Analyst

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d1an00350j

A pragmatic eLCR for an ultrasensitive detection of methicillin-resistant *Staphylococcus aureus* in joint synovial fluid: superior to qPCR†

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For periprosthetic joint infection (PJI) patients, an early and rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in joint synovial fluid is of great significance for receiving timely treatment and avoiding side effects. In clinical practice, the methods for detecting MRSA include the culture-based method and the PCR-based *mecA* gene detection method with fluorescent readout. However, the culture-based method requires up to 3–7 days for incubation and elaborative screening. The PCR-based molecular diagnosis, due to its high sensitivity, improves the detection time but sacrifices cost and gives false-positive results. Herein, a ligation chain reaction (LCR)-based electrochemical biosensor was developed to detect the *mecA* of MRSA with the advantages of rapidity, accuracy and low cost. In this system, an integrated dsDNA labeled with thiol and biotin at both terminals is generated only in the presence of the target DNA after LCR, followed by immobilization of the integrated dsDNAs on the bovine serum albumin (BSA)-coated gold electrode, and then the streptavidin horseradish peroxidase (SA-HRPs) is specifically bound to the biotin labels *via* biotin–streptavidin interaction, generating the catalytic amperometric readout. Impressively, the developed method achieved the detection of rare *mecA* in the joint synovial fluid of PJI patients (417–666 copies as quantified by qPCR). The proposed electrochemistry-based method is highly convenient for the point-of-care testing and was comparable with PCR in sensitivity, but superior in selectivity (single-base differentiation) and cost (nanomolar DNA probe consumption and simple device), demonstrating its huge potential in clinical applications for MRSA diagnosis.

Received 27th February 2021,
Accepted 8th April 2021

DOI: 10.1039/d1an00350j

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1. Introduction

Periprosthetic joint infections (PJI) of the hip and knee are disastrous complications that occur in approximately 1 to 2% of patients after total joint arthroplasties.[1,2] The management of PJI may require long-term antibiotic suppression, surgical debridement, one-stage or two-stage revision, resection arthroplasty, arthrodesis, or amputation.[3] Most PJI are caused by Gram-positive cocci, for example, *Staphylococcus* species.[4] Some researchers have reported that methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for up to 74% of PJIs.[5,6] The traditional method for detecting MRSA is the culture-based method, which includes the processes of microorganism growth on plates and colony counting.[7,8] However, it is difficult to isolate a sufficient quantity of MRSA from an intraoperative synovial fluid sample as they are usually embedded in a biofilm; this process also requires a long incubation time, which compels the use of broad-spectrum antibiotics before the culture results are known, resulting in the antibiotic-related adverse effects and increased overall cost of treatment.[6,9,10] Thus, it is necessary to develop a fast, convenient and sensitive method for rapid and early detection of MRSA in synovial fluid.

Recent studies have demonstrated the advantage of detecting the specific gene-*mecA* for the rapid identification of MRSA.[11–13] *MecA* encodes penicillin binding protein 2a that is responsible for the biosynthesis of bacterial cell walls and

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†Electronic supplementary information (ESI) available: Protocol of Gel electrophoresis, protocol of DNA extraction, sequencing and qPCR, Table S1 about the detail of calculated *mecA* gene copies. See DOI: 10.1039/d1an00350j

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shows significantly lower affinity for the β-lactam antibiotic, leading to drug resistance.[14] Molecular diagnosis methods such as polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) have been used for *mecA* detection with excellent sensitivity and rapidity.[15–17] Due to the mechanism of oligo primer extension, it is possible to amplify one *mecA* copy to 100 000 or millions of copies in a few hours for qualitative and quantitative analysis, but this primer extension-based amplification has difficulty discriminating a single-base mismatched sequence, resulting in false-positive results.[18] Additionally, the PCR method is based on a fluorescent readout, which relies on expensive equipment and high input of the DNA probes, making it difficult to implement as a routine diagnostic tool at all levels of medical institutions. Therefore, establishing a highly specific, low-cost method for MRSA identification is still urgent.

Recently, ligase chain reaction (LCR), an amplification technique, has been developed for gene detection and showed high specificity in discriminating one-base variation among DNA sequences.[19–21] Instead of using one pair of primers, as in the case of PCR, LCR uses two pairs of oligonucleotide primers to perform ligation-based exponential amplification. Each pair of oligonucleotide probes consists of two probes that are ligated only when they are hybridized to a perfectly matched target sequence. The generated ligation products can in turn serve as templates for the ligation reaction in subsequent thermal cycles. In this way, multiple DNA probes can be linked when one target strand is present, so the specific DNA sequences can be easily detected by analyzing the amplified ligation products. As the electrochemical readout method has the inherent advantages of portability, simplicity, rapidity and low cost, our group has recently made attempts to establish a ligation chain reaction-based electrochemical biosensor (eLCR) and has obtained satisfactory results.[22,23]

In the present study, we first researched ten different variants of the *mecA* gene using GenBank and a common conserved sequence (46 nucleotides) was selected as the target DNA. Then, an LCR-based electrochemical assay was developed to specifically and sensitively detect *mecA* from MRSA in the joint synovial fluid of PJI patients. In this system, two short double stranded DNAs (dsDNAs) labeled with a thiol tag or a biotin tag were employed. These two short dsDNAs were integrated into a dsDNA which contained thiol and biotin at both terminals in the presence of the target DNA through LCR and the integrated dsDNA was immobilized onto a gold electrode through Au–S bonds. In order to guarantee the precise and specific assembly of the dsDNA, a BSA-based DNA probe carrier platform developed by our group was employed.[24] Afterward, streptavidin horseradish peroxidase (SA-HRP) was specifically bound to the biotin label *via* the biotin–streptavidin interaction, generating a catalytic amperometric readout.[24] Under the optimal experimental conditions, the developed eLCR showed excellent specificity and a wide linear range from 1.0×10^{-13} to 1.0×10^{-8} M, spanning 5 orders of magnitude, toward the *mecA* target and the limit of detection was estimated to be 200 aM. Furthermore, this assay was applied to detect *mecA* in the joint synovial fluid of PJI patients with a satisfactory result.

