JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2004, p. 5041–5046 Vol. 42, No. 11
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.11.5041–5046.2004

Evaluation of the BD PHOENIX Automated Microbiology System for Detection of Methicillin Resistance in Coagulase-Negative Staphylococci

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Received 14 April 2004/Returned for modification 31 May 2004/Accepted 24 July 2004

The new BD PHOENIX automated microbiology system (Becton Dickinson Diagnostic Systems, Sparks, Md.) is designed for automated rapid antimicrobial susceptibility testing and identification of clinically relevant bacteria. In our study, the accuracy and speed of the BD PHOENIX oxacillin MIC determination for detecting methicillin resistance was evaluated for 200 clinical isolates of coagulase-negative staphylococci (CoNS). Compared to *mecA* PCR, the BD PHOENIX system detected methicillin resistance with a sensitivity of 99.2%. According to the actual NCCLS oxacillin MIC breakpoint of ≥0.5 μg/ml, the specificity was only 64.9%, attributable to false-positive results in 26 *mecA*-negative strains, including 16 non-*Staphylococcus epidermidis* strains. Alternative oxacillin breakpoints of ≥1, ≥2, and ≥4 μg/ml resulted in increased specificities of 83.8, 94.6, and 100% and high sensitivities of 99.2, 99.2, and 96.7%, respectively. Similarly, NCCLS broth microdilution oxacillin MICs exhibited a sensitivity of 100% but a low degree of specificity. However, the previous oxacillin MIC breakpoint of ≥4 μg/ml performed with a sensitivity of 98.4% and a specificity of 98.7%. BD PHOENIX oxacillin MIC results were available after 9 h for 40.5% of the examined CoNS strains and were completed after 17 h. Our results revealed the high reliability of the BD PHOENIX system as a phenotypic method for detection of resistance to oxacillin in *mecA*-positive CoNS. However, for the improvement of specificity, reevaluation of the optimal oxacillin MIC breakpoint for CoNS appears to be necessary.

Coagulase-negative staphylococci (CoNS) are major nosocomial pathogens ranking today among the five most frequently isolated organisms in nosocomial sepsis and infections related to foreign biomaterials, like intravascular and peritoneal dialysis catheters, cerebrospinal fluid shunts, prosthetic heart valves, and prosthetic joints, vascular grafts, cardiac pacemakers, and intraocular lenses (20, 26, 27, 35, 41, 43).

Frequent multiple antibiotic resistance, and especially the high prevalence of clinical CoNS exhibiting methicillin resistance, has turned treatment of CoNS infection into a therapeutic challenge (2, 10). Methicillin resistance is mediated by an additional low-affinity penicillin binding protein 2a (PBP2a) encoded by the *mecA* gene (4).

For critically ill patients, vancomycin is the empirical therapy until methicillin resistance is ruled out. However, antistaphylococcal penicillins are the drugs of choice because of unexcelled activity and low toxicity and in order to reduce the possibility of the emergence of vancomycin-resistant CoNS, *Staphylococcus aureus*, and enterococci (6, 7, 31, 36, 37, 45). Reliable and rapid detection of methicillin resistance is thus indispensable for such "curtailed" antibiotic therapy of patients with CoNS infections. Detection of *mecA* by PCR is very sensitive and is considered the reference method (33). Alternatively, detection of PBP2a as a marker of methicillin resistance has shown promising results with CoNS and is therefore also recommended (1, 15–17, 25, 33, 44, 48). In contrast, phenotypic detection of methicillin resistance in CoNS is difficult due to the heterogeneous expression of *mecA* (6, 30, 40, 47). Commonly used tests rely on modified culture conditions to enhance the expression of resistance and take up to 48 h for reliable results (30, 40, 47).

Rapid availability of antimicrobial susceptibility data is likely to result in curtailed antibiotic therapy (9, 42), and lower mortality rates have been reported (9). Some automated systems for susceptibility testing offer the advantage of rapid reporting and improved detection of unusual resistance phenotypes, which is likely to significantly improve intra- and interlaboratory reproducibility. Additionally, financial benefits may result from reduced laboratory costs, fewer diagnostic tests performed, and decreased length of stay for patients in an intensive care unit (3, 9, 12).

