Real-Time PCR Assay for Detection of blaZ Genes in Staphylococcus aureus Clinical Isolates

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The Clinical and Laboratory Standards Institute recommends consideration of blaZ gene testing for cases of serious Staphylococcus aureus infection. Conventional PCR methods have demonstrated superior sensitivity and specificity to phenotypic tests. To our knowledge, this is the first description of real-time PCR detection of the blaZ gene.

Staphylococcus aureus bacteremia is a cause of significant morbidity and mortality, with 30-day mortality rates of up to 27% (1). Although rates of S. aureus penicillin susceptibility are generally low, ranging from 5% to 20% (2,3), penicillin remains the treatment of choice for penicillin-susceptible S. aureus (PSSA) based on in vitro data indicating increased susceptibility to penicillin over penicillinase-resistant β-lactams (3–5).

Penicillin resistance in S. aureus is manifest predominantly via the production of β-lactamase encoded by the blaZ gene (6), with four variants of the β-lactamase (A, B, C, and D) identified using immunologic methods and substrate profile techniques (7,8). A number of phenotypic methods for the detection of β-lactamase production in Staphylococcus species were investigated (9–15), but compared to detection of the blaZ gene by PCR, all phenotypic methods had a sensitivity of less than 72% (15,16). This has led to the Clinical and Laboratory Standards Institute (CLSI) recommending that blaZ gene detection be considered for PSSA isolates from cases of serious infection requiring penicillin therapy (4).

Various PCR methods have been described for the detection of blaZ gene in Staphylococcus species (5,16), but to date there are no published real-time PCR methods. We describe a real-time PCR assay for the detection of the blaZ gene in S. aureus.

One hundred nonduplicate S. aureus bloodstream isolates (BSI) collected between September 2011 and December 2012 by the PathWest Laboratory Medicine WA Queen Elizabeth II Medical Centre Department of Microbiology were included in this study. Of these, 50 isolates were susceptible to penicillin and 50 resistant to penicillin when tested by Kirby-Bauer disc diffusion according to CLSI guidelines (17). An additional 28 nonduplicate S. aureus isolates which had the presence or absence of the blaZ gene determined by microarray analysis (Alere Technologies, Jena, Germany), as previously described (18), were also included. Of these, 14 isolates had blaZ detected by microarray, and 14 isolates did not. Previously characterized blaZ type strains and β-lactamase strain variants were used as positive controls. These included the following: type A, PC1(pI254) and NCTC 9789; type B, 22260 and ST79/741; type C, 3804(pI3804), RN9(pI147), and V137; and type D, FAR10.

Colonies of S. aureus located at the penicillin zone edge of a 10-μg penicillin disc on Mueller-Hinton agar (Oxoid, Ltd., Hampshire, United Kingdom) were tested with nitrocefin-imregnated discs (BBL; BD, USA) according to the manufacturer’s instructions. Zone edge assessment was made on all isolates and recorded as being either a sharp “cliff” edge suggestive of β-lactamase production or a tapered “beach” edge suggestive of absence of β-lactamase production. Interpretation of phenotypic tests was performed by two independent observers without reference to the PCR results. Although interobserver variability has been demonstrated previously in regard to interpretation of zone edge characteristics (15), in this study no interobserver discrepancies were demonstrated.

Bacterial DNA from S. aureus isolates cultured on horse blood agar for 24 h at 37°C in room air was extracted as follows. Two to five loopfuls of S. aureus were suspended in 1 ml sterile demineralized water and boiled for 10 min, and the suspension was centrifuged for 2 min. From this, 8 μl of the supernatant was used with 12 μl of either conventional or real-time PCR amplification mix.

The conventional blaZ PCR was performed as described previously (16). The real-time blaZ PCR primer and probe sequences were designed in-house using Primer Express software (Applied Biosystems, USA). Fluorophore-labeled oligonucleotide probes were synthesized by Applied Biosystems (MGB probes), and primers were synthesized by Integrated DNA Technologies (Fisher Biotec, Australia). Sequences for the blaZ gene available from GenBank were obtained by BLASTn search using accession no. FR714929 and aligned prior to primer and probe design. Anomalous bases were used in the primers and probe where necessary to allow detection of genetic variants of the blaZ target. The reaction mix contained PCR buffer (Applied Biosystems, USA), 4 mM MgCl2, 0.2 mM deoxynucleoside triphosphates (Sigma, USA), 0.75u AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), 0.2 μM forward primer (5'-GCTTTAAAA GAACATTGAGGCCTCA-3'), 0.2 μM reverse primer (5'-CCAC CGATYTCTTTATATATATTT-3'), and 0.2 μM TaqMan probe (5'-FAM-AGTGATAATACGCAAACAAMBNNFQ-3', where FAM is 6-carboxyfluorescein). The amplification was performed using RotorgeneQ real-time thermocyclers (Qiagen, Australia).
were run under the following conditions: 95°C for 10 min, followed by 50 cycles of 12 s at 94°C, 15 s at 55°C, and 20 s at 72°C. Probe emission signals were acquired during the 20-s extension step of the cycling program.

Amplified DNA produced by the conventional and real-time PCR was sequenced using the AB BigDye Terminator version 3.1 sequencing kit on the AB 3130xl genetic analyzer (Applied Biosystems). Sequences were compared to those of the \textit{blaZ} gene available in GenBank using BLASTn software.