2. Experimental

Reagents and materials

Bovine serum albumin (BSA) was supplied by Sangon Biotech (Shanghai) Company Limited (Shanghai, China). The 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was obtained from Neogen (USA) in the form of a ready-to-use reagent (Enhanced K-Blue TMB Substrate, H_2O_2 included). Streptavidin horseradish peroxidase (SA-HRP) was purchased from Roche Diagnostics (Mannheim, Germany). Ampligase® thermal stable ligase and 10× reaction buffer were obtained from Epicentre Technologies (Madison, USA). The buffer solutions employed in this study were as follows: the TE buffer for dissolving the synthetic oligonucleotides was 10 mM trihydroxymethyl aminomethane hydrochloride (Tris-HCl) (pH 8.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 M sodium chloride (NaCl); the dilution buffer for the DNA probes was 10 mM Tris-HCl (pH 8.0); the washing buffer was 10 mM phosphate buffer (PB) (pH 7.4); and the dilution buffer for SA-HRP was 10 mM PB with 0.1 M sodium chloride (abbreviated as PBS) (pH 7.4) containing 0.25% BSA. All chemicals were of at least analytical grade. All solutions were prepared with Milli-Q water (18 MΩ cm resistivity) from a Millipore system.

GenBank (https://www.ncbi.nlm.nih.gov/genbank/) was used to find the variants of *mecA* sequences, which were then subjected to multiple sequence alignment analysis to identify a common conserved sequence as the target DNA in this study. All of the DNA oligonucleotides were synthesized and purified by Sangon Biotech (Shanghai) Company Limited (Shanghai, China) and their sequences were as follows.

Capture probe (DNA probe modified with SH-C6 at 5′ terminal, CP):

5′-SH-TTTTTTTTTTGAGTTGAACCTGGTGAAGTTGTAA-3′

Signal probe (DNA probe modified with PO_4^- at 5′ terminal and biotin at 3′ terminal, SP):

5′-PO_4^--TCTGGAACTTGTTGAGCAGAGG-biotin-3′

Half-target 1 (HT1):

5′-CCTCTGCTCAACAAGTTCCAGA-3′

Half-target 2 (DNA probe modified with PO_4^- at 5′ terminal, HT2):

5′-PO_4^--TTACAACTTCACCAGGTTCAACTC-3′

MecA target (Full-matched sequence):

5′-CCTCTGCTCAACAAGTTCCAGATTACAACTTCACCAGGTTCAACTC-3′

One base-mismatched sequence:

5′-CCTCTGCTCAACAAGTTCCAGA**G**TACAACTTCACCAGGTTCAACTC-3′

Two base-mismatched sequence:

5′-CCTCTGC**G**CAACAAGTTCCAGA**G**TACAACTTCACCAGGTTCAACTC-3′

Scrambled sequence:

5′-GTTCCGACTACTGCCTCACCCATATCGTCAATCTTCTCGAGGACTG-3′

The underlined bold nucleotide is the mutation base.

The primers used for PCR amplification are described as follows.

Forward primer: 5′-ATAAAGGCTATAAAGATGATGCAGT-3′

Reverse primer: 5′-CATCGTTACGGATTGCTTCAC-3′

The primers used for qPCR are described as follows.

Forward primer labeled with SYBR green I (RTP-F):

5′-GAAAAATGATTATGGCTCAGGTAC-3′

Reverse primer labeled with SYBR green I (RTP-R):

5′-CATATCTTGTAACGTTGTAACCACC-3′

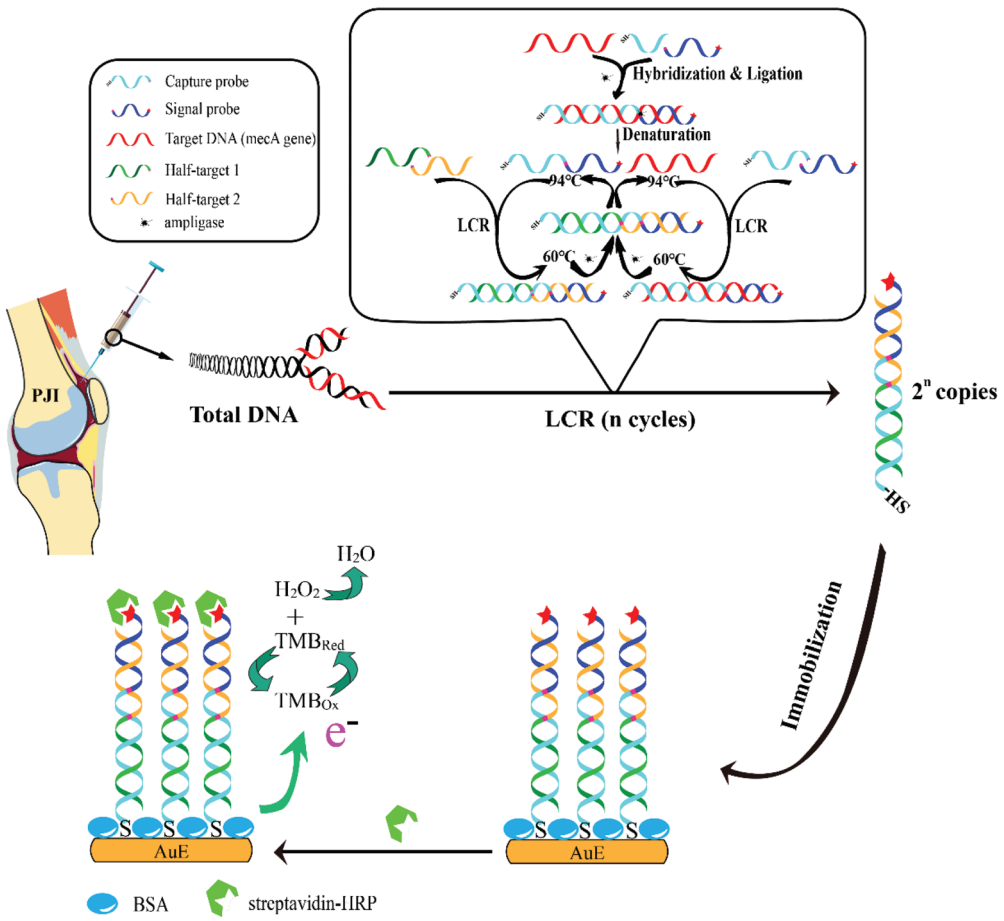
A methicillin-resistant *Staphylococcus aureus* strain (MRSA), a methicillin-sensitive *Staphylococcus aureus* strain (MSSA), an *Escherichia coli* strain and a *Candida albicans* strain were purchased from American Type Culture Collection (ATCC, Mannasse, Va., USA). Additionally, the clinical joint fluid samples of seven PJI patients were obtained by joint puncture from the Department of Orthopedics of the First Affiliated Hospital of Fujian Medical University. It must be noted that informed consent was obtained to collect samples from the patients. This study strictly followed the ethical guidelines of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the hospital.

