Evaluation of a Real-Time *blaZ* PCR Compared to Penicillin Disk Zone-Edge Test for Detection of Beta-Lactamase Producing *Staphylococcus aureus*

363

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Revised Abstract

Background: The 2012 CLSI document M100-S22 describes a new penicillin disk disk zone-edge test for detecting β-lactamase producing *S. aureus*. Our hospital has a relatively high rate of penicillin susceptible *S. aureus* (18%) as determined by an in-house developed real-time PCR method to detect the *blaZ* gene in susceptible isolates. The aim of this study was to compare the accuracy of in-house Real-time (RT) PCR test to the new penicillin disk zone-edge test.
Methods: 100 clinical isolates of *S. aurues* from different patients with penicillin MICs of <=0.12 µg/ml and a ten sample challenge set, provided by the Centers for Disease Control (CDC), were tested by both methods to detect β-lactamase positive isolates. The penicillin disk zone-edge was performed as described by CLSI (M100-S22). The penicillin disk zone-edge test was interpreted as positive for β-lactamase production only if full sized colonies were forming a sharp edge. Any amount of tapered/fuzzy edge was interpreted as negative. An in-house RT-PCR with a single primer set, using SYBR green chemistry for melting curve assessment, was performed on colonies. A melting curve showing peaks with intensity of 0.5 or higher was considered positive.
Results: Of 110 isolates, 104 (94.5%) gave congruent results with both methods. The sensitivity of *blaZ* PCR was 100% (9/9). All nine positive isolates had narrow melting peaks of intensities ≥2.0. The specificity of *blaZ* PCR was 94.1% (95/101). Of six isolates with discrepant results, five showed broad melting peaks. If a narrow peak was included in the positivity criteria, the specificity of *blaZ* PCR would be 99% (100/101).
Conclusion: Using the penicillin disk zone-edge as the gold standard, our in-house real-time *blaZ* PCR detected all the β-lactamase producing *S. aureus*. However, further testing was needed to resolve PCR-positive, penicillin disk zone-edge negative results. Inclusion of the melting peak narrowness, height(H) to width(W) ratio, in the positivity criteria increased the specificity of *blaZ* PCR to 99%

Background

S. aureus is one of the most common bacterial infections. Penicillin is still a very effective treatment if the isolate does not produce β-lactamase and the patient is not penicillin allergic. Penicillin can be a good choice for the long term treatment of endocarditis and osteomylitus. We wanted to confirm that our in-house PCR would detect the *blaZ* gene.

Material and Methods

- Training set: 40 clinical isolates of *S. aureus* with penicillin MICs of >0.12, i.e. produce β-lactamase, were tested by RT-PCR to refine interpretive criteria of melting peaks.
- Validation set: 100 clinical isolates of *S. aureus* with penicillin MICs of <=0.12 were tested by penicillin disk zone-edge and RT-PCR with a single primer set, using SYBR green chemistry for melting curve assessment.
- 10 sample challenge set, provided by CDC was tested by both methods

Results

Table 1a: Sensitivity of in-house RT *blaZ* PCR in the training set.

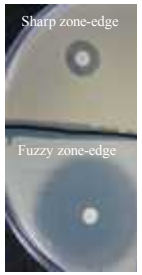
		pen. MIC >0.12 (β-lactamase producers)	
		positive	negative
blaZ PCR	positive	40 100% sensitivity	NA
	negative	0	NA

- Of the 40 Pen R *S. aureus*, 34 had melting peak intensities >2.0. The remaining 6 had melting peak height to width ratio of 0.6 or greater.

Table 1b: Training set – H:W ratio of the six melting peaks with intensities <2.0

	Pen R *S.aureus* melting peaks		
Isolates	height (H)	width (cm) (W)	Ratio H:W
PRSA1	1.65	2.25	0.73
PRSA5	0.74	1.1	0.67
PRSA19	1.04	1.6	0.65
PRSA30	1.06	1.7	0.62
PRSA36	1.33	1.8	0.74
PRSA38	1.43	1.9	0.75

Results cont.



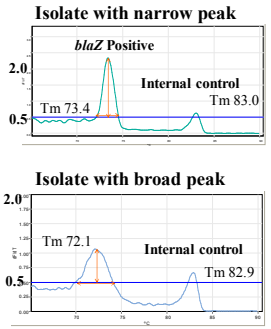


Table 2: Sensitivity and Specificity of in-house *blaZ* RT-PCR compared to pen. disk zone-edge when only using positivity criteria of melting peak intensity ≥0.5.

		pen. disk zone-edge	
		positive	negative
blaZ PCR	positive	9 100% sensitivity	6
	negative	0	95 94% specificity

Table 3a: Evaluation of the five broad peak isolates H:W ratio.

	Broad melting peaks		
Isolate #	height (H)	width (cm) (W)	Ratio H:W
4	1.47	5.3	0.28
49	0.55	1.8	0.31
50	1.07	4.8	0.22
68	1.81	6.4	0.28
71	1.9	5.9	0.32

Table 3b: Sensitivity and Specificity of in-house *blaZ* RT-PCR when narrow melt peak criteria is included.

		pen. disk zone-edge	
		positive	negative
blaZ PCR	positive	9 100% sensitivity	1
	negative	0	100 99% specificity

Final Positivity Criteria:
- Melting peak intensity ≥2.0
- If melting peak intensity is≥0.5, <2.0, the H:W ratio must be >0.5

Conclusions

- **Our in-house real time blaZ PCR is a sensitive (100%) and specific (99%) method for detecting β-lactamase producing *S. aureus*, as compared to penicillin disk zone-edge test, when positivity criteria includes melting peak intensity of >2 and for intensities between 0.5 and 2, a H:W ratio of ≥0.5.**

- **Either the penicillin disk zone-edge test or the H:W ratio could be used to resolve melting peak intensities less than 2.**