchronized such that all traces start at the moment of sample introduction in culture media. It is clear that the duration of lag phase regularly increases as the *E. coli* cell concentration decreases. There is a linear relationship between the logarithmic value of cell concentration in the capture sample and the phase lag as measured by D_t. Over the concentration range between 2.5×10^3–2.5×10^8 CFU·mL^{-1}, the correlation coefficient for the regression line (r) is found to be 0.9958 (Fig. 2e), similar to those obtained from optical [32] and electrochemical [18] measurements. This indicates that the viable *E. coli* concentration in capture samples may be evaluated from the experimental measurement of the magnitude of D_t. We find as a practical matter that the precision of the D_t measurement, exhibited by the error bars in Fig. 2c, is superior to those of traditional methods [1–3, 23]. When the *E. coli* concentration in capture sample was 2.5×10^2 CFU·mL^{-1}, no growth curve was found after 16 h of incubation/monitoring. Capture samples at concentrations of 5.0×10^2, 1.0×10^3, 1.5×10^3 and 2.0×10^3 CFU·mL^{-1} were also tested to measure the reproducibility of D_t. Obtained relative standard deviation of measurements (RSDs) are ≥19.2%. This suggests that under these conditions, the LOD is 2.5×10^3 CFU·mL^{-1}. Using 2.5×10^6 CFU·mL^{-1} *E. coli* cells as a standard concentration, repeated measurements show that the RSD is 4.6% ($n = 5$). We demonstrate that the viability of unknown samples can be measured from a standard calibration that relates the lag parameter D_t to the log of cells concentration in capture samples. Such a regression produces errors that are less than 5%. Our results show that the MAS@CS method can be used to quantify viable *E. coli* cells in pure water samples.

A 2.5×10^8 CFU·mL^{-1} capture sample of *S. aureus* cells was mixed with 1 g·L^{-1} aptamer S-functionalized magnetic beads, obtaining bead-cell complexes that were then measured by the CS. The growth curve obtained was also S-shaped (Fig. 2b), being similar to that of *E. coli* except that D_t shifted to a considerably longer delay interval (~520 min compared to ~320 min). As is found for *E. coli*, no S-shaped growth curve can be observed from the negative controls. This is in good agreement with the results obtained from SEM images. As is also seen for *E. coli*, the duration of lag phase on the growth curves linearly increases with the decrease of the log of *S. aureus* concentration in the capture samples (Fig. 2d). Over the concentration range of 4.1×10^3–4.1×10^8 CFU·mL^{-1}, there is a linear relationship between the log of *S. aureus* cell concentration and the D_t ($r^2 = 0.9963$, Fig. 2f). Further experiments show that under these conditions, the LOD is 4.1×10^3 CFU·mL^{-1}. Given such high correlation coefficients for linear regression formulae for both *E. coli* and *S. aureus*, we conclude that the viable cell density of unknown samples can be determined based on D_t from growth curves, in combination with standard calibrations. The derived cell densities are in good agreement with those obtained by the colony counting method. Our results suggest that the MAS@CS method can be used to determine a wide variation of viable bacterial populations in unknown samples based on their particular growth kinetics.

Interestingly, simultaneous identification/capture of *E. coli* and *S. aureus* was accomplished using magnetic beads containing both E- and S-type aptamers. This was followed by monitoring their growth in LB medium using the CS method. Details are shown in the Electronic Supplementary Material.

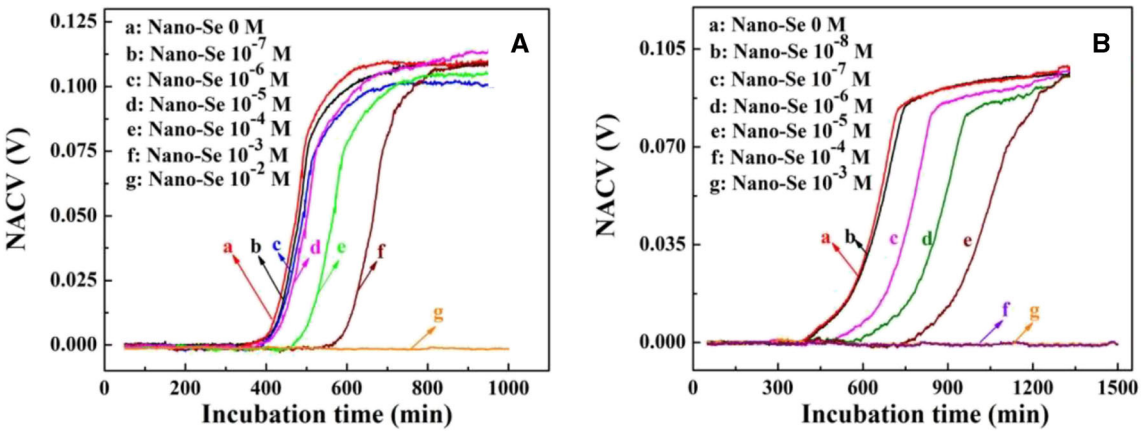


Fig. 3 Growth curves of viable *E. coli* (**a**) and *S. aureus* (**b**) captured on the magnetic beads in the presence of nano-Se particles at different particle concentrations. For preparing bead-cell complexes, the bacterial cells concentration in all capture samples was 10^6 CFU·mL^{-1}