Homogeneous ligation chain reaction

The LCR was conducted in 50 μL of reaction solution containing 10× reaction buffer, 2 U Ampligase, 30 nM of the four types of probes (CP, SP, HT1, HT2) and various amounts of target (synthetic DNA or genomic DNA extracted from freshly cultured bacteria and synovial fluid) in a 2720 thermal cycler (Applied Biosystems). The mixture underwent the following thermal cycle conditions: initial denaturation at 94 °C for 5 min, followed by 32 cycles of 2 min ligation at 60 °C and 1 min denaturation at 94 °C. After the thermal cycling, 2.5 μL of 0.2 M PB buffer (pH 7.4) was added to the amplification products.

Preparation of the electrochemical biosensor

The gold electrodes were physically polished with 1.0, 0.3 and 0.05 μm alumina powders in succession and then underwent electrochemical cleaning to obtain a smooth interface in accordance with a previous study.[25] The cleaned electrodes were immersed in 0.1% BSA at room temperature for 15 min. Subsequently, the BSA-modified electrodes were rinsed with washing buffer, blown dry with nitrogen and incubated in the solution of LCR products at room temperature for 1 h. After being washed and dried again, 3 μL of SA-HRP (0.5 U mL^{-1}) was dropped onto the assembled electrode surface at room temperature for



Scheme 1 Schematic illustration of the double-stranded DNA-assembled electrochemical biosensor using ligase chain reaction amplification.

15 min. Finally, the electrodes were extensively rinsed with ultrapure water and subjected to electrochemical measurements.

Electrochemical measurement

The amperometric experiments were carried out on a CHI 760E electrochemical workstation (Chenhua Instrument Company, Shanghai, China) equipped with a conventional three-electrode system consisting of a conventional Au disk working electrode (2 mm in diameter), an Ag/AgCl reference electrode, and a Pt wire auxiliary electrode. Cyclic voltammetry (CV) and amperometric *I–T* curves were used to measure the electrochemical signal of the enzymatic catalytic reaction in the TMB substrate. CV was carried out at a scan rate of 100 mV s^{-1}. Amperometric detection was performed at a fixed potential of 0.1 V and the current of electrocatalysis reduction was measured 100 s after the HRP redox reaction reached a steady state.

3. Results and discussion

The working principle of the developed eLCR for *mecA* detection

The strategy for the detection of *mecA* on the basis of LCR amplification with electrochemical readout is schematically illustrated in Scheme 1. The system consists of four short single-stranded DNAs (ssDNA probes) (CP, SP, HT1, HT2) and the DNA ligase Ampligase. CP and SP, which are both complementary to the target *mecA*, functioned as the anchoring unit and the reporter unit, respectively. HT1 and HT2 were each half of the target and were designed to achieve exponential amplification. In the presence of the DNA target, CP and SP were hybridized to it at 60 °C and covalently joined by Ampligase to form a ligated product (CP-SP). When heated to 94 °C, the duplex was denatured to release the DNA target and the ligated product. When the temperature was reduced to 60 °C again, the DNA target and the ligated product could be used as templates to ligate CP and SP, and HT1 and HT2, respectively, forming two ligated products (CP-SP and HT1–HT2). Therefore, by repeating thermal cycles at 94 °C for 30 s and 60 °C for 2 min, the ligated products (CP-SP and HT1–HT2) were exponentially amplified. It should be noted that the 3′-OH of CP and HT1 could be linked with the adjacent 5′-PO_4 of SP and HT2 in the catalysis of Ampligase only when the two probes were perfectly complementary with the target DNA, guaranteeing the high selectivity of the LCR. After LCR, a large number of ligated products (CP-SP and HT1–HT2) were naturally paired to form dsDNA of CP-SP/HT1–HT2. The generated dsDNAs with thiol labels at their 5′ end were immobilized in the gaps between BSA molecules on the gold surface *via* Au–S bonds. Afterward, the SA-HRPs were specifically bound to the

