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Aptamer-based fluorometric assay for direct identification of methicillin-resistant *Staphylococcus aureus* from clinical samples

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ABSTRACT

Accurate and rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) is of important clinical significance. In this study, a novel aptamer-based fluorometric assay was developed for detection of MRSA in clinical samples by coupling with immunomagnetic separation. The *S. aureus* cells in clinical specimens were enriched by magnetic separation. Following lysis by staphylococcal lysin, the PBP2a proteins were released from *S. aureus* cells and detected by the aptamer-based fluorometric assay. Without lengthy period of bacteria cultivation in the traditional susceptibility testing, this test has an overall testing time of only 2 h with the detection limit of 2.63×10^3 and 1.38×10^3 CFU/mL in PBS and spiked nasal swab, respectively. Since it is simple, rapid and sensitive, this method could be used for the detection of MRSA in various clinical samples.

1. Introduction

Staphylococcus aureus, the leading cause of bacterial infections, causes a range of serious illnesses from skin infection with mild symptoms to bacteremias associated with high mortality and morbidity (de Kraker et al., 2011; Reddy et al., 2017). Due to the abuse of antibiotics, infections with methicillin resistant *Staphylococcus aureus* (MRSA) are increasing and have become one of the important nosocomial and community acquired pathogenic bacteria (Yang et al., 2016). Rapid detection of MRSA directly from clinical specimens is of great importance to obtain information about antimicrobial drug sensitivity to guide antimicrobial therapy to reduce mortality (Ibrahim et al., 2017). Methicillin resistance is mediated by the *mecA* gene, which is carried by a mobile genetic element-staphylococcal cassette micro chromosome (SCCmec) (Ito et al., 2007) and codes for PBP2a (penicillin binding protein 2a), a substitute for other PBPs in cross-linking of peptidoglycan chains. This PBP has a low affinity to β-lactams and therefore enables staphylococci survival even in high concentrations of β-lactam antibiotics such as methicillin (Nonhoff et al., 2012). Thus, identification of the *mecA* gene or its product PBP2a are used as additional testing for rapid demonstration of methicillin resistance (CLSI, 2018).

In recent decades, many conventional approaches including culture-based susceptibility tests (cefoxitin disk diffusion, MIC determination by agar dilution and the automated Vitek 2 Compact system) (Nonhoff, 2012, Wolk et al., 2009), PCR and other DNA sensing systems for detection of *mecA* gene (Chen et al., 2017; Lin et al., 2017; Liu et al., 2016; Luo et al., 2017; Nawattanapaiboon et al., 2015; Nawattanapaiboon et al., 2016; Ning et al., 2018; Shi et al., 2015; Watanabe et al., 2015), enzyme-linked immunoabsorbent assay (ELISA) (Yamada et al., 2013) and latex agglutination assay (Abrok et al., 2018) for the detection of PBP2a have been used for determining the presence of MRSA. However, these methods have particular disadvantages. The culture-based susceptibility tests are simple and cost effective, but the time-consuming culture steps make them not suitable for rapid diagnosis of MRSA (Palavecino, 2014). PCR is highly sensitive and has high specificity; however, the activity of polymerase is easily influenced by clinical sample constituents and special equipment and skillful technicians are also required (Yan et al., 2017). In immunological methods, such as ELISA and latex agglutination assay, antibodies are usually used as capture or signal detection molecules. In contrast with most other bacteria, most *S. aureus* cells produce protein A molecules on their surface, which can strongly bind to the Fc fragment of mammalian IgG antibodies (Yan te al., 2017). For this reason, detection of MRSA by

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conventional methods employing mammalian IgG antibodies is liable to yield false-positive results. To minimize these unwanted effects of protein A, PBP2a needs to be extracted from the bacterial membranes of MRSA or PBP2a-specific chicken IgY antibody, which has a structurally different Fc region and lower reactivity with protein A must be used for detection of PBP2a from whole cell lysate samples (Yamada et al., 2013). Other drawbacks for antibody based immunological methods, such as high cost of production, low stability, and difficulties associated with purification and modification of antibodies limit their application in MRSA diagnosis (Shandordizadeh et al., 2017).

