International Journal of Food Microbiology 243 (2017) 64–69

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Rapid detection of methicillin-resistant *Staphylococcus aureus* in pork using a nucleic acid-based lateral flow immunoassay

Hongwei Zhang [a,b,1], Luyao Ma [b,1], Lina Ma [b], Marti Z. Hua [b], Shuo Wang [a,*], Xiaonan Lu [b,*]

[a] Key Laboratory of Food Nutrition, Safety Ministry of Education of China, Tianjin University of Science and Technology, Tianjin 300457, China
[b] Food, Nutrition and Health Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 11 November 2016
Accepted 9 December 2016
Available online 11 December 2016

Keywords:
MRSA
mecA
Lateral flow immunoassay
PCR
Food safety

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as one of the leading causes of food poisonings worldwide. Due to the high prevalence and extensive challenges in clinical treatment, a rapid and accurate detection method is required to differentiate MRSA from other *S. aureus* isolated from foods. Since the methicillin resistance of *S. aureus* is due to the acquisition of the *mecA* gene from staphylococcal chromosome cassette, the presence of the *mecA* gene is interpreted as a marker for the identification of MRSA. In this study, a low-cost lateral flow immunoassay (LFI) strip was used to detect the *mecA* amplicons subsequent to polymerase chain reaction (PCR). The specificity of this PCR-LFI assay was tested between MRSA and methicillin-susceptive *S. aureus*. Both the test line and control line were shown up on the LFI strip for MRSA, whereas only the control line developed for methicillin-susceptive *S. aureus*. The detection limit of PCR-LFI assay was 20 fg for genomic DNA (100 times more sensitive than gel electrophoresis) and 2×10^0 CFU per 100 g of pork products after enrichment at 37 °C for 48 h. The total detection time of using LFI was 3 min, which was faster than the conventional electrophoresis (~45 min). With the performance of PCR-LFI, 7 out of 42 *S. aureus* isolates were identified to be MRSA from imported pork products, which was consistent to the standardized minimum inhibitory concentration assay. This *mecA*-based PCR-LFI strip can be used for rapid and accurate detection of MRSA isolated from commercial pork products.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most commonly identified antibiotic-resistant pathogen worldwide (Grundmann et al., 2006). MRSA is resistant to several antibiotics, such as methicillin, oxacillin, nafcillin, dicloxacillin, amoxicillin, cephalexin, and meropenem. Thus, this microbe can impose significant challenges to clinical treatment (Green et al., 2012). MRSA was first described in 1961 and spread globally in the early 1990s (DeLeo et al., 2010). MRSA produces heat-stable enterotoxins and is responsible for almost all staphylococcal-associated food poisonings, such as vomiting, retching, diarrhea, and headache. Besides, MRSA can cause several non-food related diseases, including skin and wound infections, pneumonia, and bloodstream infections leading to sepsis or even death (DeLeo et al., 2010). In the United Sates, MRSA causes about 80,000 life-threatening infections and 11,000 deaths per year, leading to an estimated annual cost of $10 billion USD. MRSA can be transmitted from infected people through skin-to-skin contact and by digesting contaminated foods (Graveland et al., 2011). Recently, livestock-associated MRSA was isolated from farms and commercial meat products in both the United States and Europe that provoked a worldwide health concern for meat handlers and consumers (Kadariya et al., 2014). Among the research studies during the past decade, the highest prevalence of MRSA from raw meat products was 11.9% in the Netherlands (De Boer et al., 2009). Several other studies in the United Sates identified 2.0–6.6% of retailed pork products that carry MRSA (Kadariya et al., 2014). Since food products contaminated with MRSA may not exhibit any spoilage appearance or bad smell, it is challenging for consumers to dispose the contaminated foods prior to consumption. Therefore, it is urgently required to develop an easy-to-use method for rapid and convenient detection of MRSA in agri-food products (*e.g.*, meat).