In our study, the accuracy and speed of the BD PHOENIX automated microbiology system (Becton Dickinson Diagnostic Systems, Sparks, Md.) for detecting methicillin resistance in CoNS were evaluated by comparing the results of *mecA* PCR and oxacillin MIC determination by broth microdilution to the results of the BD PHOENIX oxacillin MIC determination for 200 clinical CoNS isolates.

(Part of this work will appear in the doctoral thesis of M. A. Horstkotte, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.)

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MATERIALS AND METHODS

Bacterial isolates. The CoNS strains in the present study ($n = 200$) comprise 14 different species (139 *S. epidermidis* strains, 16 *S. haemolyticus* strains, 10 *S. hominis* strains, 9 *S. saprophyticus* strains, 6 *S. capitis* strains, 4 *S. lugdunensis* strains, 4 *S. warneri* strains, 4 *S. xylosus* strains, 2 *S. schleiferi* strains, 2 *S. cohnii* strains, 1 *S. chromogenes* strain, 1 *S. simulans* strain, 1 *S. arlettae* strain, and 1 *S. kloosii* strain). All of the strains belong to a collection of clinical CoNS isolated in 1997 and 1998 at the University Hospital Hamburg-Eppendorf. Species identification was performed by using Gram stain morphology, the catalase test, the clumping factor test, and the ID 32 Staph system (bioMérieux, Marcy l' Etoile, France). The strains were characterized for the presence of PBP2a using a commercially available latex agglutination test (Denka Seiken Co., Niigata, Japan) (15) and have been used for evaluation of the new automated VITEK2 system (14). The strains were kept at −80°C in microbank tubes (Pro Lab Diagnostics, Richmond Hill, Ontario, Canada). *S. aureus* ATCC 29213 (*mecA* negative) and *S. aureus* ATCC 43300 (*mecA* positive) were used for quality control.

PCR for *mecA*. PCRs were performed as described previously (15, 27).

BD PHOENIX antimicrobial susceptibility testing. Methicillin resistance was detected by oxacillin MIC determination (range, ≤0.25 to ≥8 μg/ml) included in the PMIC/ID-6 test panel for gram-positive bacteria (reference no. 448513; Becton Dickinson) of the BD PHOENIX system according to the manufacturer's instructions. Briefly, each isolate was subcultured twice on Columbia agar (Difco, Becton Dickinson) containing 5% sheep blood for 24 h at 37°C before being tested. The BD PHOENIX identification (ID) broth was inoculated with bacterial colonies of the respective isolate adjusted to a 0.5 McFarland standard using a CrystalSpec Nephelometer (Becton Dickinson Diagnostic Systems). An aliquot of 25 μl of the ID suspension was subsequently inoculated into the antimicrobial susceptibility test broth (Becton Dickinson), and 1 drop of the redox indicator resazurin was added. The self-inoculating combination panels were filled, sealed, and loaded into the BD PHOENIX system, where kinetic measurements of both colorimetric and fluorescent signals were collected every 20 min. The results for the BD PHOENIX oxacillin MIC determination were recorded after every hour up to 9 h for each strain. Strains for which the oxacillin MICs were 0.5 to 2 μg/ml or with divergent oxacillin MIC results compared to the PCR for *mecA* were retested.

Broth microdilution. Each isolate was subcultured twice on Columbia agar containing 5% sheep blood at 37°C before being tested. Oxacillin MIC results were determined according to the guidelines of the NCCLS (33) with an inoculum of 5×10^5 CFU/ml in cation-adjusted Mueller-Hinton broth (Difco) supplemented with 2% NaCl. The polystyrol microtiter plates with U-shaped wells (Greiner, Kremsmünster, Austria) were incubated at 35°C and read after 24 h.

RESULTS

mecA was detected in 124 of 200 (62%) CoNS strains, with 99 of 140 (70.7%) *mecA*-positive *S. epidermidis* strains and 25 of 60 (41.7%) *mecA*-positive non-*S. epidermidis* strains.

A BD PHOENIX oxacillin MIC of ≥8 μg/ml was reported for 115 of 123 *mecA*-positive strains. The BD PHOENIX oxacillin MICs for four *mecA*-positive strains were 4 μg/ml, whereas those for two *mecA*-positive *S. epidermidis* strains and one *mecA*-positive *S. saprophyticus* strain were 2 μg/ml. The oxacillin MIC of only one *mecA*-positive *S. epidermidis* strain was ≤0.25 μg/ml (Tables 1 and 2).

