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Molecular recognition strategy for detection and antimicrobial susceptibility testing of *Staphylococcus aureus* by utilizing teicoplanin and porcine IgG as indicator molecules

Yong He[a,b], Yingran Wang[a], Yanli Shi[a], Zhifeng Fu[a,*]

[a] *Key Laboratory of Luminescence and Real-Time Analytical Chemistry (Ministry of Education), College of Pharmaceutical Sciences, Southwest University, Chongqing, 400716, China*
[b] *Department of Pharmacy, Affiliated Hospital of Zunyi Medical College, Zunyi, 563000, China*

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ABSTRACT

Timely and accurate anti-infective therapy significantly benefits treatment of various life-threatening bacterial infectious diseases. Unfortunately, owing to the lack of selectivity for recognizing bacterial species, most of the reported phenotypic antimicrobial susceptibility testing protocols suffer from time-consuming bacterial isolation and identification. In this work, a highly-selective molecular recognition-based sandwich fluorimetric assay was developed to rapidly detect *Staphylococcus aureus* (*S. aureus*), and furtherly to assess its susceptibility to antibiotics. Immobilized porcine IgG was utilized to capture *S. aureus* through the selective interaction between Fc fragment of porcine IgG and protein A in the cell wall of *S. aureus*. Fluorescein isothiocyanate-labeled teicoplanin was adopted as the signal tracer utilizing the binding behavior between teicoplanin and D-Ala-D-Ala moieties in the peptidoglycans of Gram-positive bacterial cell wall. *S. aureus* could be detected within a wide linear rang of 1.0×10^3 to 1.0×10^7 CFU mL^{-1}. Subsequently, this protocol utilizing porcine IgG and teicoplanin as the indicator molecules was utilized to assess the antimicrobial susceptibility of *S. aureus* after the bacteria were treated with antibiotics. The minimum inhibitory concentrations of penicillin, cefoxitin, clindamycin, trimethoprim/sulfamethoxazole and erythromycin were estimated to be >0.25,<4, <0.25, <2/38 and <0.5 μg mL^{-1}, respectively. The whole process for antimicrobial susceptibility testing can be completed within 4 h since it avoided extra time-consuming isolation and identification of bacterial species.

1. Introduction

Pathogenic bacteria is continually causing serious global health crisis. Bacterial infectious diseases are associated with increasing morbidity, mortality and substantial healthcare costs [1]. Bacterial detection and assessment of antimicrobial susceptibility are crucial points for the treatment of infectious diseases in clinic [2]. Therefore, numerous researches have been focused on developing rapid, accurate and sensitive detection and antimicrobial susceptibility testing (AST) protocols for pathogenic bacteria.

As the gold standard for bacterial detection and AST, traditional bacterial culture approaches show high standardization, good reliability and ideal repeatability. However, bacterial detection requires isolation and identification processes typically consuming 1–2 days [3,4]. The subsequent AST process performed with disk diffusion and broth dilution [5] demands another bacterial growth in the culture media added with antibiotics, which also last no less than 1–2 days [6]. Very long consumed time of these bacterial culture-based approaches results in irrational empiric therapy and accelerates development of bacterial resistance. Some other bacterial growth-based methodologies are recently developed to shorten the consumed time of AST, such as optical fiber biosensor [7,8], surface plasmon resonance [9], electrochemical biosensor [10–12], flow cytometry [13], fluorimetric assay [14,15] and microfluidic devices combined with colorimetric [16] and fluorescent [17] detections. However, since they often lack selectivity for the recognition of given bacterial species, they also consume long time for pretreatment process of bacterial isolation, identification and enrichment.

Alternatively in clinical laboratory, polymerase chain reaction (PCR)-based protocols combined with fluorimetric detection have been widely adopted for bacterial detection and AST because they

* Corresponding author.
E-mail address: fuzf@swu.edu.cn (Z. Fu).

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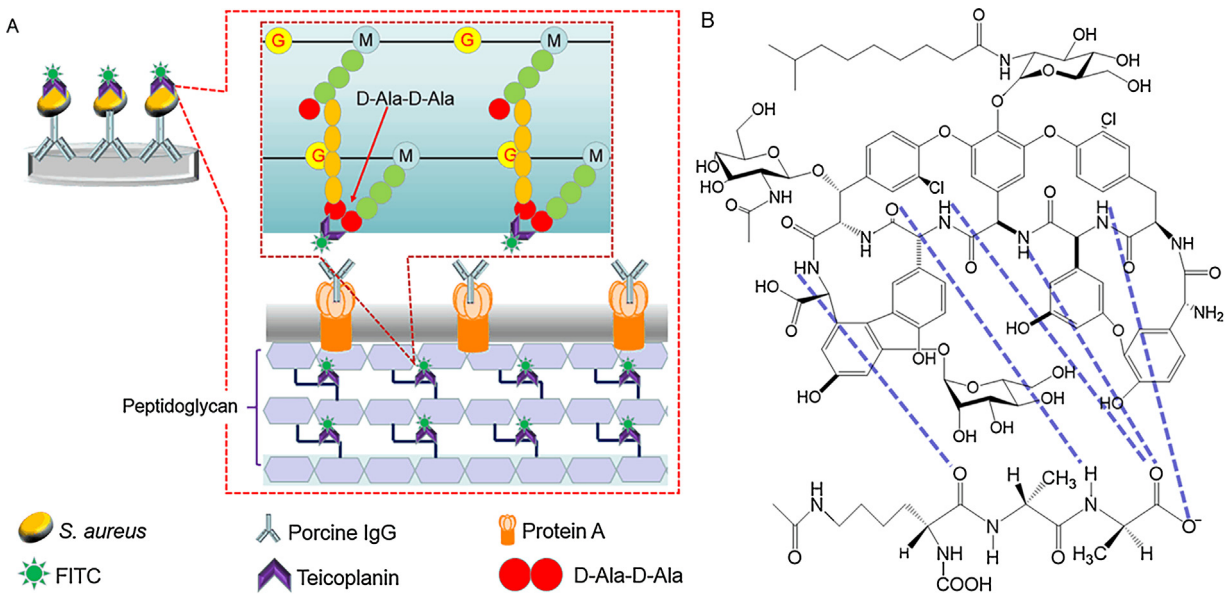