Aberrant growth behavior conveys information when there is mixed species interference. Moreover, the particular growth patterns become meaningful for revealing physiological interactions between symbiotic bacteria (as in biofilm formation) during growth of mixed populations.

Inhibitory effect of nano-selenium on growth of bacteria cells captured on magnetic beads

Figure 3a shows the growth curves of viable *E. coli* captured on the magnetic beads in the presence of nano-Se at different concentrations. At a concentration lower than 10^{-5} M, the nanoparticles exhibit almost no perceptible effect on the duration of lag phase. By contrast, their presence at concentrations of 10^{-7} M and above is sufficient to significantly alter the onset of exponential growth of *S. aureus*, as demonstrated by the shift of D_t (Fig. 3b). These results indicate that *E. coli* is less sensitive to this particular nanoparticle antibiotic than *S. aureus*. It is likely, especially in the case of gram-negative *E. coli*, that there is strong electrostatic repulsion between the nano-Se particles and the outer bacterial membrane that has a net negative charge originating from the anionic glycolipids and outer phospholipid membrane compositions. However, in the case of *S. aureus*, the gram-positive peptidoglycan present in the outer wall may be electrostatically attractive to nano-Se particles [33]. Using a batch of magnetic beads, on which both *E. coli* and *S. aureus* are captured, we obtained growth curves in the presence of nano-Se particles at different concentrations. At a concentration lower than 10^{-4} M, there are both viable *E. coli* and viable *S. aureus* that expand their populations. While, at a concentration of 10^{-4} M and above, there is present only the typical S-shaped curve corresponding to a multiplying *E. coli* population, suggesting that growth of *S. aureus* is inhibited completely (Fig. S5). These results confirm that *S. aureus* cells are more susceptible to this nanoparticle antibiotic than *E. coli* cells.

Understanding the toxicity of bactericidal nanoparticles towards microorganisms is of concern and significance to material sciences, as well as microbiology. Up to now, the growth-based analysis remains the gold standard to characterize the susceptibility of organisms to toxins [6]. However, it is difficult to perform such analyses using automated optical instruments in the presence of nanoparticles, since some properties such as adventitious adsorption, optical absorption, scattering, and fluorescence, interfere with real-time monitoring. The CS method, fortunately, appears to circumvent most of these experimental difficulties.

Direct antimicrobial susceptibility testing of bacterial cells captured on magnetic beads

Capture scenarios using magnetic beads plus aptamers has previously been reported for the rapid identification of target bacteria [4, 27]. However, using magnetic bead capture to test susceptibility of various phenotypes has heretofore not been possible, especially with commercial turbidity-based instruments [34]. Figure 4a shows the growth curves of *E. coli* captured on magnetic beads in the presence of chloramphenicol over the concentration range of 0.001–0.500 μg·mL^{-1}. As anticipated [16], we obtain a minimal inhibitory concentration (MIC, ≥ 0.500 μg·mL^{-1}). Over the concentration range below the MIC, the growth rate decreases with increasing antibiotic concentration, while the duration of the lag phase becomes extended in a dose dependent manner. The effects of chloramphenicol on the growth of *E. coli* are in good agreement with those reported previously [15]. In the case of *S. aureus*, similar features are observed (Fig. 4b), with a slightly higher value of MIC (≥ 0.750 μg·mL^{-1}) than for *E. coli*. Our experiments suggest the feasibility of using the CS for direct metabolic

