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Comparison of Susceptibility Testing Methods with *mecA* Gene Analysis for Determining Oxacillin (Methicillin) Resistance in Clinical Isolates of *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus* spp.

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Ninety-nine clinical staphylococcal isolates (58 coagulase-negative *Staphylococcus* spp. [CoNS] and 41 *Staphylococcus aureus* isolates) were evaluated for susceptibility to oxacillin. The following susceptibility testing methods, media, and incubation conditions were studied: agar dilution by using Mueller-Hinton (MH) medium (Difco) supplemented with either 0, 2, or 4% NaCl and incubation at 30 or 35°C in ambient air for 24 or 48 h; disk diffusion by using commercially prepared MH medium (Difco) and MH II agar (BBL) and incubation at 35°C in ambient air for 24 or 48 h; and agar screen (spot or swab inoculation) by using commercially prepared agar (Remel) or MH agar (Difco) prepared in-house, each containing 4% NaCl and 6 μg of oxacillin/ml (0.6-μg/ml oxacillin was also studied with MH agar prepared in-house for the agar swab method and CoNS isolates) and incubation at 35°C in ambient air for 24 or 48 h for swab inoculation and at 30 or 35°C in ambient air for 24 or 48 h for spot inoculation. The results for these methods were compared to the results for *mecA* gene detection by a PCR method. Given the ability to support growth and the results for susceptibility testing (the breakpoint for susceptible isolates was ≤2 μg/ml), the best methods for CoNS isolates were (i) agar dilution by using MH medium supplemented with 4% NaCl and incubation at 35°C for 48 h (no growth failures were noted, and sensitivity was 97.6%) and (ii) agar screen (swab inoculation) by using MH medium prepared in-house supplemented with 4% NaCl and containing 0.6 μg oxacillin/ml and incubation at 35°C for 48 h (one isolate that did not carry the *mecA* gene did not grow, and the sensitivity was 100%). All but one (agar dilution without added NaCl and incubation at 30°C for 48 h) of the methods tested revealed all oxacillin-resistant *S. aureus* isolates, and no growth failures occurred with any method. If the breakpoint for susceptibility was lowered to ≤1 μg/ml for agar dilution methods, more CoNS isolates with oxacillin resistance related to the *mecA* gene were detected when 0 or 2% NaCl agar supplementation was used. Only one CoNS isolate with *mecA* gene-associated resistance was not detected by using agar dilution and MH medium supplemented with 4% NaCl with incubation for 48 h. When the breakpoint for susceptibility was decreased 10-fold (from 6.0 to 0.6 μg of oxacillin per ml) for the agar swab screen method, fully 100% of the CoNS isolates that carried the *mecA* gene were identified.

Despite guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS) for the testing of susceptibility to oxacillin for staphylococci, the optimal phenotypic method for detecting methicillin (oxacillin) resistance remains controversial. The objective of the present study was to determine which of the following susceptibility test methods, performed by using recommended or modified NCCLS guidelines, best detected oxacillin resistance: agar dilution, disk diffusion, and agar screen (swab or spot inoculation). The results for these methods were compared to PCR detection of the *mecA* gene for 58 clinical isolates of coagulase-negative *Staphylococcus* spp. (CoNS) and 41 clinical isolates of *Staphylococcus aureus*.

MATERIALS AND METHODS

Ninety-nine clinical isolates (58 CoNS isolates and 41 *S. aureus* isolates) and four control strains (*S. aureus* ATCC 25923 [lacking *mecA*], *S. aureus* MC 205 [a Mayo Clinic isolate lacking *mecA*], *Staphylococcus epidermidis* ATCC 27626 [*mecA* positive], and *S. aureus* MC 206 [a Mayo Clinic isolate, *mecA* positive]) were evaluated. All of the clinical isolates were obtained from human specimens submitted to the Mayo Clinical Microbiology Laboratory. No two isolates were from the same patient, and no isolates were part of nosocomial outbreaks. All staphylococcal isolates and ATCC control strains were screened for the presence of the *mecA* gene by using a modification of a previously described multiplex PCR method (6). The following PCR primers were used for amplification of the *mecA* gene: mec449F, 5′-AAA CTA CGG TAA CAT TGA TCG CAA C-3′, and mec761R, 5′-CTT GTA CCC AAT TTT GAT CCA TTT G-3′. Primers specific to staphylococcus 16S rRNA, i.e., 16S 387F, 5′-CGA AAG CCT GAC GGA GCA AC-3′, and 16S 914R, 5′-AAC CTT GCG GTC GTA CTC CC-3′, were used in the multiplex PCR to provide a positive control for target amplification. The PCR mix contained 200 μM deoxynucleotide triphosphates, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 μM $MgCl_2$, 10% glycerol, 200 μM *mec* primers, 50 μM 16S rRNA primers, and 0.025 U of Ampli*Taq* DNA polymerase (PE Applied Biosystems, Foster City, Calif.) per μl. Target DNA (2 μl) was added to 48 μl of mix and then thermocycled for 30 cycles of 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s. PCR amplicons were analyzed by gel electrophoresis. With this modification, a 313-bp fragment of the *mecA* gene and a 528-bp fragment of the 16S rRNA gene unique to staphylococci are amplified. By this analysis, 30 (52%) of 58 CoNS clinical isolates and 17 (42%) of 41 *S. aureus* clinical isolates were shown to carry the *mecA* gene.