Methicillin resistance of *S. aureus* is due to the acquisition of the *mecA* gene from staphylococcal chromosome cassette (Archer et al., 1994). The *mecA* gene encodes penicillin binding protein 2A, which has low affinity to β-lactam antibiotics and enables transpeptidase activities in the presence of β-lactams, thus endowing the cells with antibiotic resistance by preventing β-lactams from inhibiting cell wall synthesis (Moellering, 2011; Wu et al., 1996). Therefore, the *mecA* gene has been widely used as a major marker for the characterization of methicillin resistance of *S. aureus* strains (Corrente et al., 2007;

* Corresponding authors.
E-mail addresses: s.wang@tust.edu.cn (S. Wang), xiaonan.lu@ubc.ca (X. Lu).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.12.003
0168-1605/

Couto et al., 2000; Felten et al., 2002; Salisbury et al., 1997; van Duijkeren et al., 2014).

Detection of antimicrobial-resistant foodborne pathogens is critical to quality control of final food products. Conventional methods for the characterization of methicillin resistance of *S. aureus* are time- consuming. Several days are required for culture enrichment and isolation, followed by performing minimum inhibitory concentration (MIC) assay that typically takes at least another 24 h. Alternatively, polymerase chain reaction (PCR)-based methods have been developed for sensitive and reliable detection of MRSA (Corrente et al., 2007; Couto et al., 2000; Felten et al., 2002; Salisbury et al., 1997; van Duijkeren et al., 2014). However, the detection and analysis of PCR products are still labor-intensive and require expensive reagents and instrument that are not appropriate for on-site detection.

Lateral flow immunoassay (LFI), also called as immunochromatographic strip test, has been recently developed and applied for the detection of foodborne pathogens (Shan et al., 2015). Gold colloidal nanoparticles are widely used in LFI because they can be conjugated with most types of antibodies in a very stable manner and produce visible colors when antibody is combined with its corresponding antigen (Shan et al., 2015). The aim of the current study was to develop a rapid, convenient, and cost-effective method for the detection of *mecA* in MRSA from pork. We performed PCR to amplify *mecA* from the genomic DNA of *S. aureus* with a pair of chemical labeled primers, followed by assembling this nano-colloidal gold-based LFI for the detection of MRSA pure culture. The customized LFI was then applied for rapid detection of MRSA in pork.

2. Materials and methods

2.1. Bacterial cultures and genomic DNA preparation

Bacteria were cultivated in 10 mL Giolitti-Cantoni Broth (Beijing Land Bridge Tech Co. Ltd., China) at 37 °C overnight with shaking. The overnight bacterial culture (1 mL) was collected and the genomic DNA was prepared using a commercial gDNA extraction kit (Promega, USA) according to the manufacturer's instruction.

2.2. Primer design for polymerase chain reaction (PCR)

The primers for PCR were designed within the highly conserved region of *mecA* using online primer design software (http://primerexplorer.jp/elamp4.0.0/index.html) according to the sequence of *mecA* in *S. aureus* (Genbank accession number KC243783.1). Forward primer (5′- TGATGCTAAAGTTCAAAAGAGT -3′) and reverse primer (5′-GTAATCTGGAACTTGTTGAGC -3′) (Lu et al., 2013) were labeled with fluorescein isothiocyanate (FITC) and biotin at the 5′ end, respectively. Both FITC and biotin are two typical labels used in numerous molecular biological applications, including immunoassays. The expected amplicon size is 210 bp.

2.3. Polymerase chain reaction (PCR)

The PCR was performed with 1.0 μL genomic DNA templates, 1.0 μL 25 pmol/μL forward primer, 1.0 μL 25 pmol/μL reverse primer, 2.0 μL dNTP (0.25 mM of each nucleotide), 5.0 μL 10× PCR buffer (100 mM Tris-HCl, 500 mM KCl), 3.0 μL 25 mM $MgCl_2$ and 1.0 μL Taq™ DNA polymerase (5.0 U/μL, TaKaRaTag™) in a total of 50 μL reaction system. The cycling amplification was conducted on a Bio-Rad T100 Thermocycler (Bio-Rad Laboratories Ltd., Mississauga, Canada) according to the following steps: initiation at 94 °C for 3 min, 5 cycles of denaturation at 94 °C for 40 s, annealing at 46 °C for 40 s and extension at 72 °C for 30 s, 30 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C for 40 s and extension at 72 °C for 30 s, and final extension at 72 °C for 8 min. A 210-bp band can be detected only if *mecA* is present in the genomic DNA. PCR products (5 μL) were separated on a 1.5% agarose gel (Biowest, Nuaillé, France) at 100 V for 30 min using Biometra Power Pack P25 (Biometra GmbH, Göttingen, Germany), followed by gel staining with Gelred (Biotium, Fremont, USA). Gel imaging was performed using a Bio-Rad Gel Imager XR^+ System (Bio-Rad Laboratories Ltd., Mississauga, Canada).