The BD PHOENIX system detected an oxacillin MIC of ≤0.25 μg/ml for 48 of 74 *mecA*-negative CoNS but oxacillin MICs of 0.5, 1, and 2 μg/ml, respectively, for 14, 8, and 4 *mecA*-negative strains. These higher oxacillin MICs were observed for 10 S. *epidermidis* strains, 6 *S. saprophyticus* strains, 4 *S. lugdunensis* strains, 1 *S. xylosus* strains, 1 *S. capitis* strain, 1 *S. haemolyticus* strain, 1 *S. arlettae* strain, 1 *S. kloosii* strains, and 1 *S. cohnii* strain (Tables 1, 2, 3, and 4).

The 29 strains for which there were BD PHOENIX oxacillin MICs ranging from 0.5 to 2 μg/ml and the 1 *mecA*-positive *S. epidermidis* strain for which there was a BD PHOENIX oxacillin MIC of ≤0.25 μg/ml were retested (Table 2). In the majority of strains, the oxacillin MIC results were within ±1 doubling dilution (Table 2).

TABLE 1. BD PHOENIX oxacillin MICs compared to *mecA* PCR on the basis of different oxacillin MIC breakpoints[a]

Oxacillin MIC breakpoint (μg/ml) and strain type	No. of CoNS strains classified as:		Sensitivity (%)	Specificity (%)
	Resistant	Susceptible		
≥0.5				
mecA-positive	122	1	99.2	64.9
mecA-negative	26	48		
≥1				
mecA-positive	122	1	99.2	83.8
mecA-negative	12	62		
≥2				
mecA-positive	122	1	99.2	94.6
mecA-negative	4	70		
≥4				
mecA-positive	119	4	96.7	100
mecA-negative	0	74		

[a] BD PHOENIX oxacillin MICs were not reported initially for 3 of 200 CoNS strains due to insufficient growth of the strains in the test panels. These three strains were not included this analysis.

For three strains, a *mecA*-negative *S. epidermidis* strain, a *mecA*-negative *S. cohnii* strain, and a *mecA*-positive *S. epidermidis* strain, no BD PHOENIX oxacillin MIC was reported initially due to insufficient growth, but on retesting, the BD PHOENIX oxacillin MICs for the *mecA*-negative and the *mecA*-positive *S. epidermidis* strains were 1 and 4 μg/ml, respectively. For the *mecA*-negative *S. cohnii* strain, no BD PHOENIX result was obtained on retesting.

According to the actual resistant NCCLS oxacillin MIC breakpoint of ≥0.5 μg/ml, the BD PHOENIX system detected methicillin resistance in CoNS with a sensitivity of 99.2% and a specificity of 64.9%. Interestingly, the previous NCCLS oxacillin MIC breakpoint of ≥4 μg/ml would have displayed an excellent specificity of 100% and a high sensitivity of 96.7%. Alternative oxacillin MIC breakpoints of ≥1 and ≥2 μg/ml both had a sensitivity of 99.2% with specificities of 83.8 and 94.6%, respectively (Table 2).

Broth microdilution testing among the 124 *mecA*-positive CoNS strains revealed oxacillin MICs of ≥8 μg/ml for 114 strains, 4 μg/ml for 8 strains, and 2 and 0.5 μg/ml, respectively, for one *S. epidermidis* and one *S. saprophyticus* strain (Tables 2, 3, and 4). This resulted in a sensitivity of 100% for the actual resistant NCCLS oxacillin MIC breakpoint of ≥0.5 μg/ml. However, the specificity reached only 13%, with an oxacillin MIC of ≤0.25 μg/ml for 10 of 76 *mecA*-negative strains, whereas the oxacillin MICs for 27, 25, and 13 strains were 0.5, 1, and 2 μg/ml, respectively. The broth microdilution oxacillin MIC for only one *mecA*-negative *S. kloosii* strain was 4 μg/ml (Tables 3 and 4). In contrast, the previous NCCLS oxacillin MIC breakpoint of ≥4 μg/ml performed with an improved specificity of 98.7% and a sensitivity of 98.4%. Alternative oxacillin MIC breakpoints of ≥1 or ≥2 μg/ml displayed a sensitivity of 99.2% and specificities of 48.7 and 81.6%, respectively.

