J Infect Chemother 22 (2016) 372–376

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Development of a rapid diagnostic method for identification of *Staphylococcus aureus* and antimicrobial resistance in positive blood culture bottles using a PCR-DNA-chromatography method

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form
16 February 2016
Accepted 17 February 2016
Available online 4 April 2016

Keywords:
Staphylococcus aureus
mecA
blaZ
Blood culture
DNA-Chromatography
Rapid diagnosis

ABSTRACT

Blood culturing and the rapid reporting of results are essential for infectious disease clinics to obtain bacterial information that can affect patient prognosis. When gram-positive coccoid cells are observed in blood culture bottles, it is important to determine whether the strain is *Staphylococcus aureus* and whether the strain has resistance genes, such as *mecA* and *blaZ*, for proper antibiotic selection. Previous work led to the development of a PCR method that is useful for rapid identification of bacterial species and antimicrobial susceptibility. However, that method has not yet been adopted in community hospitals due to the high cost and methodological complexity. We report here the development of a quick PCR and DNA-chromatography test, based on single-tag hybridization chromatography, that permits detection of *S. aureus* and the *mecA* and *blaZ* genes; results can be obtained within 1 h for positive blood culture bottles. We evaluated this method using 42 clinical isolates. Detection of *S. aureus* and the resistance genes by the PCR-DNA-chromatography method was compared with that obtained via the conventional identification method and actual antimicrobial susceptibility testing. Our method had a sensitivity of 97.0% and a specificity of 100% for the identification of the bacterial species. For the detection of the *mecA* gene of *S. aureus,* the sensitivity was 100% and the specificity was 95.2%. For the detection of the *blaZ* gene of *S. aureus*, the sensitivity was 100% and the specificity was 88.9%. The speed and simplicity of this PCR-DNA-chromatography method suggest that our method will facilitate rapid diagnoses.

1. Introduction

Blood culture is an essential test for infectious disease clinics, and the rapid characterization of the detected bacteria contributes to the prognosis of patients [1].

When gram-positive coccoid cells are detected in blood cultures, it is important to determine whether the isolate is *Staphylococcus aureus* or another species of staphylococcus. If the organism is *S. aureus*, proper antibiotic selection by the clinician requires the early determination of whether the strain is methicillin-resistant *S. aureus* (MRSA) or a β-lactamase-producing strain [2].

In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used in making diagnoses, since this method can provide results quickly. MALDI-TOF MS can be obtained within minutes when starting from an individual colony, and or within only 30 min starting from a positive blood culture bottle [3]. The concordance rate for the identification of blood culture-positive samples between the conventional culture method and MALDI-TOF MS was good at 83.3%–96.6% for gram-negative bacilli, but it was only 31.8%–64.8% for gram-positive bacteria [3]. Although MALDI-TOF MS can be used to quickly identify bacterial species, the method cannot be used to concurrently obtain information on susceptibility to antimicrobials.

A useful PCR method has been developed to quickly provide information on the antimicrobial susceptibility and species identification of bacteria [4,5], but this PCR method has not yet been adopted by community hospitals in Japan. Adoption has been

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http://dx.doi.org/10.1016/j.jiac.2016.02.013
1341-321X/

Table 1
List of primers used.

Targeted gene	Primer name	Sequence (5′–3′)
S. aureus (*ftsY*)	Forward *ftsY*	Biotin-GAACAAGGTCAAGACAAATTAGA
	Reverse *ftsY*	AntiTag4-CTTCACTCAACTTGCGTGGC
mecA	Forward *mecA*	Biotin-CCAGGAATGCAGAAAGACC
	Reverse *mecA*	AntiTag3-CTCATATGCTGTTCCTGTATTG
blaZ	Forward *blaZ*	Biotin-CCTAAGGGCCAATCTGAACC
	Reverse *blaZ*	AntiTag2-GTGAAACCGCCAAGAGTGTA
Internal control	Forward IPC	Biotin-CGGGAAATCGTGCGTGAC
	Reverse IPC	AntiTag7-ATGGTGATGACCTGGCCG

IPC: Internal Positive Control.

impeded by the fact that equipment and reagents for real-time PCR are expensive and that gel electrophoresis (needed to detect the PCR amplicon) is cumbersome.

To address these problems, the single-tag hybridization (STH) chromatographic printed array strip (PAS) has been developed [6]. The principle of STH chromatographic PAS is to detect the tagged PCR amplicons bound to the anti-tag printed on the strip [6]. The STH chromatographic PAS method permits the detection of multiple PCR amplicons simultaneously. The STH chromatographic PAS method does not require gel electrophoresis and can be carried out in a low-cost conventional PCR machine, permitting rapid and economical detection.

In this study, we developed a rapid and simple PCR-DNA-chromatography method based on the STH chromatographic PAS method for identifying *S. aureus* and for concurrently detecting the *mecA* and *blaZ* genes for use when gram-positive coccoid cells are observed in positive blood culture bottles.