Aptamers, known as chemical antibodies, are single-stranded DNA or RNA that bind to a wide range of molecules with high specificity and affinity, of which dissociation constants (Kd) for their target are found to be pico- to nanomolar (Gu et al., 2014), due to their specific three-dimensional structure (Cheng et al., 2016; Toh et al., 2015). A panel of ssDNA aptamers specific to *S. aureus* had been obtained by a systematic evolution of ligands by exponential enrichment (SELEX) procedure and applied to probing *S. aureus* (Cao et al., 2009; Chang et al., 2013; Stoltenburg et al., 2015). Compared with traditional antibodies, aptamers are relatively stable and are easier to prepare and modify (Cheng et al., 2016), which make them alternatives to antibodies as recognition agents in *S. aureus* sensors. Aptamer-based systems have been developed with different detection methods including fluorescence (He et al., 2014; Huang et al., 2015; Huang et al., 2014; Maeng et al., 2012; Shangguan et al., 2015; Zuo et al., 2013), colorimetry (Hong et al., 2015; Stoltenburg et al., 2016; Yuan et al., 2014a; Yuan et al., 2014b) and electrochemistry (Abbaspour et al., 2015; Chang et al., 2013; Jia et al., 2014; Reich et al., 2017; Zelada-Guillen et al., 2012) for detection of whole cells of *S. aureus* and its toxins such as *S. aureus* enterotoxin A (SEA), SEC1 (Huang et al., 2015), SEB (Degrasse, 2012) and alpha toxin (Hong et al., 2015). However, the application of an aptamer targeting PBP2a as a membrane protein specific for MRSA is rarely described. To date, two studies have been published relating to the selection of DNA aptamers against PBP2a using the recombinant transpeptidase domain of PBP2a as SELEX target (Ma, 2013; Tang et al., 2016). A panel of aptamers with high affinity and specificity to PBP2a have been successfully selected and applied in the dot immune filtration assay for detection of recombinant PBP2a (Ma, 2013; Tang et al., 2016), but the application of these aptamers for identification of MRSA in clinical samples has not yet been reported.

In this study, the aptamer M23 (Kd = 21.9 nM, containing 4 stem-loop structures) reported by Ma (Ma, 2013) was selected to develop an aptamer-based FRET assay for MRSA detection using a fluorophore-labeled aptamer and a complementary quencher-labeled oligonucleotide. The hybridization between the two ssDNAs is in competition with the aptamer-target interaction, resulting in changes in fluorescence intensity. The assay was combined with an immunomagnetic separation step specific for Protein A-expressing *S. aureus* cells before the detection step. To the best of our knowledge, this is the first report of MRSA detection in clinical samples based on a specific aptamer against PBP2a.

2. Experimental

2.1. Materials and methods

S. aureus strains N315 (MRSA) and CCTCC AB91118 (methicillin sensitive *Staphylococcus aureus*, MSSA), which were used as positive and negative controls, respectively, *Escherichia. coli* O157:H7(NCTC 129007)and *Staphylococcus epidermidis* (ATCC 12228) were kindly provided by Professor Hongping Wei, from Wuhan Institute of Virology, Chinese Academy of Sciences. A total of 30 *Staphylococcus* spp. were isolated from 30 different clinical samples (sputum, pus, throat swab and secretions of eye, skin, vulvae, vagina, cervical or reproductive tract) at the affiliated hospital of Weifang Medical University (Weifang, China). Prior approval for using these clinical staphylococcus isolates had been obtained from the ethics committee of Weifang Medical University. The isolates were identified as *S. aureus* by VITEK 2 g positive (GP) identification card (bioMe'rieux, France). Methicillin resistance in *S. aureus* was also tested by the automated Vitek 2 Compact system with AST-GP card and evaluated by the Advanced Expert System (AES). The information and Vitek 2 minimal inhibitory concentration (MIC) of Oxacillin (OXA) results of clinical staphylococcus isolates are listed in Table 1.