For susceptibility testing, the information about the media used (including whether each medium was prepared in-house), oxacillin concentration, incubation parameters, and susceptibility interpretive guidelines is shown in Table 1. Recent studies suggest that oxacillin susceptibility testing of CoNS isolates at lower breakpoints may more reliably detect oxacillin resistance encoded by the *mecA* gene (5, 17, 19, 24). Therefore, as part of the agar screen evaluation for CoNS isolates, we used a Mueller-Hinton (MH) plate containing 0.6 μg of oxacillin per ml in addition to a standard MH plate containing 6.0 μg of oxacillin per ml. Also, the results for the agar dilution were interpreted with ≤1-μg/ml

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concentration of oxacillin regarded as the breakpoint for susceptibility (analysis 2, Table 1).

RESULTS

Results for CoNS isolates are displayed in Table 2, and those for *S. aureus* isolates are displayed in Table 3. Based on the ability to support growth and the results for susceptibility testing, the best phenotypic methods for detecting *mecA* gene-encoded oxacillin resistance for CoNS isolates were agar dilution by using MH agar (Difco) supplemented with 4% NaCl and incubation at 35°C in ambient air for 48 h (there were no growth failures, and sensitivity was 96.7%) and agar screen (swab inoculation) by using MH medium (Difco) prepared in-house supplemented with 4% NaCl and containing 0.6 μg of oxacillin/ml (there was one growth failure, and sensitivity was 100%). The single CoNS isolate that failed to grow for the agar screen plate did not carry the *mecA* gene. This isolate grew poorly or not at all for all methods tested. The 96.7% sensitivity for the former method resulted from one very major error; one isolate that carried the *mecA* gene was interpreted as susceptible regardless of whether a susceptibility breakpoint of ≤2 μg/ml or of ≤1 μg/ml was used. However, when this isolate was evaluated by the agar screen by using the swab inoculation, 20 colonies grew on the plate with oxacillin concentration of 6.0 μg/ml and 60 colonies grew on the plate with oxacillin concentration of 0.6 μg/ml.

All methods, with the exception of one (agar dilution without added NaCl with incubation at 30°C for 48 h), correctly identified all *S. aureus* isolates with *mecA*-encoded oxacillin resistance; no growth failures occurred with any method. Varying the temperature for incubation (30 or 35°C) had little effect on results for both *S. aureus* and CoNS isolates.

DISCUSSION

Numerous studies have been conducted to determine optimal methods for phenotypic detection of oxacillin (methicillin) resistance among clinical isolates of staphylococci. Table 4 summarizes a number of these studies; only recent reports for commercial (including automatic) systems are included as many of the early reports indicated inferior performance for these methods. Oxacillin (methicillin) resistance for *S. aureus* isolates can be reliably detected by a variety of phenotypic methods. As shown in Table 4, several studies have demonstrated that 100% of oxacillin (methicillin)-resistant *S. aureus* test isolates were detected by either broth dilution (20), agar dilution (6, 8, 22), agar spot screen (9, 20), gradient diffusion (Epsilometer test) (22), or disk diffusion (4, 8, 12, 21) method or by the automated API-Plus system (bioMerieux) (21). For these methods, different concentrations of NaCl in media (0 to 4%) or varying incubation times (24 or 48 h) had little effect.

