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Detection and Prevalence of Penicillin-Susceptible *Staphylococcus aureus* in the United States in 2013

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Using *blaZ* PCR as the "gold standard," the sensitivities of CLSI penicillin zone edge and nitrocefin-based tests for β-lactamase production in *Staphylococcus aureus* were 64.5% and 35.5%, respectively, with specificity of 99.8% for both methods. In 2013, 13.5% of 3,083 *S. aureus* isolates from 31 U.S. centers were penicillin susceptible.

Penicillinase-producing strains of *Staphylococcus aureus* emerged in the 1940s and by the 1970s represented 70 to 85% of the *S. aureus* population (1). Four types of *blaZ* genes (A to D) have been associated with penicillinase production in *S. aureus* (2). A study conducted in Germany demonstrated the sensitivity of nitrocefin-based testing was unacceptably low (36%) for penicillinase detection compared to *blaZ* PCR (3). Since 2012, the Clinical and Laboratory Standards Institute (CLSI) has recommended the penicillin zone edge test (4) to screen *S. aureus* isolates with a susceptible penicillin MIC (≤0.12 μg/ml) or disk zone (≥29 mm) for β-lactamase production (5).

There are limited data regarding the detection and prevalence of penicillin-susceptible *S. aureus* using a molecular method as the reference standard. The objectives of this national study were (i) to evaluate multiple phenotypic methods for β-lactamase detection in *S. aureus* using *blaZ* PCR as the "gold standard" and (ii) to determine the prevalence of penicillin-susceptible *S. aureus* in the United States.

As part of a national surveillance program, laboratories were asked to send 100 clinically significant *S. aureus* isolates to the University of Iowa. Isolates were recovered from specimens received during June to December 2013. Susceptibility testing using the CLSI broth microdilution method (5, 6) and *mecA* PCR were performed as previously described (7) on the 3,083 isolates received from 31 centers. The predominant specimen sources were 61% wound, 18% blood, 10% lower respiratory tract, 4% tissue, and 2% sterile body fluid. Classification as methicillin-susceptible *S. aureus* (MSSA) was based on a negative *mecA* PCR result. All isolates with a susceptible penicillin MIC (≤0.12 μg/ml) were assessed for β-lactamase production using *blaZ* PCR, penicillin zone edge, and induced nitrocefin-based testing. PCR to amplify a 355-bp region of the *blaZ* gene was followed by sequencing to classify positive strains as type A, B, C, or D as previously described (8). The *blaZ* type was also determined for a subset of penicillin-resistant isolates ($n = 51$). The penicillin zone edge test was performed on Mueller-Hinton agar using a 10-U penicillin disk following CLSI guidelines (5). After 16 to 18 h of incubation in ambient air, a sharp zone edge was interpreted as positive and a fuzzy zone as negative for β-lactamase production. Nitrocefin-based testing was performed on induced growth taken from the zone margin surrounding a 10-U penicillin disk.

TABLE 1 *blaZ* PCR results for 448 MSSA isolates with susceptible penicillin MICs[a]

Penicillin MIC (μg/ml)	No. of isolates	No. (%) of isolates:	
		blaZ positive	*blaZ* negative
≤0.015	1	0 (0)	1 (100)
0.03	24	0 (0)	24 (100)
0.06	370	14 (3.8)	356 (96.2)
0.12	53	17 (32.1)	36 (67.9)
Total	448	31 (6.9)	417 (93.1)

[a] Susceptibility was defined as a MIC of ≤0.12 μg/ml.

TABLE 2 Phenotypic test results for 31 *blaZ*-positive isolates with susceptible penicillin MICs

Penicillin MIC (μg/ml)	No. of isolates	No. (%) of isolates detected by:	
		Penicillin zone edge	Inducible nitrocefin test
0.06	14	4 (28.6)	3 (21.4)
0.12	17	16 (94.1)	8 (47.1)
Total	31	20 (64.5)	11 (35.5)

As expected, all 1,387 *mecA*-positive strains had penicillin MICs in the resistant range (≥0.5 μg/ml; 80% at >16 μg/ml). There were 448 isolates (14.5% of all isolates, 26.4% of MSSA isolates) with a susceptible penicillin MIC of ≤0.12 μg/ml. Thirty-one of the 448 isolates (6.9%) were *blaZ* positive (Table 1) and represented 32.1% of 53 isolates with a penicillin MIC of 0.12 μg/ml and 3.8% of 370 isolates with a penicillin MIC of 0.06 μg/ml. None of the 25 isolates with a penicillin MIC of ≤0.03 μg/ml contained *blaZ*.