Comparison of BD PHOENIX oxacillin MICs and broth microdilution oxacillin MICs revealed that the MICs were

TABLE 2. CoNS strains for which BD PHOENIX oxacillin MIC is 0.5 to 2 μg/ml or is discrepant compared to *mecA* PCR

Strain no.	Species	Oxacillin MIC (μg/ml)			*mecA* PCR
		BD PHOENIX		Broth microdilution	
		Initially	On repeat testing		
2	*S. epidermidis*	0.5	≤0.25	0.25	Negative
12	*S. epidermidis*	0.5	≤0.25	0.5	Negative
15	*S. epidermidis*	0.5	No growth	2	Negative
174	*S. epidermidis*	0.5	≤0.25	0.5	Negative
199	*S. haemolyticus*	0.5	0.5	0.125	Negative
64	*S. capitis*	0.5	0.5	2	Negative
8	*S. lugdunensis*	0.5	0.5	2	Negative
89	*S. lugdunensis*	0.5	≤0.25	0.5	Negative
175	*S. lugdunensis*	0.5	0.5	0.5	Negative
7	*S. saprophyticus*	0.5	0.5	1	Negative
9	*S. saprophyticus*	0.5	0.5	1	Negative
48	*S. saprophyticus*	0.5	0.5	1	Negative
177	*S. saprophyticus*	0.5	0.5	1	Negative
95	*S. xylosus*	0.5	0.5	1	Negative
1	*S. epidermidis*	1	1	0.25	Negative
55	*S. epidermidis*	1	≤0.25	0.25	Negative
207	*S. epidermidis*	1	0.5	1	Negative
173	*S. arlettae*	1	1	2	Negative
90	*S. kloosii*	1	1	4	Negative
16	*S. lugdunensis*	1	0.5	2	Negative
3	*S. saprophyticus*	1	1	2	Negative
4	*S. saprophyticus*	1	1	0.5	Negative
50	*S. epidermidis*	2	0.5	1	Negative
52	*S. epidermidis*	2	≤0.25	0.5	Negative
99	*S. epidermidis*	2	≤0.25	0.5	Negative
60	*S. cohnii*	2	2	2	Negative
102	*S. epidermidis*	≤0.25	≤0.25	8	Positive
73	*S. epidermidis*	2	≥8	4	Positive
204	*S. epidermidis*	2	1	2	Positive
96	*S. saprophyticus*	2	≥8	0.5	Positive

within ±1 doubling dilution (essential agreement) for the majority of the examined strains (Table 3 and 4).

Analysis of the time to results of the BD PHOENIX system revealed that oxacillin MIC results were available after 8 and 9 h in 18.5 and 40.5% of the strains, respectively (Table 5). After 6 or 7 h, *mecA*-positive strains predominated, but after 8 h, the proportions of *mecA*-positive CoNS detected as resistant (19.4%) and *mecA*-negative CoNS reported as susceptible (17.1%) were about equal (Table 5). However, after 9 h, *mecA*-negative CoNS reported as susceptible outweighed *mecA*-positive CoNS reported as resistant, with 47.4 versus 36.3% (Table 5). BD PHOENIX oxacillin MIC testing was completed after 17 h (Table 5).

DISCUSSION

The BD PHOENIX system provides automated antimicrobial susceptibility testing and identification of clinically relevant bacteria and has been evaluated for the identification of *Burkholderia cepacia* complex isolates and the detection of

TABLE 3. One-to-one comparison of NCCLS broth microdilution oxacillin MIC results with BD PHOENIX oxacillin MIC results in *mecA*-positive CoNS strains[a]

BD PHOENIX oxacillin MIC (μg/ml)	No. of strains at NCCLS broth microdilution oxacillin MIC (μg/ml) of:					
	≤0.25	0.5	1	2	4	≥8
≥8					6	109
4					1	3
2		1		1	1	
1						
0.5						
≤0.25						1

[a] BD PHOENIX oxacillin MICs were not reported initially for 3 of 200 CoNS strains due to insufficient growth of the strains in the test panels. These three strains were not included this analysis.