In contrast, NaCl supplementation, incubation time, and in one instance, inoculum were important parameters for expression of oxacillin (methicillin) resistance for CoNS isolates. As shown in Table 4, detection of oxacillin (methicillin) resistance was best achieved (sensitivity, 97 to 100%) if the NaCl concentration in medium was 4 to 5% (4, 20, 22, 24), the incubation time was 48 h rather than 24 h (4, 17, 22, 24), and/or a larger inoculum was used (14). Furthermore, when these parameters were applied, non-broth-based methods (agar spot [4, 20] or swab [4, 24] screens, disk diffusion [14, 17], or gradient diffusion [Epsilometer test] [4, 22]) had the highest accuracy. Of interest, two studies reported by the same group of investigators concluded that 2%, and not 4%, NaCl supplementation produced the best results for phenotypic detection of oxacillin resistance when agar dilution, broth dilution, and

TABLE 1. Susceptibility test methods used in this study

Method	Media[a]	Oxacillin concn	Inoculum	Incubation	Interpretive guidelines
Agar dilution	MH (Difco) with 0% NaCl	1 and 2 μg/ml	10^4 CFU/spot	30 and 35°C for 24 and 48 h (used for all media)	Analysis 1: susceptible, ≤2 μg/ml; resistant, ≥4 μg/ml Analysis 2: Susceptible, ≤1 μg/ml; Resistant, ≥2 μg/ml
	MH (Difco) with 2% NaCl				
	MH (Difco) with 4% NaCl				
Disk diffusion	MH (Difco)	1 μg disk	Swab, a McFarland standard equal to 0.5 to 1	35°C for 24 and 48 h (used for both media)	Susceptible, zone diameter of ≥13 mm; Intermediate, zone diameter of 11 to 12 mm; resistant, zone diameter of ≤10 mm
	MH II (BBL)				
Agar screen					
Swab	MH agar with 4% NaCl (Remel and Difco)	6 μg/ml (0.6 μg/ml for Difco only)	Swab, McFarland standard of 0.5 to 1	35°C for 24 and 48 h	Analysis 1: Resistant, >1 colony growth at 6 μg/ml Analysis 2: Resistant, >1 colony growth at 0.6 μg/ml (for Difco swab only)
Spot	MH (Difco) with 4% NaCl		10^4 CFU/spot	30 and 35°C for 24 and 48 h	

[a] All media prepared in-house except Remel.

TABLE 2. Comparison of susceptibility testing methods to *mecA* gene analysis for 58 CoNS isolates

Method	Medium	Incubation temp (°C)	Incubation time (h)	No. of isolates					Sensitivity (%)	Specificity (%)	No growth on control
				mecA positive	*mecA* negative	S[a]	I[a]	R[a]			
Agar dilution	0% NaCl	30	24	27	27	41[b]/34[c]		13[b]/20[c]	48.1[b]/59.3[c]	100[b]/85.2[c]	4
			48	29	27	42[b]/36[c]		14[b]/20[c]	48.3[b]/62.1[c]	100[b]/96.3[c]	2
		35	24	29	28	43[b]/36[c]		14[b]/21[c]	48.3[b]/65.5[c]	100[b]/92.9[c]	1
			48	30	28	40[b]/35[c]		18[b]/23[c]	60.0[b]/70.0[c]	100[b]/92.9[c]	0
	2% NaCl	30	24	28	27	34[b]/31[c]		21[b]/24[c]	75.0[b]/85.7[c]	100[b]/100[c]	3
			48	29	28	34[b]/32[c]		23[b]/25[c]	79.3[b]/86.2[c]	100[b]/100[c]	1
		35	24	30	28	39[b]/35[c]		19[b]/23[c]	63.3[b]/76.7[c]	100[b]/100[c]	0
			48	30	28	35[b]/30[c]		23[b]/28[c]	76.7[b]/93.3[c]	100[b]/100[c]	0
	4% NaCl	30	24	26	26	28[b]/27[c]		24[b]/25[c]	92.3[b]/92.3[c]	100[b]/96.1[c]	6
			48	29	28	32[b]/31[c]		25[b]/26[c]	86.2[b]/86.2[c]	100[b]/96.4[c]	1
		35	24	29	27	31[b]/30[c]		25[b]/26[c]	86.2[b]/89.7[c]	100[b]/100[c]	2
			48	30	28	28[b]/28[c]		28[b]/30[c]	96.7[b,d]/96.7[c]	96.4[b,d]/96.4[c]	0
Disk diffusion	Difco	35	24	29	27	22	6	28	90.0	74.1	2
			48	30	27	20	4	33	93.3	66.7	1
	BBL	35	24	29	27	23	2	31	86.2	74.1	2
			48	29	27	20	3	33	93.1	70.4	2
Agar screen											
Swab inoculation	Remel	35	24	30	28	46		12	40.0	100	—[e]
			48	30	28	33		25	83.3	100	—[e]
	Difco	35	24	29[f]/27[g]	27[f]/23[g]	27[f]/35[g]		29[f]/14[g]	93.1[f]/51.8[g]	92.6[f]/100[g]	2[f]/1[g]
			48	30[f]/27[g]	27[f]/23[g]	25[f]/22[g]		32[f]/26[g]	100[f]/96.3[g]	92.6[f]/100[g]	1[f]/1[g]
Spot inoculation	Remel	30	24	30	28	44		14	46.7	100	—[e]
			48	30	28	39		19	63.3	100	—[e]
		35	24	30	28	38		20	66.7	100	—[e]
			48	30	28	38		20	66.7	100	—[e]
	Difco	30	24	26	26	30		22	84.6	100	6
			48	29	28	33		24	82.3	100	1
		35	24	29	27	36		20	70.0	100	2
			48	30	28	32		26	83.3	96.4	0