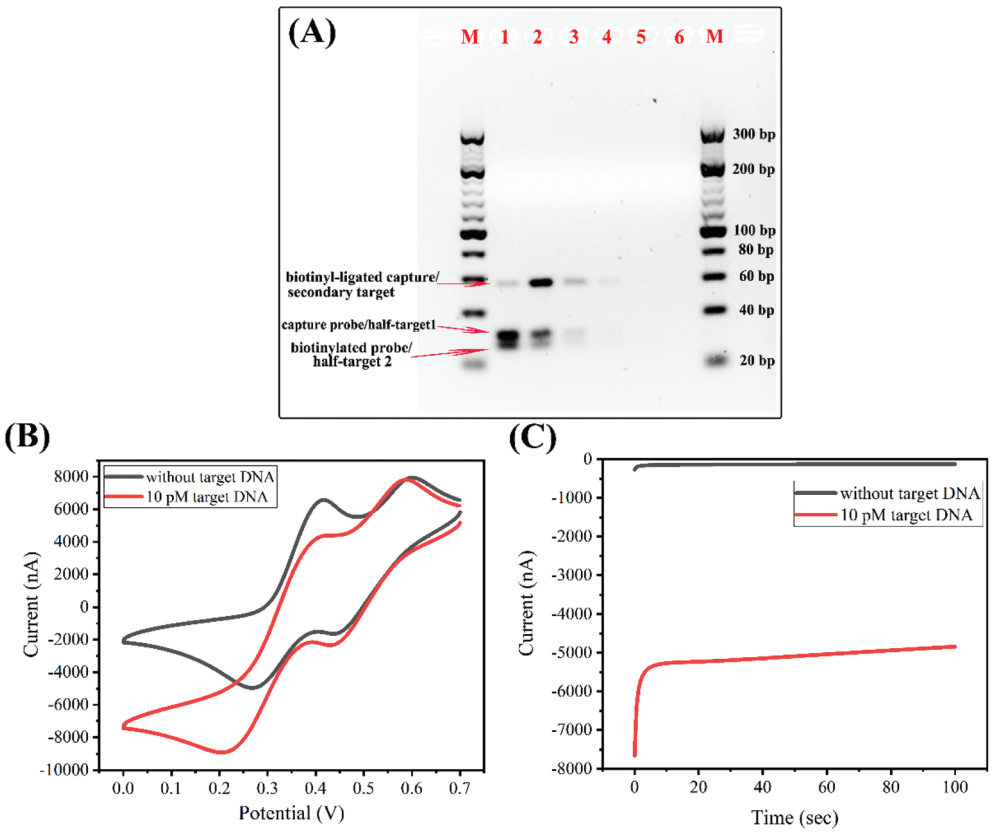


Fig. 1 (A) 3.5% agarose gel electrophoresis of different LCR products. Lane M: 300 base pair (bp) DNA ladder; Lane 1: without *mecA* target; Lane 2: with *mecA* target; Lanes 3–6: Lane 2 products diluted by 3, 9, 27, or 81 times, respectively. The concentration of DNA probe employed was 2.0 μM. (B) Cyclic voltammograms and (C) amperometry for the developed eLCR in the absence (black curve) and presence (red curve) of 10 pM target DNA.

biotin labels *via* biotin–streptavidin interaction, leading to the catalytic amperometric readout. In the absence of the *mecA* target, SP could not be ligated with CP, resulting in no catalytic current.

Characterization and feasibility of the eLCR

The LCR products were characterized by agarose gel electrophoresis and electrochemical measurements (CV and amperometry), the results of which are shown in Fig. 1. As shown in Fig. 1A, in the absence of the DNA target, only the short dsDNAs (S-dsDNA) of CP/HT1 and SP/HT2 presented as two bright signal bands of around 30 bp (Lane 1). However, due to interactional collisions among the four short ssDNA probes (CP, SP, HT1, HT2) during LCR, non-specific amplification was observed, presenting as a blurred band of around 60 bp as shown in Lane 1. In contrast, in the presence of the DNA target, CP and SP were hybridized with the DNA target and ligated by Ampligase to generate long dsDNA (L-dsDNA), resulting in a bright band of around 60 bp after LCR. The two blurred bands of around 30 bp could be explained by the decreased amounts of S-dsDNA (CP/HT1 and SP/HT2) due to substrate consumption in the process of forming L-dsDNA (Lane 2). From these two bands, we can conclude that successful amplification was achieved by the proposed LCR. Electrochemical measurements were also used to characterize the LCR products. As shown in Fig. 1B, compared with a blank sample without the DNA target, the sample with the DNA target showed an apparent increase in the HRP-catalyzed reduction peak at 0.2 V in the presence of 10 pM target DNA, demonstrating that LCR products templated by *mecA* were generated and the SA-HRP was attached to the generated product to produce an electrochemical current signal. Also, a significant increase in the amperometric current by about 5000 nA was observed in the presence of 10 pM target DNA (Fig. 1C), while there was only 200 nA in the absence of target DNA.

In order to further investigate the detectability of LCR products using agarose gel electrophoresis, the final LCR products were diluted by 3 times (Lane 3), 9 times (Lane 4), 27 times (Lane 5) and 81 times (Lane 6) and the results showed that the bands disappeared after the 9-times dilution. From these results, we find that the proposed electrochemical DNA biosensor was at least 3 orders of magnitude (10 pM *vs.* 100/9 nM) more sensitive than agarose gel electrophoresis in detecting the amplified LCR products, indicating its great potential for detection of trace amounts of DNA.