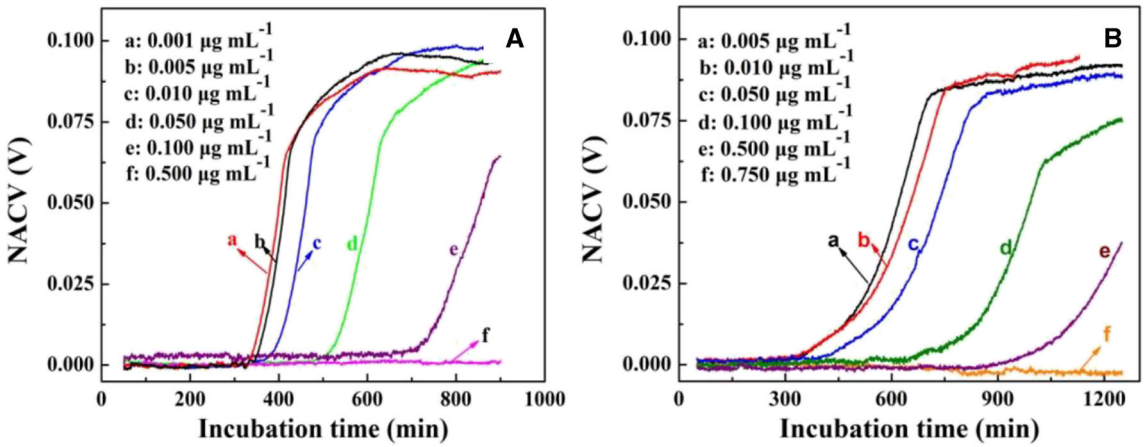


Fig. 4 Growth curves of *E. coli* (**a**) and *S. aureus* (**b**) captured on the magnetic beads in the presence of chloramphenicol at different concentrations. For preparing bead-cell complexes, the bacterial cells concentration in all capture samples was 10^7 CFU·mL^{-1}

testing, requiring no auxiliary pretreatment step post capture to remove the capture agents.

Determination of viable *E. coli* and *S. aureus* in spiked tap water

The sensitivity, repeatability, and accuracy of the MAS@CS method were also evaluated by spiking serial dilutions of bacterial cells in tap water samples. Commonly, the sensitivity to determine bacterial load from such sources is lower for real aqueous samples than for similar samples extracted from pure water or buffer [6]. Accordingly, we established new calibration plots for the two bacteria species in spiked tap water samples (details are shown in Electronic Supplementary Material). Our results show that over the range between 2.3×10^4–2.3×10^8 CFU·mL^{-1} of *E. coli*, the best linear regression equation obtained is D_t (min) = −101.4278 log [*E. coli* (CFU·mL^{-1})] + 1291.4143, with a LOD of 2.3×10^4 CFU·mL^{-1} ($r^2 = 0.9764$). Over the range between 4.0×10^3–4.0×10^8 CFU·mL^{-1} of *S. aureus*, the regression equation to determine bacterial load is D_t (min) = −74.8024 log[*S. aureus* (CFU·mL^{-1})] + 1298.0751, with a LOD of 4.0×10^3 CFU·mL^{-1} ($r^2 = 0.9929$).

Using spiked samples with known concentrations of bacteria, recovery tests were conducted to evaluate the accuracy of the above regression formulae by comparing the regression formula calculated results to those obtained by the colony counting method (typical photographs of bacterial colonies are shown in Fig. S10). As summarized in Table S1, over the concentration range between 10^4 and 10^7 CFU·mL^{-1}, the accuracy and precision of the results obtained by the CS method are marginally superior to the classical manual method of colony counting for measuring viable *E. coli*. They are vastly superior in terms of reducing time and reagent costs needed to similar results. There is no significant difference between bacterial loads obtained by the CS method and that by the colony counting method for viable *S. aureus* over the concentration range of 10^3–10^7 CFU·mL^{-1}. These results suggest that the MAS@CS method can be used for quantifying viable bacterial cells in actual environmental samples. However, no *S. aureus* or *E. coli* was detected in any unspiked tap water sample we surveyed ($n = 6$).

Conclusion

A novel method for determining viable *E. coli* and *S. aureus* population densities in water samples was developed. Target bacteria were specifically captured and separated by means of aptamer-functionalized magnetic beads. Magnetic beads were then directly transferred to the multichannel CS. Viable bacterial cells were quantified by monitoring their growth kinetics in a non-invasive manner. The presence of micro/nanomaterials (e.g., magnetic beads) does not interfere with the automated measurement of bacterial growth. This distinct feature makes it possible to perform direct antimicrobial susceptibility testing of bacteria captured on magnetic beads. This method is superior to the automated method based on optical density [16], opening up a new avenue for developing analytical platforms for widespread quantitative bacteriology that are user-friendly.

The method proposed here has several merits in comparison with existing nanomaterial-based electrochemical methods, specifically in simplicity and accuracy (as listed in Table S2). However, improvement in determination sensitivity appears to require further developments, via reduction of analyte volume and increase of incubation time. We note that, contrary to the results reported previously [23], aptamer S is slightly cross-reactive with *E. coli*. This should therefore be taken into consideration when one employs this aptamer to measure low concentrations of *S. aureus*. In addition, the applicability of the method needs to be demonstrated further in analyzing more complex, realistic samples (e.g. spiked food and urine).

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Compliance with ethical standards

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

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