[a] S, susceptible; I, intermediate; R, resistant.
[b] Susceptibility breakpoint, ≤2 μg/ml.
[c] Susceptibility breakpoint, ≤1 μg/ml.
[d] One isolate was interpreted as susceptible but was *mecA* positive; this isolate was interpreted as resistant by the agar screen swab inoculation method with Difco medium prepared in-house (60 colonies at 0.6 μg/ml; 20 colonies at 6.0 μg/ml).
[e] No control plate available.
[f] Oxacillin concentration, 0.6 μg/ml.
[g] Oxacillin concentration, 6.0 μg/ml.

gradient diffusion testing were compared (1, 10). The same group of organisms was used in each study; however, the reference standards were broth microdilution (MH medium with 2% NaCl and incubation for 24 h at 35°C) for one study (1) and identification of the *mecA* gene for the other study (10). However, for neither of these studies was the incubation period extended beyond 24 h.

The results of our evaluation for *S. aureus* isolates are in agreement with those of many of the studies summarized in Table 4. That is, *mecA* gene-associated resistance is reliably detected by a variety of phenotypic methods for which varying NaCl supplementation or incubation time has little effect. In our study, a 24-h incubation period was sufficient for *mecA*-associated resistance in *S. aureus* isolates for all of the methods we tested; extending the incubation time to 48 h frequently resulted in decreased specificities. These decreases in specificity were minor and may have occurred as the result of a decrease in the bioactivity of antimicrobial in the test media over time but could relate to mechanisms associated with oxacillin (methicillin) resistance not involving the *mecA* gene. These mechanisms might include hyperproduction of β-lactamase, production of penicillin binding proteins other than PBP2a encoded by *mecA*, which have decreased affinity for methicillin or related compounds, enzymes which inactivate methicillin, or as yet undiscovered mechanisms (3). Geha and colleagues (6) noted that among 228 clinical *S. aureus* isolates, 44 were methicillin resistant by agar dilution and disk diffusion techniques. Forty of these isolates carried the *mecA* gene as assessed by PCR; three of the remaining four isolates were demonstrated to be hyperproducers of β-lactamase. Kolbert and colleagues (15) noted that among 147 consecutive clinical *S. aureus* isolates, 28 were resistant by using a disk diffusion method. Fourteen of those isolates possessed the *mecA* gene; the remaining 14 isolates did not carry the *mecA* gene and were felt to be hyperproducers of β-lactamase.

The results of our study for CoNS isolates corroborate the results of several studies summarized in Table 4 in which similar media and incubation times were used. That is, oxacillin (methicillin) resistance encoded by the *mecA* gene, by an agar-based method, is best detected by using MH medium supplemented with ≥4% NaCl and incubation for 48 h (4, 20, 22, 24).

Current NCCLS recommendations for oxacillin susceptibility testing of staphylococci by using the agar dilution method specify the use of MH medium supplemented with 2% NaCl

TABLE 3. Comparison of susceptibility testing methods to *mecA* gene analysis for 41 *S. aureus* isolates

Method	Medium	Incubation temp (°C)	Incubation time (h)	No. of isolates					Sensitivity (%)	Specificity (%)
				mecA positive	*mecA* negative	Susceptible	Intermediate	Resistant		
Agar dilution	0% NaCl	30	24	17	24	24		17	100	100
			48	17	24	24		17	94.1	95.8
		35	24	17	24	24		17	100	100
			48	17	24	24		17	100	100
	2% NaCl	30	24	17	24	24		17	100	100
			48	17	24	23		18	100	95.8
		35	24	17	24	24		17	100	100
			48	17	24	24		17	100	100
	4% NaCl	30	24	17	24	22		19	100	95.8
			48	17	24	24		17	100	100
		35	24	17	24	24		17	100	91.7
			48	17	24	20		21	100	83.3
Disk diffusion	Difco	35	24	17	24	24	0	17	100	100
			48	17	24	12	2	27	100	41.7
	BBL	35	24	17	24	24	0	17	100	100
			48	17	24	14	4	23	100	58.3
Agar screen										
Swab inoculation	Remel	35	24	17	23	23[a]		17	100	100
			48	17	23	23[a]		17	100	100
	Difco	35	24	17	24	24		17	100	100
			48	17	24	24		17	100	100
Spot inoculation	Remel	30	24	17	24	24		17	100	100
			48	17	24	23		18	100	95.8
		35	24	17	24	24		17	100	100
			48	17	24	24		17	100	100
	Difco	30	24	17	24	24		17	100	100
			48	17	24	24		17	100	100
		35	24	17	24	24		17	100	100
			48	17	24	24		17	100	100