Optimization of the eLCR

In order to achieve high sensitivity and specificity in the proposed eLCR, key parameters, such as hybridization/ligation temperature, probe concentration, amount of Ampligase and cycle number, were carefully investigated and optimized. As shown in Fig. 2A, in the presence of 10 pM target *mecA*, the

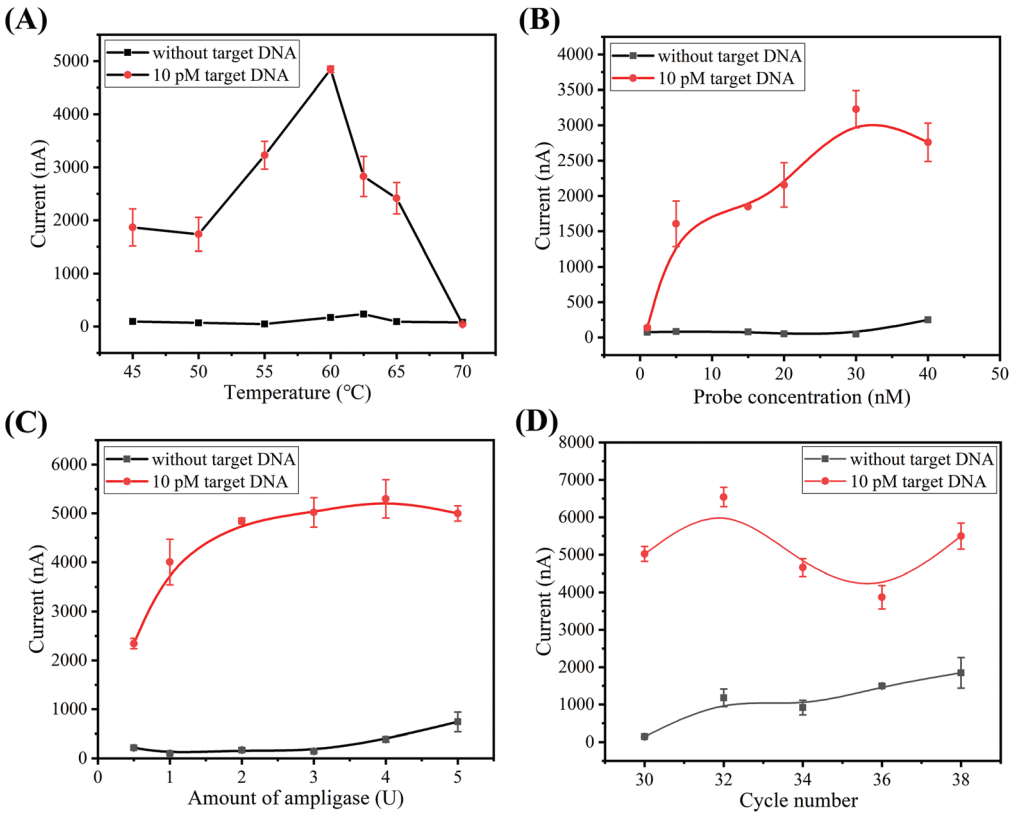


Fig. 2 Effects of (A) hybridization temperature, (B) probe concentration, (C) amount of Ampligase and (D) cycle number on the signal of blank (without target DNA) and 10 pM target DNA samples. The error bars represent the standard deviation of three repeated experiments.

current intensity increased markedly with the increase of hybridization temperature from 50 °C to 60 °C and reached a maximal value at 60 °C before dropping rapidly. In the absence of the *mecA* target, there were no significant changes in the current signal with increased temperature. Thus, 60 °C was selected for the hybridization/ligation temperature. Probe concentration affected the amplification efficiency of the LCR and accounted for the production of nonspecific signals. Therefore, concentrations from 0 to 100 nM were subsequently investigated. As indicated in Fig. 2B, in the presence of 10 pM *mecA*, the current intensity increased sharply with the increase of probe concentration from 0 to 30 nM. However, in the absence of *mecA*, the current signal increased when the probe concentration was over 30 nM. These phenomena could be explained by the high probe concentration increasing the probability of random collisions among ssDNA probes, leading to non-target dependent amplification. Therefore, 30 nM was selected as the optimum probe concentration. The amount of Ampligase and the cycle number were also optimized, as shown in Fig. 2C and D. Taking the best signal-to-background ratio (S/B) and best time cost into account, 2 U of Ampligase and 32 cycles were selected for subsequent experimental studies.

Performance of the eLCR

Under the optimal conditions, the sensitivity of the eLCR was evaluated. As demonstrated in Fig. 3A, the current signal increased linearly with the increasing concentration of *mecA* from 100 fM to 10 nM. The regression equation was obtained by using the logarithmic value of *mecA* concentration ($\lg C_{DNA}$) as the abscissa and the peak current (I/nA) as the ordinate; the equation was $I = 1067.72 \lg C_{DNA} + 15\,888.68$ ($R = 0.9947$). As shown in Fig. 3B, the limit of detection (LOD) was estimated to be 200 aM ($>3\sigma$, where σ is the standard deviation of the blank sample, $n = 3$). Therefore, the proposed eLCR was highly sensitive and had a wide dynamic detection range of more than 6 orders of magnitude which could be attributed to the amplification of biotinyl-ligated capture probes during LCR and the