Fig. 1. (A) Schematic illustration of the principle of sandwich fluorimetric assay for *S. aureus* detection. (B) Binding behavior between TEI and D-Ala-D-Ala moieties in peptidoglycans of *S. aureus* cell wall.

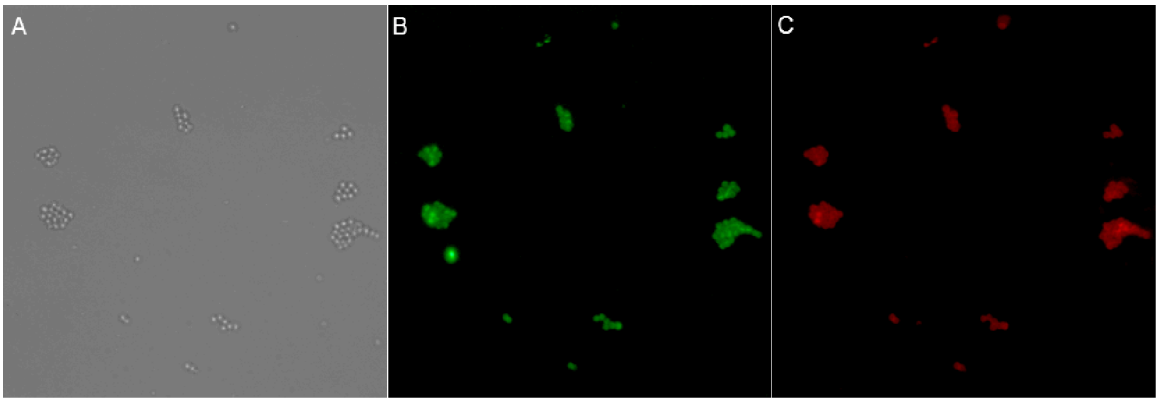

Fig. 2. Fluorescence microscope image of stained *S. aureus*. (A) Bright field, (B) green fluorescence channel, (C) red fluorescence channel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

don't need time-consuming bacterial culture [18–21]. Nevertheless, they usually require complicated molecular manipulation and well-trained personnel. Furthermore, due to the demand for precise information of resistant gene, PCR-based protocols are often restricted by resistant gene mutation and sometimes only applied to limited antibiotics [22]. Recently, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MS) has attracted increasing interest in bacterial detection and AST [23], which is based on highly-sensitive MS detection of marker proteins in the target bacteria and resistant strains. However, sometimes MS data are too complicated to be elucidated and the data pool still needs to be greatly improved in the future [24].

Molecular recognition mode utilizing antibodies [25,26], bacteriophages [27], aptamers [28,29] and polypeptides [30] as the indicator molecules to recognize bacteria has drawn great concern in bacterial detection as it can specifically isolate the target bacterial from complicated matrix. However, these biological indicator molecules are always limited by high cost, ease to deactivity and varied performance among batches. Teicoplanin (TEI), a very powerful glycopeptide antibiotic, can anchor D-Ala-D-Ala moieties in the peptidoglycans of Gram-positive bacterial cell wall through five hydrogen bonds [31]. As a commercially available small-molecular antibiotic, this agent perfectly avoids the above mentioned problems frequently encountered by biomolecular agents. Its main drawback is the lack of enough selectivity for a given bacteria as it shows broad-spectrum antibiotic activity against most Gram-positive bacteria. Fortunately, protein A highly expressed in the cell wall of *S. aureus* can specifically recognize the Fc fragment of IgG [32]. Meanwhile, bacteriophage tail fiber protein has been reported to specifically recognize the lipopolysaccharide of *Escherichia coli* (*E. coli*) [33], and bacteriophage endolysin has been proven to specifically bind with the ligand in the cell wall of *Bacillus cereus* (*B. cereus*) [34]. Thus combinational usage of TEI and other indicator agents shows promise in the development of highly selective and low-cost sandwich detection protocols for various pathogens.

In our previous work, we utilized TEI immobilized on magnetic beads as recognition molecule and rat IgG2a tagged with horseradish peroxidase as the signal tracer to detect *Streptococcus mutans* [35]. In order to investigate the feasibility of molecular recognition strategy for bacterial AST, microplate-immobilized porcine IgG was adopted to capture *S. aureus*, and fluorescein isothiocyanate (FITC)-labeled TEI was adopted as the signal tracer. The