[a] One isolate not tested.

and incubation at 35°C in ambient air for 24 h (18). In contrast, NCCLS recommendations indicate that the oxacillin agar screen method should be used only for *S. aureus* isolates and that for this method, MH medium should be supplemented with 4% NaCl (not 2% NaCl) and incubation should be in ambient air for 24 h (18). The findings of the present study indicate that the medium specified for the agar screening method (MH medium with 4% NaCl) should also be used for the agar dilution method. Furthermore, the present study supports the use of the agar screen plate for CoNS isolates as well as *S. aureus* isolates.

In our study, extension of the incubation period to 48 h for CoNS, but not for *S. aureus* isolates, improved sensitivity regardless of the test method used. Like *S. aureus* isolates, specificity decreased for CoNS isolates when the incubation period was extended to 48 h and was most pronounced for disk diffusion methods. Like *S. aureus* isolates, CoNS isolates that are *mecA* negative but phenotypically resistant to oxacillin may possess other mechanisms for resistance. Geha and colleagues (6) noted that among 272 CoNS isolates, 148 were methicillin resistant and all of these possessed the *mecA* gene. However, Kolbert and associates (15) noted that among 253 consecutive clinical CoNS isolates, 128 of 130 *mecA*-positive isolates were resistant by disk diffusion. Thirteen additional isolates that were resistant by disk diffusion did not possess *mecA*, and three of these were shown to be β-lactamase hyperproducers.

We also observed that for agar screen methods and testing of CoNS isolates, media prepared in-house performed better than commercially prepared media. All commercially prepared media were used for testing prior to the expiration dates. We are unsure as to the reasons for the better performance of media prepared in-house. Unlike for media prepared in-house, we were unable to determine growth failures due to the unavailability of growth control plates (i.e., plates, provided by the manufacturer, that do not contain oxacillin). Undetected growth failures would have been misinterpreted as indicating susceptibility.

It has been suggested that the growth of heteroresistant subpopulations of staphylococci may be enhanced by using a cooler incubation temperature (i.e., 30 rather than 35°C) (13). However, our results showed that varying the temperature of incubation from 30 to 35°C had little effect.

The oxacillin susceptibility breakpoints currently recommended by the NCCLS for dilution testing methods are ≤2 μg/ml for *S. aureus* and <0.25 μg/ml for CoNS. The lower breakpoint for CoNS (compared with that for *S. aureus*) is a recent recommendation (18). Our study supports this recommendation. Specifically, we have demonstrated that for agar dilution and testing of CoNS isolates (analysis 2, Table 1), a lower susceptibility breakpoint of ≤1 μg of oxacillin/ml (instead of ≤2 μg of oxacillin/ml) permitted the detection of more CoNS isolates with *mecA*-associated resistance (Table 2).

To our knowledge, our study is the first to evaluate lower breakpoints for an agar screen method. Indeed, if the breakpoint for susceptibility for CoNS isolates was decreased 10-fold, from ≤6 μg/ml to 0.6 μg/ml, 100% sensitivity was

TABLE 4. Summary of reported studies evaluating phenotypic methods for detection of oxacillin (methicillin) resistance in staphylococci[a,b]