The conventional PCR method was verified by testing with the positive-control reference strains and the clinical isolates previously characterized by microarray analysis. All positive-control reference strains had \textit{blaZ} detected by both conventional PCR and real-time PCR. For these isolates, all had either no zone around the penicillin disc or a cliff zone edge, and all had a positive nitrocefin test; results are shown in Table 1. For the 50 phenotypically penicillin-susceptible \textit{S. aureus} BSI, the sensitivity, specificity, and positive and negative predictive values of the real-time PCR, the nitrocefin disc test, and the characterization of zone edge, compared to those of the conventional PCR reference method, are shown in Table 1. Two of these had \textit{blaZ} detected by both the conventional and real-time PCR methods, and the results were confirmed by sequencing. Sequences of the 2 isolates demonstrated 100% homology with \textit{blaZ} types in GenBank (accession no. DQ016067 and GQ980074). There was no genetic mutations identified that would account for the discrepant phenotypic and genotypic results; however, expression of the \textit{blaZ} gene may be affected by mutations in DNA encoding promoter or repressor regions (23). These 2 isolates had a negative nitrocefin test, and one of the isolates had a penicillin zone edge characterized as beach edge and the other a cliff edge. All of the remaining 48 BSI that did not have \textit{blaZ} detected by PCR had phenotypic test results consistent with the absence of β-lactamase.

The real-time PCR for detection of the \textit{blaZ} gene was developed in response to current CLSI recommendations. Conventional PCRs have previously been described and have been used as

\begin{table}
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\caption{Phenotypic and real-time PCR tests compared to conventional PCR for detection of \textit{β-lactamase} in \textit{S. aureus} isolates}
\begin{tabular}{lllllll}
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Testing method and result by \textit{S. aureus} isolate & No. of positive tests & No. of negative tests & \% sensitivity & \% specificity & PPV (\%)$^a$ & NPV (\%)$^b$ \\
\hline
\textbf{Penicillin-sensitive (by disc diffusion) \textit{S. aureus} BSI} & & & & & & \\
\textit{(n = 50)} & & & & & & \\
Nitrocefin disc test & 0 & 50 & 0 & 100 & 0 & 96 \\
Penicillin zone edge$^c$ & 1 & 49 & 50 & 100 & 100 & 98 \\
Real-time PCR & 2 & 48 & 100 & 100 & 100 & 100 \\
Conventional PCR & 2 & 48 & & & & \\
\textbf{Penicillin-resistant (by disc diffusion) \textit{S. aureus} BSI} & & & & & & \\
\textit{(n = 50)} & & & & & & \\
Nitrocefin disc test & 50 & 0 & 100 & & 100 & \\
Penicillin zone edge$^c$ & 50 & 0 & 100 & & 100 & \\
Real-time PCR & 50 & 0 & 100 & & 100 & \\
Conventional PCR & 50 & 0 & & & & \\
\textbf{\textit{S. aureus} clinical isolates, \textit{blaZ} status characterized} & & & & & & \\
\textbf{by microarray \textit{(n = 28)}} & & & & & & \\
Nitrocefin disc test & 14 & 14 & 100 & 100 & 100 & 100 \\
Penicillin zone edge$^c$ & 14 & 14 & 100 & 100 & 100 & 100 \\
Real-time PCR & 14 & 14 & 100 & 100 & 100 & 100 \\
Conventional PCR & 14 & 14 & & & & \\
\textbf{\textit{S. aureus} \textit{blaZ}-positive reference strains \textit{(n = 6)}} & & & & & & \\
Nitrocefin disc test & 6 & 0 & 100 & & 100 & \\
Penicillin zone edge & 6 & 0 & 100 & & 100 & \\
Real-time PCR & 6 & 0 & 100 & & 100 & \\
Conventional PCR & 6 & 0 & & & & \\
\textbf{All \textit{S. aureus} isolates tested \textit{(n = 134)}} & & & & & & \\
Nitrocefin disc test & 70 & 64 & 97.2 & 100 & 100 & 96.9 \\
Penicillin zone edge$^c$ & 71 & 63 & 98.6 & 100 & 100 & 98.4 \\
Real-time PCR & 72 & 62 & 100 & 100 & 100 & 100 \\
Conventional PCR & 72 & 62 & & & & \\
\hline
$^a$ PPV, positive predictive value. \\
$^b$ NPV, negative predictive value. \\
$^c$ A beach edge indicates lack of \textit{β-lactamase} (negative test), whereas a cliff edge indicates its presence (positive test). 
\end{tabular}
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the gold standard for β-lactamase detection when comparing phenotypic tests (6, 13, 16, 24, 25). The advantages of the real-time PCR over conventional PCR include a faster turnaround time, less specimen handling with subsequent reduced workload and risk of specimen contamination, lower cost, and equivalent sensitivity (100%) and specificity (100%). These advantages would facilitate more routine testing of the blaZ gene in accordance with CLSI recommendations. The clinical impact of introducing routine real-time PCR testing will require further study.

To our knowledge, this is the first published study to describe a real-time PCR for the detection of the blaZ gene in S. aureus. This real-time PCR is able to detect all four variants of S. aureus β-lactamase facilitating rapid and accurate confirmation of the presence of the blaZ gene in S. aureus clinical isolates that test penicillin susceptibility by phenotypic methods.

ACKNOWLEDGMENTS

The reference S. aureus type strains and β-lactamase strain variants used as positive controls were provided by D. Kernodle, Vanderbilt University, Nashville, TN, USA. The 28 clinical S. aureus isolates which had the presence or absence of the blaZ gene determined by microarray analysis were provided by G. Coombs, PathWest Laboratory Medicine WA, Royal Perth Hospital Department of Microbiology.

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