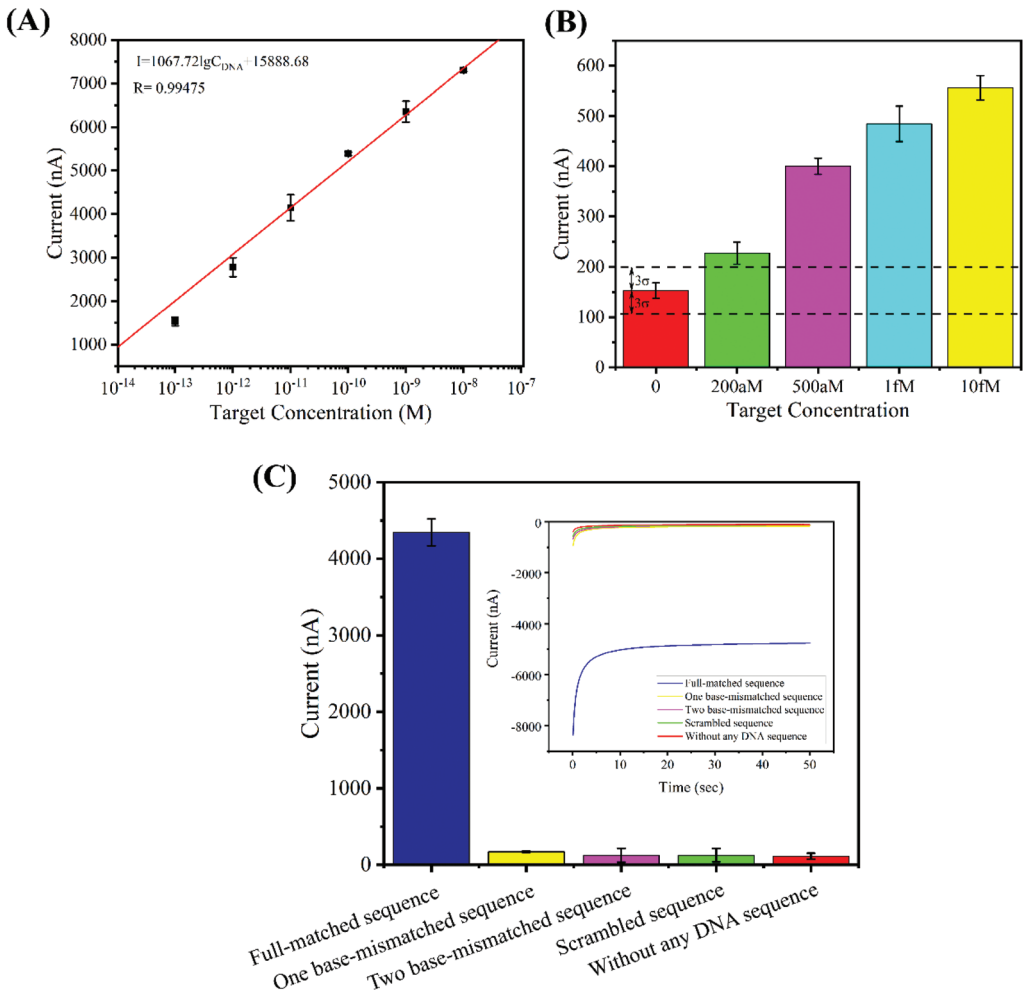


Fig. 3 (A) Calibration plot of the amperometric current *versus* logarithmical concentration of target DNA (from 1.0×10^{-13} to 1.0×10^{-8} M). (B) Amperometric response of the biosensor in the presence of 0, 200 aM, 500 aM, 1 fM, and 10 fM target DNA. (C) Selectivity of the biosensor in analyzing the fully matched target DNA, one base-mismatched sequence, two base-mismatched sequence and fully scrambled sequence at 1 pM. The inset shows the current-time curves of different targets. Mean values and standard deviations were obtained from three independent experiments.

effective inhibition of nonspecific adsorption of the DNA probes *via* BSA-based carrier platform.[22] In addition, on the basis of three independent measurements of 10 pM *mecA* with three different electrodes, the relative standard deviation (RSD) was calculated to be 1.2%, indicating the high reproducibility of this sensor.

In addition to sensitivity, specificity is another vital performance characteristic for DNA assays. In this study, mismatched target DNAs, including a one base-mismatched sequence, a two base-mismatched sequence and a fully scrambled sequence, were employed. As shown in Fig. 3C, under the optimal conditions, the current intensity from the fully matched DNA target was significantly higher than the others; the current intensity for the scrambled sequence was similar to that of the blank sample without target DNA. These results showed that the eLCR had good specificity in distinguishing single-base variations of the *mecA* target due to the two adjacent oligonucleotides of each pair of partial probes being ligated by Ampligase only when they are hybridized with a perfectly matching target sequence at the junction.[23] Thus, the developed eLCR holds great potential for detecting single nucleotide polymorphisms (SNPs) in complex real samples.

Real sample assay

The clinical joint synovial fluid samples of PJI patients were collected from the Orthopedic Department of the First Affiliated Hospital of Fujian Medical University. The samples were cultured on blood agar plates for 3–7 days, then drug susceptibility testing was performed to identify MRSA according to oxacillin minimum inhibitory concentration (MIC) ≥ 4 μg mL^{-1} or cefoxitin MIC ≥ 8 μg mL^{-1}; this whole process was lengthy and tedious. In this work, we present an eLCR to