Author(s) (reference)	Organism, no. of isolates	Reference standard	Test methods[c]	% of isolates detected				% Overall agreement	Notes
				S. aureus		CoNS			
				Resistant	Susceptible	Resistant	Susceptible		
Thornsberry and McDougal (20)	*S. aureus*, 45; CoNS, 12	None (all isolates previously characterized as methicillin susceptible or resistant)	Broth dilution: CSMH with 2% NaCl; 24 h at 35°C	100		100			Increasing NaCl concentration to 5% resulted in better growth than that with 2% NaCl for resistant isolates; for susceptible isolates 5% NaCl resulted in higher MICs; 10% NaCl inhibited growth
			Agar spot screen: MH with 4% NaCl; 24 h at 35°C	100		100			
Hindler and Inderlied (8)	*S. aureus*, 10	None (all isolates previously characterized as methicillin resistant)	Disk diffusion: MH; 16 to 24 h at 35°C, reincubated additional 24 h at 30°C	100					Different commercial sources of MH were evaluated and differences in results were noted, especially for agar dilution
			Agar dilution: MH; 16 to 18 h at 35°C						
Hansen and Pope (7)	*S. aureus*, 161	None (all isolates previously characterized as methicillin susceptible or resistant)	Broth screen: CSMH with 2% NaCl; ~5 h at 35°C	96.9	100				Broth test vials were placed on a rotary shaker for 5 h at 35°C; tetrazolium salt respiratory indicator was added, and vials were observed for the development of a pink color after 30 min of additional incubation
Coudron et al. (4)	*S. aureus*, 95; CoNS, 175	Agar dilution (MH with 4% NaCl; 24 and 48 h incubation at 35°C) or broth dilution (CSMH with 2% NaCl; 24 and 48 h incubation at 35°C)	Disk diffusion: MH with 10-μg/ml methicillin and 0% NaCl						Comprehensive study which evaluated an array of different concentrations of NaCl and different incubation periods
			18 h	35	100	15	100		
			48 h	61	96	45	100		
			4% NaCl						
			18 h	89	98	88	100		
			48 h	100	76	95	100		
			Agar swab screen: MH with 10-μg/ml methicillin and 0% NaCl						
			24 h	87	100	46	100		
			48 h	89	100	94	92		
			4% NaCl						
			24 h	91	100	95	97		
			48 h	94	100	98	90		
			Agar spot screen: MH with 10-μg/ml methicillin and 4% NaCl						
			24 h	89	98	96	97		
			48 h	89	96	99	95		

Hindler and Warner (9)	*S. aureus*, 109	Broth microdilution: CSMH with 2% NaCl, 24 h at 35°C	Agar spot screen: MH with 4% NaCl, 24 h at 35°C	90–100[e]	100				MH media from five different commercial sources were evaluated. Results varied according to the source of MH medium.
Milne et al. (16)	*S. aureus*, 50; CoNS, 135	None (all isolates previously characterized as methicillin susceptible or resistant)	Disk Diffusion, 10-μg/ml methicillin:						Other media (Diagnostic Sensitivity Test agar, Sensitest agar, and Isosensitest agar) tested but results were worse than those for COL and MH agars.
			COL agar and 0% NaCl, 30°C						
			18 h	90	95	66	93		
			40 h	97	63	81	83		
			COL agar and 5% NaCl, 35°C						
			18 h	90	63	90	94		
			40 h	97	63	92	93		
			MH agar and 0% NaCl, 30°C						
			18 h	77	100	50	93		
			40 h	84	100	75	97		
			MH agar and 5% NaCl, 35°C						
			18 h	74	89	32	97		
			40 h	87	63	50	96		
Huang et al. (10)	*S. aureus* and CoNS, 223; no differentiation among species	*mecA* gene	Agar dilution: MH agar, 24 h at 35°C						The authors concluded that 2% NaCl is required for agar-based dilution methods; however, incubation time was limited to 24 h.
			0% NaCl					78	
			2% NaCl					83	
			4% NaCl					71	
			5% NaCl					72	
			Broth microdilution: CSMH, 24 h at 35°C						
			0% NaCl					64	
			2% NaCl					94	
			5% NaCl					91	
			E-test: MH, 24 h at 35°C						
			0% NaCl					71	
			2% NaCl					78	
			4% NaCl					69	
			5% NaCl					66	
Baker et al. (1)	*S. aureus* and CoNS, 223 (no differentiation among species)	Broth microdilution: MH with 2% NaCl, 24 h at 35°C	Agar dilution: MH, 24 h at 35°C						The authors recommended 2% NaCl supplementation for both broth and agar dilution; the presence of *mecA* gene was also determined in isolates. The best correlation with *mecA* and results for broth microdilution was achieved using MH supplemented with 2% NaCl.
			0% NaCl					89	
			2% NaCl					96	
			4% NaCl					88	
			5% NaCl					87	