TABLE 4. One-to-one comparison of NCCLS broth microdilution oxacillin MIC results with BD PHOENIX oxacillin MIC results in *mecA*-negative CoNS strains[a]

BD PHOENIX oxacillin MIC (μg/ml)	No. of strains at NCCLS broth microdilution oxacillin MIC (μg/ml) of:					
	≤0.25	0.5	1	2	4	≥8
≥8						
4						
2		2	1	1		
1	2	1	1	3	1	
0.5	2	4	5	3		
≤0.25	6	19	17	6		

[a] BD PHOENIX oxacillin MICs were not reported initially for 3 of 200 CoNS strains due to insufficient growth of the strains in the test panels. These three strains were not included this analysis.

TABLE 5. Time course of BD PHOENIX oxacillin MIC results in CoNS[a]

CoNS	No. (%) of strains with reported oxacillin MIC results at (h):					
	5	6	7	8	9	17
All	1 (0.5)	8 (4)	21 (10.5)	37 (18.5)	81 (40.5)	197 (98.5)
mecA positive	1 (0.8)	8 (6.5)	18 (14.5)	24 (19.4)	45 (36.3)	123 (99.2)
mecA negative	0 (0)	0 (0)	3 (3.9)	13 (17.1)	36 (47.4)	74 (97.4)

[a] BD PHOENIX oxacillin MICs were not reported initially for 3 of 200 CoNS strains due to insufficient growth of the strains in the test panels. These three strains were not included this analysis.

extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* species (5, 22, 38). Recently, the BD PHOENIX system has been tested with a variety of gram-positive bacteria. There was good agreement of oxacillin MIC results for 161 CoNS strains between the BD PHOENIX system and broth microdilution as the reference method (11). However, detailed information about discrepant strains was lacking and *mecA* PCR was performed only for strains already categorized as resistant by the oxacillin MIC, rendering exact determination of sensitivity and specificity difficult. The time until detection of methicillin resistance was not addressed.

In our study, the new BD PHOENIX system proved to be a highly reliable phenotypic method for detecting *mecA*-positive CoNS strains as resistant to oxacillin. Based on the current NCCLS oxacillin MIC breakpoint of ≥0.5 μg/ml, the BD PHOENIX oxacillin MIC exhibited an excellent sensitivity of 99.2% but a rather low specificity of 64.9% (Table 1). These findings were supported by the broth microdilution results, which exhibited an excellent sensitivity of 100% but a specificity of 13%. However, evaluation of the two oxacillin MIC determination methods with higher breakpoints would have shown a favorable performance.

In 1999, the NCCLS lowered the oxacillin MIC breakpoint for CoNS from ≥4 to ≥0.5 μg/ml (33, 40). Subsequently, several investigators found high sensitivity of the new breakpoint for *S. epidermidis*, *S. hominis*, and *S. haemolyticus* but described reduced specificity for *S. saprophyticus*, *S. cohnii*, *S. xylosus*, *S. warneri*, and *S. lugdunensis* strains (13, 19, 25, 34, 40). Concordantly, in our study, six *S. saprophyticus* strains, four *S. lugdunensis* strains, one *S. xylosus* strain, one *S. cohnii* strain, one *S. capitis* strain, one *S. arlettae* strain, and one *S. kloosii* strain were *mecA* negative, but the BD PHOENIX oxacillin MICs for the strains ranged from 0.5 to 2 μg/ml. The classification of these 15 isolates was confirmed by broth microdilution results (Table 2).

Similarly, decreased specificity of the actual breakpoint was described for the Vitek automated system (bioMérieux), predominantly due to *mecA*-negative *S. saprophyticus* and *S. lugdunensis* and to a minor extent to *S. cohnii*, *S. sciuri*, and *S. capitis* strains (18, 25, 29, 34, 46). The new VITEK 2 system (bioMérieux) showed high sensitivity (14, 21, 23, 24), and concordance between *mecA* PCR and VITEK 2 oxacillin MICs was observed for almost all *S. epidermidis*, *S. haemolyticus*, and *S. hominis* strains (14, 23). However, the specificities varied considerably, between 97 and 80%, due to false-positive results, especially for *mecA*-negative *S. saprophyticus*, *S. cohnii*, and *S. lugdunensis* strains (14, 23).