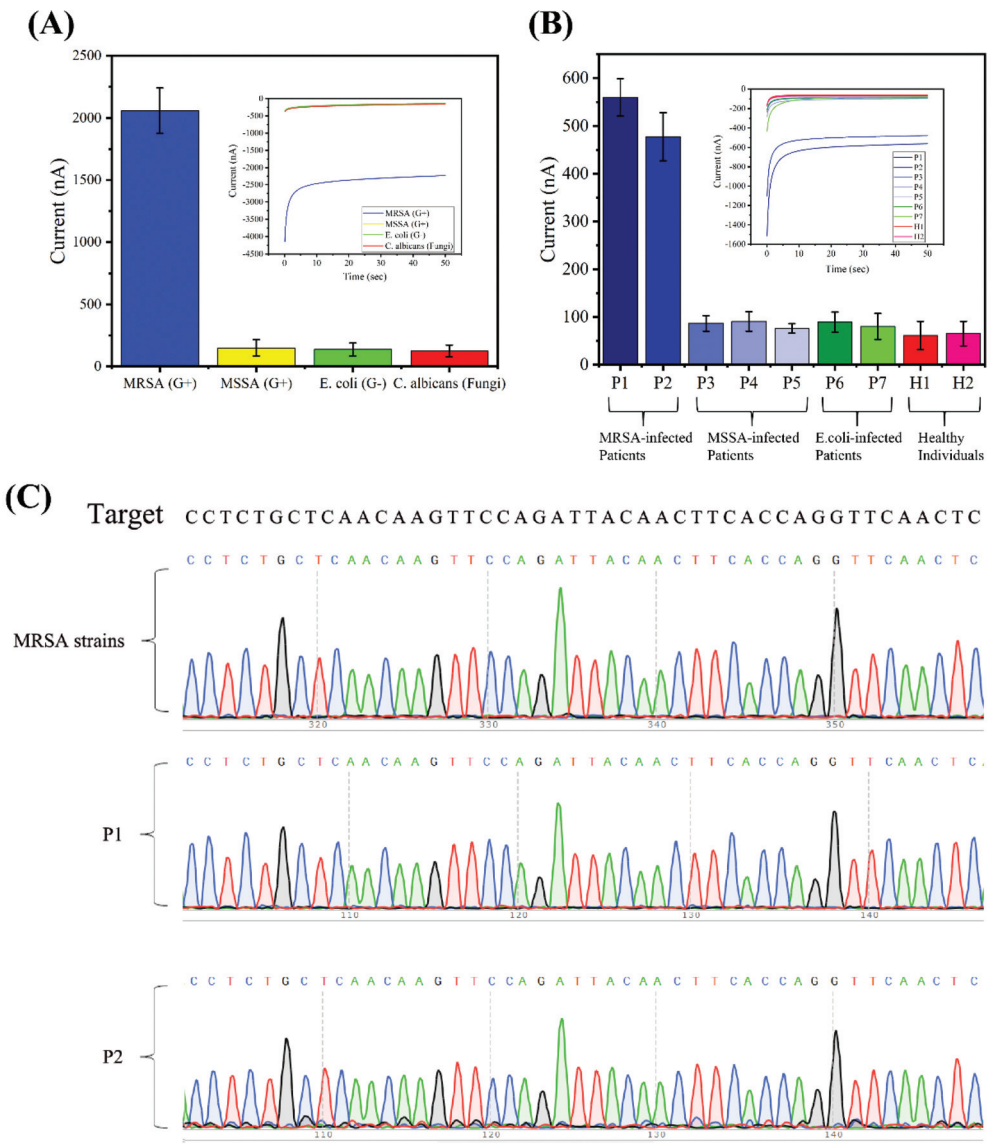


Fig. 4 (A) Amperometric response of the biosensor to the cultured strain samples. (B) Amperometric response of the biosensor to the real clinical samples. (C) Sequencing map of cultured strain and clinical samples.

directly detect *mecA* in DNA extracts obtained from the joint synovial fluid of PJI patients to identify MRSA. First, the feasibility of the developed eLCR in real sample assay was explored in genomic DNA extracts from a cultured MRSA strain, with a MSSA strain, *Escherichia coli* strain and *Candida albicans* strain as negative controls, as they are commonly reported microorganisms in joint synovial fluid infections. As presented in Fig. 4A, in the presence of 0.1 μL of DNA extracts, the current signal from the MRSA strain was ~2059 nA, which was significantly higher than those from the control groups, demonstrating the powerful capability and high accuracy of the developed eLCR in detecting the *mecA* target in a complex system. Further, the eLCR was used for the detection of MRSA in the joint synovial fluid of PJI patients (seven samples: P1 and P2 were MRSA-infected cases, P3–P5 were MSSA-infected cases, while P6 and P7 were *Escherichia coli*-infected cases diagnosed by the Clinical Laboratory Department in our hospital); also, two healthy subjects (H1 and H2) were used as controls. As shown in Fig. 4B, the current signals from P1 and P2 were over 420 nA, while the other seven samples (P3, P4, P5, P6, P7, H1 and H2) were below 150 nA, indicating that P1 and P2 were MRSA-infected cases according to the calibration curve shown in Fig. 3. This result was fully consistent with the clinical diagnostic results. To further validate the results, a sequencing experiment was conducted for the cultured strain and the clinical samples. As indicated in Fig. 4C, the employed 48-nt