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TABLE 4—*Continued*

Author(s) (reference)	Organism, no. of isolates	Reference standard	Test methods[c]	% of isolates detected				% Overall agreement	Notes
				S. aureus		CoNS			
				Resistant	Susceptible	Resistant	Susceptible		
			E-test: MH, 24 h at 35°C						
			0% NaCl					88	
			2% NaCl					97	
			4% NaCl					90	
			5% NaCl					88	
			Broth microdilution: MH, 24 h at 35°C						
			0% NaCl					87	
			4% NaCl					92	
			5% NaCl					91	
Knapp et al. (14)	*S. aureus*, 67; CoNS, 47 (all species were *S. epidermidis* and all were oxacillin resistant)	All isolates determined to be resistant by broth microdilution: CSMH with 2% NaCl, 24 h at 35°C; heterogeneous population analysis and oxacillin agar screen method: MH with 4% NaCl, 24 h	Disk diffusion, MH, 35°C						
			Inoculation 10^7 CFU/plate						
			24 h	82		87			
			48 h	97		89			
			Inoculation, 10^9 CFU/plate						
			24 h	88		100			
			48 h	97					
			Vitek GPS-SA card software 6.1 and 7.1	98		100			
Woods et al. (23)	*S. aureus*, 92; CoNS, 103	Microscan 24 h MIC panels. Discrepants resolved by oxacillin agar screening: MH with 4% NaCl, 24 h at 35°C	Microscan rapid panel	97		72			22 of 100 SCN isolates did not grow in the rapid panels.
Geha et al. (6)	*S. aureus*, 228; CoNS, 272	*mecA* gene	Agar dilution (MH with 0% NaCl, 48 h at 30°C) confirmed by disk diffusion (MH with 0% NaCl, 24 h at 35°C)	100		93			3 of 4 *S. aureus* isolates that were *mecA*-negative were resistant by agar dilution/disk diffusion method. These isolates were demonstrated to be hyperproducers of β-lactamase. No CoNS isolates were *mecA* negative and resistant by agar dilution/disk diffusion method.
York et al. (24)	CoNS, 140	*mecA* gene	Broth microdilution: CSMH with 2% NaCl at 35°C						Authors concluded that a lower breakpoint for resistance (2 μg/ml) than recommended by the NCCLS (4 μg/ml) would improve results for the microdilution method.
			24 h						
			48 h			50			
						77			
			Disk diffusion: MH at 35°C						
			24 h			84			
			48 h			82			
			Agar swab screen: MH with 4% NaCl at 35°C						
			24 h			70			
			48 h			100			

Wallet et al. (21)	*S. aureus*, 57; CoNS, 100	*mecA* gene	Disk diffusion: MH, 24 h at 35°C	100	91 (91)[d]		*S. epidermidis* and SCN isolates were separately analyzed.
			Automated API-plus system (bioMerieux): MH with 5% NaCl, 24 h at 35°C	100	86 (85)[d]		
			BBL Crystal MRSA ID system (Becton-Dickinson)				
			4 h	92	79 (81)[d]		
			6 h	96	86 (88)[d]		
			Agar dilution: MH 2% of NaCl, 24 h at 35°C	96	88 (88)[d]		
Mulder (17)	CoNS, 319	*mecA* gene	Disk diffusion: MH				
			Methicillin, 5 μg, 48 h at 30°C		84		Disk diffusion results best with MH agar supplemented with 2% NaCl and oxacillin (5 μg disks). The results for the E-test were interpreted by using 2 μg/ml as the breakpoint for oxacillin resistance.
			Oxacillin, 1 μg, 24 h at 35°C		95		
			Oxacillin, 5 μg/ml, 24 h at 35°C		86		
			Disk diffusion: MH with 2% NaCl				
			Methicillin, 5 μg, 48 h at 30°C		96		
			Oxacillin, 1 μg, 24 h at 35°C		96		
			Oxacillin, 5 μg, 24 h at 35°C		99		
			E-test: MH with 2% NaCl, 48 h at 35°C		99.6		
Baker and Tenover (2)	*S. aureus*, 54; CoNS, 22	Broth microdilution: CSMH with 2% NaCl, 24 h at 35°C	Alamar colorimetric broth microdilution: MH with saline diluent, 24 h at 35°C			97.9	63% of isolates were resistant by reference methods.
Weller et al. (22)	*S. aureus*, 44; CoNS, 120	*mecA* gene	Agar dilution:				
			Methicillin				
			COL with 5% NaCl, 48 h at 35°C	100	83		
			IS with 5% NaCl, 48 h at 35°C	67	77		
			E-test:				
			Methicillin				
			COL with 5% NaCl, 48 h at 35°C	100	81		
			IS with 5% NaCl, 48 h at 35°C	100	63		
			Oxacillin				
			COL with 5% NaCl, 48 h at 35°C	95	97		
			IS with 5% NaCl, 48 h at 35°C	90	17		