It is notable that in our study BD PHOENIX oxacillin MICs of 0.5, 1, and 2 μg/ml were reported for four, three, and three *mecA*-negative *S. epidermidis* strains, respectively, as well as for one *mecA*-negative *S. haemolyticus* strain (Table 2). Broth microdilution would have classified 4 of these 11 isolates as susceptible (Table 2). Essential agreement between the oxacillin MICs in the two methods was recorded for four of the remaining seven strains, which all had to be classified as resistant (Table 2). The 10 (7.2%) *mecA*-negative *S. epidermidis* strains for which the oxacillin MICs were ≥0.5 μg/ml, out of a total of 139 *S. epidermidis* strains, represent a proportion similar to that in another study, which found 2 of 37 (5.4%) *S. epidermidis* strains for which the oxacillin MICs were ≥0.5 μg/ml (30).

For epidemiological reasons, study populations vary in their species compositions and strains differ in their phenotypic expression of methicillin resistance. Consequently, a range of oxacillin MIC breakpoints for resistance have been proposed. Until 1999, the NCCLS recommended an oxacillin MIC breakpoint for resistance in all staphylococci of ≥4 μg/ml, but ≥2 (47) or ≥1 (8, 30) μg/ml was advocated as more appropriate for CoNS. An even lower oxacillin MIC breakpoint of ≥0.5 μg/ml, suggested by two other investigators (28, 40), was adopted for CoNS by the NCCLS in 1999.

The inoculum size is crucial for oxacillin MIC determination. Despite adherence to the NCCLS protocol, the inoculum was clearly below the target concentration of 5×10^5 CFU/ml for all CoNS species except *S. lugdunensis* in a large multicenter study (33, 40). Interestingly, the recorded mean numbers of CFU per milliliter were lower for *S. epidermidis*, *S. hominis*, and *S. haemolyticus* than for *S. saprophyticus*, *S. capitis*, and *S. lugdunensis* (40), which correlates with the higher oxacillin MICs described in several studies for the last three species despite their being *mecA* negative.

Subtle changes in the test conditions, e.g., the source of the Mueller-Hinton medium, have been reported to affect oxacillin MIC results for CoNS (40). The BD PHOENIX antimicrobial susceptibility test broth contains 0.01% Tween 80, which may explain some discrepancies in oxacillin MIC results.

Mechanisms of oxacillin resistance independent of *mecA* may be responsible for oxacillin MIC results of 0.5 to 2 μg/ml for *mecA*-negative strains. Alterations in PBPs other than PBP2a have been described in *S. haemolyticus* and *S. saprophyticus* (39) and have been suggested as one explanation for this phenomenon (40). Alternatively, *S. saprophyticus* and *S. lugdunensis*, and probably certain other CoNS species, might inherently display a higher oxacillin MIC despite lacking *mecA*.

To improve the specific detection of methicillin resistance in CoNS, exclusion of *S. saprophyticus* and *S. lugdunensis* strains was recommended (25), and this proposal was adopted in part by the NCCLS, which no longer recommends routine oxacillin susceptibility testing of *S. saprophyticus* isolated from urine (33).

However, this issue cannot be fully resolved by the exclusion of *S. saprophyticus* and *S. lugdunensis* strains or by the recommendation of the NCCLS to additionally analyze CoNS strains other than *S. epidermidis* which cause severe infections for the presence of either PBP2a or *mecA* and to consider strains lacking these markers as susceptible. PCR for *mecA* is both laborious and costly, whereas false-positive PBP2a latex agglutination tests have been observed for *mecA*-negative *S. warneri*, *S. lugdunensis*, *S. simulans*, and *S. hominis* strains but not *S.*

epidermidis strains (1, 15, 16, 44, 48). The question of whether penicillinase-resistant penicillins can eradicate severe infections due to *mecA*-negative CoNS for which the oxacillin MICs are 0.5 to 2 μg/ml is unresolved. Clinical trials with strains of *S. lugdunensis*, which is now regarded as an important pathogen in native valve endocarditis (32), or *S. saprophyticus* causing complicated urinary tract infection are needed.

In our study, all CoNS strains for which the BD PHOENIX oxacillin MICs were 0.5 or 1 μg/ml did not harbor *mecA*, in contrast to three out of seven strains for which the oxacillin MIC was 2 μg/ml. In another study, *mecA* PCR results and the presence of PBP2a have been found to be identical in these CoNS strains (15). We conclude that CoNS strains for which the critical BD PHOENIX oxacillin MIC is 2 μg/ml should be examined for the presence of PBP2a or *mecA*. Further investigations are warranted to clarify whether differential, species-specific oxacillin MIC breakpoints of ≥2 μg/ml for *S. saprophyticus* and *S. lugdunensis* (19, 40) and ≥0.5 μg/ml for *S. epidermidis* and other CoNS species are more appropriate.