Phenotypic β-lactamase screening test results for the 31 *blaZ*-positive isolates are shown in Table 2. One *blaZ* PCR-negative isolate (penicillin MIC, 0.12 μg/ml) was positive by both phenotypic tests. The sensitivity and specificity of penicillin zone edge testing were 64.5% and 99.8%, respectively. The sensitivity and

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TABLE 3 *blaZ* types detected in *S. aureus* isolates with susceptible and resistant penicillin MICs

Penicillin MIC (μg/ml)	No. (%) of isolates:		No. of isolates with *blaZ* type detected			
	Tested for *blaZ*	*blaZ* positive	A	B	C	NT
Susceptibility						
0.015	1 (100)					
0.03	24 (100)					
0.06	370 (100)	14 (3.4)[a]	2	2	8	2
0.12	53 (100)	17 (32.1)[b]	6	2	8	1
Resistance						
0.25	8 (14.8)	8 (100)	5		3	
0.5	1 (0.7)	1 (100)	1			
1	10 (3.0)	10 (100)	3	3	4	
2	4 (4.5)	4 (100)	2	1	1	
4	10 (3.8)	10 (100)	2		8	
8	11 (2.6)	11 (100)	3	1	7	
16	3 (1.6)	3 (100)	1		1	1
>16	4 (0.4)	4 (100)		1	3	
Total	499	82	25	10	43	4

[a] The *blaZ* types of the 10 isolates with a penicillin MIC of 0.06 μg/ml and not detected by the penicillin zone edge test were types A ($n = 2$), B ($n = 1$), C ($n = 6$), and nontypeable (NT [$n = 1$]).
[b] The *blaZ* type of the isolate with a penicillin MIC of 0.12 μg/ml and not detected by the penicillin zone edge test was type A.

specificity of the induced nitrocefin testing were 35.5% and 99.8%, respectively. These findings are similar to those from a German study that included 197 *S. aureus* isolates with Vitek 2 penicillin-susceptible results and reported sensitivities of 71.4% for penicillin zone edge and 35.7% for nitrocefin testing with *blaZ* PCR as the reference standard (3). A small Australian study assessed 50 *S. aureus* isolates that appeared penicillin susceptible by disk diffusion but only found 2 *blaZ*-positive isolates for evaluation of phenotypic methods (with one isolate detected by penicillin zone edge and neither detected by nitrocefin testing) (9). A larger Australian evaluation analyzing 157 isolates that appeared penicillin susceptible by agar dilution (38 were *blaZ* positive) noted 89% sensitivity and 100% specificity for the CLSI zone edge test (10). The EUCAST zone edge test incorporating a lower-concentration (1-U) penicillin disk provided 100% sensitivity and specificity for penicillinase detection in the Australian study (10). Lack of a CLSI quality control range and limited availability of the 1-U penicillin disk hinder further investigation of the EUCAST zone edge test. A U.S. study evaluating 105 isolates that appeared penicillin susceptible by disk diffusion found 10 (9.5%) to possess *blaZ* and noted variability among four readers of the CLSI penicillin zone edge test (with only 60% of those 10 strains having a sharp edge reported by all readers) (11).

The distribution of β-lactamase types detected in the present study among *blaZ*-positive isolates with a penicillin MIC of ≤0.12 μg/ml was 26% A, 13% B, and 52% C. (Three isolates did not correspond to a known type [NT].) The distribution was similar for isolates with higher penicillin MICs (Table 3). A 2009 study analyzing 98 MSSA isolates reported no *blaZ* for 13%, type A β-lactamase for 26%, type B for 15%, and type C for 46% (8). A South Korean study found type A *blaZ* in 17%, type B in 20%, type C in 53%, and type D in 1% of 220 MSSA isolates (12). Cefazolin failures have been reported for MSSA infections caused by type A *blaZ* isolate demonstrating an inoculum effect (8), but other retrospective studies have only found treatment success (12). Prospective studies are needed to determine if screening MSSA isolates for a cefazolin inoculum effect has clinical utility.

A correlation of penicillin zone edge test performance with *blaZ* type was not apparent in the present study. The 11 isolates not detected by the zone edge method represented all of the *blaZ* types (37% of the 8 type A, 25% of the 4 type B, 38% of the 16 type C, and 33% of the 3 nontypeable [NT] isolates).

In conclusion, the CLSI penicillin zone edge method detected penicillinase production among 45% more *blaZ*-positive *S. aureus* isolates than nitrocefin-based testing. The failure of the CLSI zone edge test to detect 35% of *blaZ*-positive isolates is concerning. This study indicates that in the United States, 13.5% of *S. aureus* isolates (24.6% of MSSA isolates) are penicillin susceptible based on negative *blaZ* testing. Many labs do not routinely test for penicillin susceptibility (13, 14). Although higher mortality has been reported for cefuroxime therapy of penicillin-susceptible MSSA bacteremia (15), superiority of penicillin over penicillinase-stable agents (e.g., dicloxacillin, nafcillin, and oxacillin) has not been proven. The limitations of phenotypic testing should be relayed to clinicians who request assessment of penicillin susceptibility. Molecular testing for *blaZ* is recommended before relying on penicillin for therapy of complicated MSSA infections.