Continued on following page

TABLE 4—*Continued*

Author(s) (reference)	Organism, no. of isolates	Reference standard	Test methods[c]	% of isolates detected				% Overall agreement	Notes
				S. aureus		CoNS			
				Resistant	Susceptible	Resistant	Susceptible		
Jarløv et al. (11)	CoNS, 359	*mecA* gene/agar dilution: COL with 4.5% NaCl, 42 to 46 h at 35°C	Disk diffusion: 42 to 46 h at 35°C						Based on the best agreement shown for the *mecA* gene assay among four agar dilution assays for determining the MIC, a COL agar assay with a heavy inoculum (10^5 to 10^6 CFU (spot) was used as the reference MIC method. A total of 19 different methods using different concentrations of oxacillin, methicillin, and different media were used for disk diffusion, but only the methods with the best performance are shown. *S. epidermidis* and CoNS isolates were separately analyzed.
			Oxacillin, 1 μg						
			MH			97 (84)[d]			
			COL with 4.5% NaCl			96 (86)[d]			
			COL			97 (86)[d]			
			DBA			98 (93)[d]			
			Beef agar with 7.5% NaCl			96 (82)[d]			
			IS			99 (81)[d]			
			IS with heavy inoculum			81 (99)[d]			
			Oxacillin 5 μg/ml						
			DBA			96 (84)[d]			
			Beef agar with 7.5% NaCl			95 (68)[d]			
Frebourg et al. (5)	*S. aureus*, 64; CoNS, 76 (All isolates carried *mecA* gene)	*mecA* gene and oxacillin agar screening: MH with 4% NaCl, 6-μg/ml oxacillin, 48 h at 35°C	E-test: MH with 2% NaCl, 24 h at 35°C	95		67			The authors note that the performance of the E-test and Vitek methods may be enhanced if the NCCLS oxacillin breakpoint for resistance is changed.
			ATB Staph (bioMerieux): MH with 5% NaCl, 24 h at 35°C	89		58			
			Rapid ATB: MH with 5% NaCl, 24 h at 35°C	77					
			Vitek GPS-503 card	95		80			
Kampf et al. (12)	*S. aureus*, 136	*mecA* gene	Disk diffusion: MS, 24 h at 36°C	100					Evaluated only *S. aureus* isolates
			Agar spot screen: 2-μg/ml oxacillin, MS, 24 h at 36°C	98					

[a] Summary includes only recent reports for commercial (including automated) systems as many of early reports indicated inferior performance for these methods.
[b] Abbreviations: MH, Mueller-Hinton medium; MS, mannitol salt medium; CSMH, cation-supplemented MH medium; COL, Columbia medium; TS, tryptic soy medium; IS, isosensitest medium; DBA, Danish blood agar.
[c] All incubations performed in ambient air; results for oxacillin unless otherwise indicated; 6 μg of oxacillin/ml was used for agar screen methods unless otherwise stated; 1-μg oxacillin disks were used for disk diffusion unless otherwise stated.
[d] The first number refers to percentage for *S. epidermidis* isolates; the second number refers to percentage for all other CoNS isolates.
[e] The percentages varied for different sources of MH medium.

achieved. York and colleagues concluded that lowering the breakpoint for susceptibility to less than 2 μg/ml would increase the sensitivity of the broth microdilution method when CoNS isolates are tested (24). Mulder demonstrated that oxacillin resistance was best detected by the E-test when less than 2 μg of oxacillin/ml was used as the breakpoint (17). Frebourg and colleagues also noted that a decrease in the breakpoint for oxacillin susceptibility should improve results for staphylococcal testing for both the E-test and the Vitek method (5). Finally, Ramotar and colleagues recently reported that among 188 CoNS clinical isolates reported as susceptible by automated methods, 16 were positive for the *mecA* gene by PCR analysis (19). For two of these isolates MICs of oxacillin were equal to 1 μg/ml, and for four isolates the MICs were 0.5 μg/ml (19). The current study suggests that a breakpoint near 0.5 μg of oxacillin/ml is more reasonable for testing CoNS isolates whether agar dilution or agar screening plates are used. As previously mentioned, current NCCLS guidelines advise use of the agar screening plate for *S. aureus* isolates but not CoNS isolates. We believe that the agar plate can be useful for detecting oxacillin resistance for CoNS isolates but that a lower concentration of oxacillin should be used for testing CoNS isolates than that used for *S. aureus* isolates.

In conclusion, we recommend that the following parameters for the agar dilution or agar screening methods be used for testing of CoNS isolates: agar dilution by using MH medium supplemented with 4% NaCl with incubation at 35°C for 48 h, and agar swab screen by using MH medium supplemented with 4% NaCl (prepared in-house) with incubation at 35°C for 48 h. The breakpoint for susceptibility should be ≤0.5 μg/ml.

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