An optimized antibiotic regimen can result in lower mortality (9, 42). Therefore, antimicrobial test results should be accessible on the day of testing. Evaluation of the speed of the BD PHOENIX system for providing oxacillin MIC results revealed at least equivalence to those of other phenotypic methods. After 9 h, results for ~41% of the CoNS strains were reported, and oxacillin MIC testing was completed after 17 h (Table 5).

ACKNOWLEDGMENTS

We thank Rainer Laufs for his continuous support. Becton Dickinson (Heidelberg, Germany) kindly provided the BD PHOENIX automated microbiology system.

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft given to D.M.

REFERENCES

1. **Andrews, J. M., F. J. Boswell, and R. Wise.** 2000. Establishing MIC breakpoints for coagulase-negative staphylococci to oxacillin. J. Antimicrob. Chemother. **45:**259–261.
2. **Archer, G. L., and M. W. Climo.** 1994. Antimicrobial susceptibility of coagulase-negative staphylococci. Antimicrob. Agents Chemother. **38:**2231–2237.
3. **Barenfanger, J., C. Drake, and G. Kacich.** 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J. Clin. Microbiol. **37:**1415–1418.
4. **Berger-Bächi, B.** 1997. Resistance not mediated by β-lactamase (methicillin resistance), p. 158–174. *In* K. B. Crossley and G. L. Archer (ed.), The staphylococci in human disease. Churchill Livingston, New York, N.Y.
5. **Brisse, S., S. Stefani, J. Verhoef, A. van Belkum, P. Vandamme, and W. Goessens.** 2002. Comparative evaluation of the BD PHOENIX and VITEK 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. J. Clin. Microbiol. **40:**1743–1748.
6. **Chambers, H. F.** 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. **10:**781–791.
7. **Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, and S. K. Fridkin.** 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N. Engl. J. Med. **348:**1342–1347.
8. **Cormican, M. G., W. W. Wilke, M. S. Barrett, M. A. Pfaller, and R. N. Jones.** 1996. Phenotypic detection of *mecA*-positive staphylococcal blood stream isolates: high accuracy of simple disk diffusion tests. Diagn. Microbiol. Infect. Dis. **25:**107–112.
9. **Doern, G. V., R. Vautour, M. Gaudet, and B. Levy.** 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J. Clin. Microbiol. **32:**1757–1762.
10. **Edmond, M. B., S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, and R. P. Wenzel.** 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. **29:**239–244.
11. **Fahr, A. M., U. Eigner, M. Armbrust, A. Caganic, G. Dettori, C. Chezzi, L. Bertoncini, M. Benecchi, and M. G. Menozzi.** 2003. Two-center collaborative evaluation of the performance of the BD PHOENIX automated microbiology system for identification and antimicrobial susceptibility testing of *Enterococcus* spp. and *Staphylococcus* spp. J. Clin. Microbiol. **41:**1135–1142.
12. **Ferraro, M. J., and J. H. Jorgensen.** 1999. Susceptibility testing instrumentation and computerized expert systems for data analysis and interpretation, p. 1593–1600. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
13. **Gradelski, E., L. Valera, L. Aleksunes, D. Bonner, and J. Fung-Tomc.** 2001. Correlation between genotype and phenotypic categorization of staphylococci based on methicillin susceptibility and resistance. J. Clin. Microbiol. **39:**2961–2963.
14. **Horstkotte, M. A., J. K. M. Knobloch, H. Rohde, S. Dobinsky, and D. Mack.** 2002. Rapid detection of methicillin resistance in coagulase-negative staphylococci with the VITEK 2 system. J. Clin. Microbiol. **40:**3291–3295.
15. **Horstkotte, M. A., J. K. M. Knobloch, H. Rohde, and D. Mack.** 2001. Rapid detection of methicillin resistance in coagulase-negative staphylococci by a penicillin binding protein 2a-specific latex agglutination test. J. Clin. Microbiol. **39:**3700–3702.