Table 1
Sample information and detection results of methicillin resistance by Vitek 2 using OXA MIC method and aptamer-based fluorometric assay.

Sample no.	Original clinical specimen	Vitek 2 MIC of OXA [a]	Detection results of aptamer-based fluorometric assay
AB91118	laboratory strain	ND [b]	MSSA
N315	laboratory strain	ND	MRSA
S41	pus	≥4	MRSA
S42	pus	≤0.25	MSSA
S43	sputum	0.5	MSSA
S44	pus	≥4	MRSA
S45	sputum	0.5	MSSA
S46	pus	0.5	MSSA
S47	secretion	≤0.25	MSSA
S48	sputum	0.5	MSSA
S49	pus	≥4	MRSA
S50	secretion	0.5	MSSA
S51	secretion	0.5	MSSA
S52	secretion	≥4	MRSA
S53	sputum	≥4	MRSA
S54	sputum	≥4	MRSA
S55	secretion	0.5	MSSA
S56	secretion	≥4	MRSA
S57	secretion	0.5	MSSA
S58	sputum	0.5	MSSA
S59	pus	≥4	MRSA
S60	sputum	0.5	MSSA
S61	sputum	≥4	MRSA
S62	sputum	≤0.25	MSSA
S63	secretion	≤0.25	MSSA
S64	throat swab	≤0.25	MSSA
S65	secretion	≤0.25	MSSA
S66	secretion	≤0.25	MSSA
S67	pus	≥4	MRSA
S68	throat swab	≤0.25	MSSA
S69	throat swab	0.5	MSSA
S70	secretion	≤0.25	MSSA

[a] , detection of methicillin resistance in *S. aureus* is achieved by Vitek 2 system using OXA MIC method with oxacillin concentrations between 0.25 and 4 μg/mL. *S. aureus* isolates with oxacillin MIC ≥ 4 μg/mL should be reported as methicillin resistant, while oxacillin MIC ≤ 2 μg/mL reported as methicillin sensitive (CLSI, 2018).

[b] , laboratory strains were not detected by Vitek 2 system.

PBP2a aptamer (5′-CCATCCACACTCCGCAAGGGTGCCCCGGGGGGCTGTTCAGCGTGGTGGTGGGATGCCGTTTTGGTCCTTAGTCTCCGTCGTCGGCTGCCTCTACAT-3′) was labeled with fluorescein FITC (F-aptamer) at the 5′ end. Three short DNA probes with DABCYL (Q-DNAs) on their 3′ ends, which would hybridize with the aptamer to quench the fluorescence, were designed. Nucleic acid sequences of the Q-DNAs were 5'-GCGGAGTGTGGATGG-DABCYL-3′(Q-DNA-1), 5'-CCTTGCGGAGTGTGG-DABCYL-3′(Q-DNA-2) and 5'-CACCCTTGCGGAGTG-DABCYL-3′(Q-DNA-3). F-aptamer and Q-DNAs were synthesized by Sangon Biotech (Shanghai, China).

The carboxy-terminal magnetic particles (C-MPs, 800 nm in diameter) were purchased from JiaYuan Quantum Dots Corporation., Ltd. (Wuhan, China). Superdex™-200 columns were obtained from GE Healthcare Life Sciences (Boston, USA). Octanoic acid, ammonium sulfate, N-(3-dimethy-laminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin Industrial Corporation (Shanghai, China). Bovine serum albumin (BSA), skim milk powder and Tween-20 were obtained from Sangon Biotech. Staphylococcal lysin was purchased from Scithera