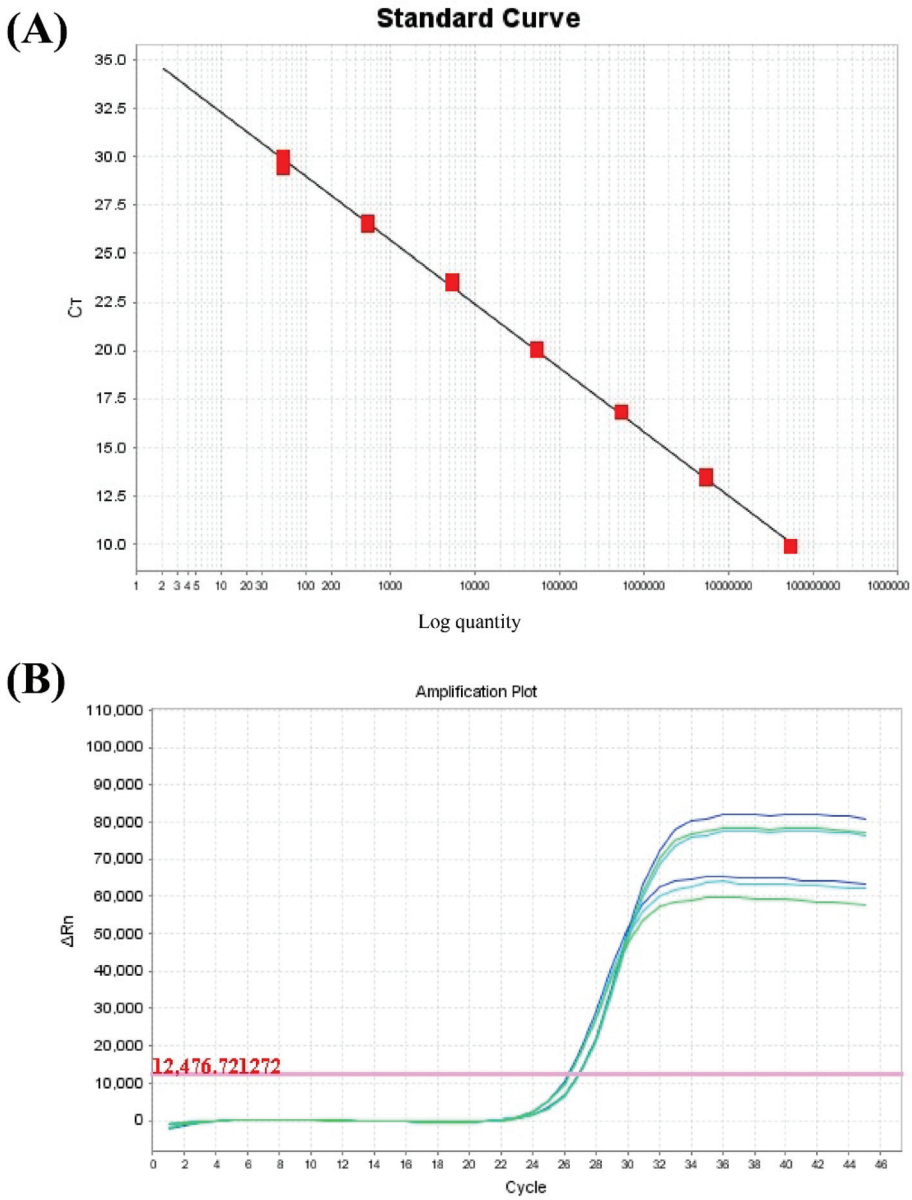


Fig. 5 (A) The calibration curve prepared with standard plasmid with gradient concentrations using qPCR. (B) Amplification curves of *mecA* in clinical samples using qPCR. Error bars show the standard deviations of measurements from three independent experiments in each sample.

Table 1 Comparison of different methods for diagnosis of MRSA infection

	Culture	qPCR	eLCR
Specificity (%)	100	72.7	100
Time consumption	3–7 days	2 hours	2 hours
Cost (¥)	150	1200	100
Equipment required	Incubator	Quantitative PCR machine	Thermal cycler

sequence of *mecA* was found in the standard MRSA strain in the position 313 nt to 358 nt. The 48-nt sequence was also found in clinical samples P1 and P2 (P1, position 101–146; P2, position 103–148), but not in the other five patients. Collectively, the results demonstrated that the developed eLCR provides precise detection of *mecA* in clinical samples with high sensitivity and specificity, indicating its considerable potential in the diagnosis of PJI patients with suspected MRSA infection.

The qPCR method was also employed to quantify *mecA* in the joint synovial fluid of PJI patients with MRSA infection. As shown in Fig. 5A, the quantification cycles of P1 and P2 were 26.32 and 26.99, respectively. Based on the standard curve (Fig. 5B) made using standard plasmid, the numbers of *mecA* in these two samples were calculated to be 666.46 and 417.78 copies (Table S1†), respectively, showing that the rare MRSA existed in the joint synovial fluid. To fully compare the performances of the developed eLCR and other methods, including culturing and qPCR, the specificity, time consumption, cost and equipment requirements for these methods are shown in Table 1. Compared with qPCR, the developed eLCR has advantages in specificity, cost and equipment requirement. Its good specificity could be attributed to the specific linkage of two adjacent oligonucleotides from each pair of partial probes by Ampligase, which only happened when the pair of probes were hybridized with a perfectly matched target at the junction. In terms of readout mode, the electrochemical readout method has inherently high sensitivity (nanomolar-level DNA consumption), as well as being a simple device without equipping a light source excitation device, so the cost could be significantly lower than that of qPCR. Furthermore, compared with the culture method, which requires a long time for the isolation and culture of MRSA, the developed eLCR is much less time-consuming. Therefore, the developed eLCR demonstrates its great potential in detecting MRSA in the joint synovial fluid of PJI patients.

4. Conclusions

In summary, this study provided an LCR-based electrochemical readout method to specifically and sensitively detect the *mecA* of MRSA in the joint synovial fluid of PJI patients. Under the optimum conditions, our approach has a wide linear range from 1.0×10^{-13} to 1.0×10^{-8} M toward the target DNA and a low limit of detection at 200 aM. Additionally, the LCR-based electrochemical biosensor exhibited considerable specificity for even single-base mutation differentiation. The developed eLCR was applied in the detection of *mecA* in a standard MRSA strain and the clinical joint synovial fluid of PJI patients and obtained satisfactory results. Considering the advantages in specificity, cost and equipment requirement, this developed eLCR holds great potential for the rapid and accurate diagnosis of MRSA infections in PJI patients and for antibiotic medication guidance in MRSA infection-related diseases.

Author contributions

Jinyuan Chen: conceptualization, writing review & editing; Hongxiang Wei: investigation, writing-original draft; Xinyu Fang: resources; Yuanqing Cai: resources; Zhenzhen Zhang: software; Yunqing Wang: data curation; Jianhua Lin: visualization; Wenming Zhang: funding acquisition; Guangxian Zhong: supervision, writing review & editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (81972021, 21904019), the Fujian Natural Science Foundation Youth Innovation Project (2019J05077), the Joint Funds for the Innovation of Science and Technology in Fujian Province (2019Y9016), University-Industry-Research Cooperation Project of Scientific and Technical Department in Fujian Province (2018Y4003), the Middle-aged Backbone Project of the Fujian Provincial Health and Family Planning Commission (2019-ZQN-55).

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