2. Materials & Methods

2.1. Selection of target genes and primers for DNA-chromatography

For the specific detection of *Staphylococcus aureus*, cell wall synthesis gene *ftsY* of *S. aureus* was selected as a target (Table 1). We designed primers that correspond to sequences conserved among 71 MRSA genotyping strains, which were kindly provided by Dr. Hiramatsu [7]. The specificity also was evaluated against the following 30 type strains of the genus *Staphylococcus*: *Staphylococcus arlettae* JNBP05238^T, *Staphylococcus auricularis* JNBP05320^T, *S. aureus* JNBP05244^T, *Staphylococcus capitis* JNBP05321^T, *Staphylococcus caprae* JNBP05324^T, *Staphylococcus carnosus* JNBP05326^T, *Staphylococcus cohnii* JNBP07989^T, *Staphylococcus condimenti* JNBP05333^T, *Staphylococcus delphini* JNBP05334^T, *Staphylococcus chromogenes* JNBP05330^T, *Staphylococcus epidermidis* JNBP05462^T, *Staphylococcus haemolyticus* JNBP05357^T, *Staphylococcus hominis* JNBP05359^T, *Staphylococcus hyicus* JNBP05371^T, *Staphylococcus intermedius* JNBP05372^T, *Staphylococcus kloosii* JNBP05374^T, *Staphylococcus lentus* JNBP05375^T, *Staphylococcus lugdunensis* JNBP05377^T, *Staphylococcus lutrae* JNBP05381^T, *Staphylococcus muscae* JNBP05382^T, *Staphylococcus pseudintermedius* JNBP05388^T, *Staphylococcus piscifermentans* JNBP05386^T, *Staphylococcus saccharolyticus* JNBP05390^T, *Staphylococcus saprophyticus* JNBP05476^T, *Staphylococcus schleiferi* JNBP05399^T, *Staphylococcus sciuri* JNBP05477^T, *Staphylococcus simulans* JNBP05404^T, *Staphylococcus succinus* JNBP05406^T, *Staphylococcus warneri* JNBP05408^T, and *Staphylococcus xylosus* JNBP05410^T. The *mecA* primers in Table 1 also were evaluated against the 71 MRSA genotyping strains provided by Dr. Hiramatsu [7]; again, specificity was confirmed using the above-listed 30 type strains of the genus *Staphylococcus*. The *blaZ* primers were selected from a published reference [8].

2.2. Blood culturing and the conventional assays for identification and antimicrobial susceptibility testing

Blood culture was performed using the BACTEC FX Blood Culture System (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan). Bacterial identification was performed using a VITEK 2 (SYSMEX-bioMérieux Co., Ltd., Tokyo, Japan), *S. aureus* Latex Test (DENKA SEIKEN Co., Ltd., Tokyo, Japan), and MALDI Biotyper Microflex LT (Bruker Daltonics K.K., Yokohama, Japan). The antimicrobial susceptibility test was carried out using the Dry Plate Eiken for the broth microdilution method (Eiken Chemical Co., Ltd., Tokyo, Japan). Susceptibility was assessed according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [9]. The β-lactamase confirmation test was performed with a Cefinase Disc (Nippon Becton Dickinson) or with a penicillin disc zone-edge test.

2.3. Sample preparation method for PCR

Harvesting of cells from blood culture bottles was carried out using the centrifugal washing method [3,10]. Ten milliliters of a blood culture was collected in a sterile tube and centrifuged at 1500 rpm for 5 min to pellet the blood cells. One milliliter of the resulting supernatant was transferred to a new tube and centrifuged at 13,000 rpm for 1 min. The supernatant then was removed and 1 ml of sterilized water was added to the precipitate to lyse the erythrocytes. The sample was centrifuged 2 to 3 times at 13,000 rpm for 1 min to remove the lysed erythrocytes. Next, 100 μl of sterile water was added to the pellet, and the resulting suspension was boiled for 5 min to lyse the staphylococci (Fig. 1).

2.4. PCR

Five microliters each of the crude lysate and 2 × EX Taq (TAKARA BIO INC., Shiga, Japan) were mixed in a PCR tube in which the primers (4 nM each) listed in Table 1 had been pre-coated and dried. A QuickBath thermal cycler (ThermoGen Ltd., Nagano, Japan) was used for rapid amplification under the following conditions: denaturation for 2 min at 97 °C, then 30 cycles of annealing and extension at 60 °C for 10 s, followed by denaturation at 97 °C for 5 s (Fig. 2). The amplification required 30 min or less.

2.5. DNA-chromatography method

After completion of the PCR, 10 μl of streptavidin-coated blue latex was added to the PCR reaction and a DNA-chromatography strip (Tohoku Bio-Array Co., Ltd., Sendai, Japan) was placed in the tube (Fig. 2). The results were read 10 min after the insertion of the DNA-chromatography strip (Fig. 3). This series of operations, from the centrifugal wash preparation protocol through the PCR-DNA-chromatography protocol, required 1 h or less.

The DNA-chromatography method is based on the STH chromatographic PAS method [6] used to amplify the target gene using primers with anti-tag or labeled by conjugation of biotin to the 5'-terminus. PCR amplicons with anti-tag bind to the tag printed on the DNA-strip. Streptavidin-blue latex particles bind to the biotin-PCR amplicons, which then are captured by a tag on the membrane; the bound products become visible after 5–10 min. Appearance of the blue line is indicative of binding of streptavidin-coated blue latex to the PCR amplicons, which are labeled with biotin on the DNA-strip. By changing the tagged primers, it is possible to detect various different genes simultaneously. Notably, this method does not require gel electrophoresis to detect the PCR amplicons. Detection sensitivity of the DNA-chromatography method is 10 times greater than that of agarose gel electrophoresis [6].