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REFERENCES

1. **Chambers HF.** 2001. The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis **7:**178–182. http://dx.doi.org/10.3201/eid0702.010204.
2. **Voladri RK, Tummuru MK, Kernodle DS.** 1996. Structure-function relationships among wild-type variants of *Staphylococcus aureus* beta-lactamase: importance of amino acids 128 and 216. J Bacteriol **178:**7248–7253.
3. **Kaase M, Lenga S, Friedrich S, Szabados F, Sakinc T, Kleine B, Gatermann SG.** 2008. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. Clin Microbiol Infect **14:**614–616. http://dx.doi.org/10.1111/j.1469-0691.2008.01997.x.
4. **Gill VJ, Manning CB, Ingalls CM.** 1981. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. J Clin Microbiol **14:**437–440.
5. **Clinical and Laboratory Standards Institute.** 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25. CLSI, Wayne, PA.
6. **Clinical and Laboratory Standards Institute.** 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—10th ed. CLSI document M7-A10. CLSI, Wayne, PA.
7. **Richter SS, Heilmann KP, Biek D, Critchley I, Diekema DJ, Doern GV.** 2011. Activity of ceftaroline and epidemiologic trends in *Staphylococcus aureus* isolates collected from 43 medical centers in the United States in 2009. Antimicrob Agents Chemother **55:**4154–4159. http://dx.doi.org/10.1128/AAC.00315-11.
8. **Nannini EC, Stryjewski ME, Singh KV, Bourgogne A, Rude TH, Corey GR, Fowler VG, Murray BE.** 2009. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible *Staphylococcus aureus*: frequency and possible cause of cefazolin treatment failure. Antimicrob Agents Chemother **53:**3437–3441. http://dx.doi.org/10.1128/AAC.00317-09.
9. **Pereira LA, Harnett GB, Hodge MM, Cattell JA, Speers DJ.** 2014. Real-time PCR assay for detection of *blaZ* genes in *Staphylococcus aureus* clinical isolates. J Clin Microbiol **52:**1259–1261. http://dx.doi.org/10.1128/JCM.03413-13.
10. **Papanicolas LE, Bell JM, Bastian I.** 2014. Performance of phenotypic tests for detection of penicillinase in *Staphylococcus aureus* isolates from Australia. J Clin Microbiol **52:**1136–1138. http://dx.doi.org/10.1128/JCM.03068-13.
11. **El Feghaly R, Stamm JE, Fritz SA, Burnham C-A.** 2012. Presence of the bla_z beta-lactamase gene in isolates of *Staphylococcus aureus* that appear penicillin susceptible by conventional phenotypic methods. Diagn Microbiol Infect Dis **74:**388–393. http://dx.doi.org/10.1016/j.diagmicrobio.2012.07.013.
12. **Chong YP, Park SJ, Kim ES, Bag KM, Kim MN, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS.** 2015. Prevalence of *blaZ* gene types and the cefazolin inoculum effect among methicillin-susceptible Staphylococcus aureus blood isolates and their association with multilocus sequence types and clinical outcome. Eur J Clin Microbiol Infect Dis **34:**349–355. http://dx.doi.org/10.1007/s10096-014-2241-5.
13. **Crane JK.** 2014. Resurgence of penicillin-susceptible *Staphylococcus aureus* at a hospital in New York State, USA. J Antimicrob Chemother **69:**280–281. http://dx.doi.org/10.1093/jac/dkt317.
14. **Chabot MR, Stefan MS, Friderici J, Schimmel J, Larioza J.** 2015. Reappearance and treatment of penicillin-susceptible *Staphylococcus aureus* in a tertiary medical centre. J Antimicrob Chemother **70:**3353–3356. http://dx.doi.org/10.1093/jac/dkv270.
15. **Nissen JL, Skov R, Knudsen JD, Ostergaard C, Schonheyder HC, Frimodt-Moller N, Benfield T.** 2013. Effectiveness of penicillin, dicloxacillin and cefuroxime for penicillin-susceptible *Staphylococcus aureus* bacteraemia: a retrospective, propensity-score-adjusted case-control and cohort analysis. J Antimicrob Chemother **68:**1894–1900. http://dx.doi.org/10.1093/jac/dkt108.
16. **Richter SS, Doern GV, Heilmann KP, Miner S, Tendolkar S, Riahi F, Diekema DJ.** 2014. Abstr 54th Intersci Conf Antimicrob Agents Chemother, abstr D-870.