2.4. Performance of LFI strip test

LFI strips used in this study were purchased from Ustar Biotech Co. Ltd. (Hangzhou, China, cat# 0003-03, Patent No. 200610003429.1). The principle of LFI strip test is demonstrated in Fig. 1. In brief, colloidal gold nanoparticles were conjugated with anti-FITC polyclonal antibody, and then sprayed onto a plain substrate pad for the construction of the sample pad. The test line and control line was coated with anti-biotin antibody and goat anti-rabbit IgG, respectively. An aliquot (3 μL) of PCR product was added onto the sample pad, followed by immersing the sample end of the strip into 97-μL developing buffer (Ustar, Hangzhou, China) in a 1.5-mL Eppendorf tube. The test results became visible in ~3 min.

2.5. Detection of S. aureus in pork products using LFI strip test

MRSA strain ATCC 29213 was cultured in Giolitti-Cantoni broth at 37 °C overnight to achieve a concentration of about 10^9 CFU/mL. Then, the culture was washed, ten-fold serially diluted, and inoculated to 25 g pork samples confirmed to be without any bacterial contamination, as determined by plating assay. Two different concentrations of the spiked samples were prepared, aiming at 2 and 20 CFU per 100 g pork product.

Twenty five grams of each inoculated pork sample was aseptically transferred to 225 mL sterilized saline water and mixed homogenously using BagMixer® 400 VW (Interscience, Breteche, France). The resultant homogenous solution (5 mL) was added into 50 mL 7.5% NaCl broth (Land Bridge Tech Co. Ltd., Beijing, China), followed by incubation with shaking at 37 °C for 24 h. A loop of the culture was then streaked onto the surface of Baird-Parker plate (Land Bridge Tech Co. Ltd., Beijing, China). After incubation at 37 °C for another 24 h, 10 random colonies with grey to jet-black color surrounded by opaque zone were selected for PCR. Briefly, the colonies were mixed with 200 μL TE buffer (10 mM Tris: 0.1 mM EDTA; pH 8.0) and 2 μL lysostaphin (2000 U/mL, Sigma) at 37 °C for 30 min, followed by boiling for another 30 min. The solution was centrifuged at 13,000 × *g* for 1 min and the supernatant was collected for PCR as aforementioned. An aliquot (3 μL) of the PCR products was detected using LFI strips. In comparison, 5 μL of the PCR products was loaded onto 1.5% agarose gel for electrophoresis and image collection. Each test was performed at least in duplicate. The colonies were further confirmed by Gram staining and coagulase activity test using rabbit plasma (Land Bridge Tech Co. Ltd., Beijing, China).

The developed LFI strip test was then applied for routine test of MRSA from 660 frozen pork products imported from Denmark. The test was performed after pre-enrichment as aforementioned and repeated in duplicate.

2.6. Minimum inhibitory concentration (MIC) test

According to the Clinical Laboratory Standards Institute (CLSI) guidelines, cefoxitin MIC test was performed using Mueller-Hinton BBL II agar with different concentrations of cefoxitin (0.5, 1, 2, 4, 8, 16, 32, 64 μg/mL). Plates were incubated in ambient air at 37 °C. Cefoxitin MICs were read after incubation for 24 h. Quality control strain was methicillin-susceptive *S. aureus* strain ATCC 43300 and the obtained MIC range for cefoxitin was 1– 4 μg/mL. According to the CLSI guidelines, MIC value of *S. aureus* less than or equal to 4 μg/mL is considered as methicillin susceptible, whereas MIC value higher than 8 μg/mL is regarded as methicillin resistant.