16. **Hussain, Z., L. Stoakes, S. Garrow, S. Longo, V. Fitzgerald, and R. Lannigan.** 2000. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. J. Clin. Microbiol. **38:**2051–2054.
17. **Hussain, Z., L. Stoakes, M. A. John, S. Garrow, and V. Fitzgerald.** 2002. Detection of methicillin resistance in primary blood culture isolates of coagulase-negative staphylococci by PCR, slide agglutination, disk diffusion, and a commercial method. J. Clin. Microbiol. **40:**2251–2253.
18. **Hussain, Z., L. Stoakes, R. Lannigan, S. Longo, and B. Nancekivell.** 1998. Evaluation of screening and commercial methods for detection of methicillin resistance in coagulase-negative staphylococci. J. Clin. Microbiol. **36:**273–274.
19. **Hussain, Z., L. Stoakes, V. Massey, D. Diagre, V. Fitzgerald, S. El Sayed, and R. Lannigan.** 2000. Correlation of oxacillin MIC with *mecA* gene carriage in coagulase-negative staphylococci. J. Clin. Microbiol. **38:**752–754.
20. **Kloos, W. E.** 1997. Taxonomy and systematics of staphylococci indigenous to humans, p. 113–137. *In* K. B. Crossley and G. L. Archer (ed.), The staphylococci in human disease. Churchill Livingston, New York, N.Y.
21. **Leclercq, R., M. H. Nicolas-Chanoine, P. Nordmann, A. Philippon, P. Marchais, A. Buu-Ho, H. Chardon, H. Dabernat, F. Doucet-Populaire, C. Grasmick, P. Legrand, C. Muller-Serieys, J. Nguyen, M. C. Ploy, M. E. Reverdy, M. Weber, and R. J. Courcol.** 2001. Multicenter evaluation of an automated system using selected bacteria that harbor challenging and clinically relevant mechanisms of resistance to antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. **20:**626–635.
22. **Leverstein-van Hall, M. A., A. C. Fluit, A. Paauw, A. T. Box, S. Brisse, and J. Verhoef.** 2002. Evaluation of the Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 automated instruments for detection of extended-spectrum beta-lactamases in multiresistant *Escherichia coli* and *Klebsiella* spp. J. Clin. Microbiol. **40:**3703–3711.
23. **Ligozzi, M., C. Bernini, M. G. Bonora, M. De Fatima, J. Zuliani, and R. Fontana.** 2002. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clin. Microbiol. **40:**1681–1686.
24. **Livermore, D. M., M. Struelens, J. Amorim, F. Baquero, J. Bille, R. Canton, S. Henning, S. Gatermann, A. Marchese, H. Mittermayer, C. Nonhoff, K. J. Oakton, F. Praplan, H. Ramos, G. C. Schito, J. Van Eldere, J. Verhaegen, J. Verhoef, and M. R. Visser.** 2002. Multicenter evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests. J. Antimicrob. Chemother. **49:**289–300.
25. **Louie, L., A. Majury, J. Goodfellow, M. Louie, and A. E. Simor.** 2001. Evaluation of a latex agglutination test (MRSA-Screen) for detection of oxacillin resistance in coagulase-negative staphylococci. J. Clin. Microbiol. **39:**4149–4151.
26. **Mack, D.** 1999. Molecular mechanisms of *Staphylococcus epidermidis* biofilm formation. J. Hosp. Infect. **43**(Suppl.)**:**S113–S125.
27. **Mack, D., K. Bartscht, S. Dobinsky, M. A. Horstkotte, K. Kiel, J. K. M. Knobloch, and P. Schäfer.** 2000. Staphylococcal factors involved in adhesion and biofilm formation on biomaterials, p. 307–330. *In* Y. H. An and R. J. Friedman (ed.), Handbook for studying bacterial adhesion: principles, methods, and applications. Humana Press, Totowa, N.J.
28. **Marshall, S. A., W. W. Wilke, M. A. Pfaller, and R. N. Jones.** 1998. *Staphylococcus aureus* and coagulase-negative staphylococci from blood stream infections: frequency of occurrence, antimicrobial susceptibility, and molecular (*mecA*) characterization of oxacillin resistance in the SCOPE program. Diagn. Microbiol. Infect. Dis. **30:**205–214.
29. **Martinez, F., L. J. Chandler, B. S. Reisner, and G. L. Woods.** 2001. Evaluation of the Vitek card GPS105 and VTK-RO7.01 software for detection of oxacillin resistance in clinically relevant coagulase-negative staphylococci. J. Clin. Microbiol. **39:**3733–3735.
30. **McDonald, C. L., W. E. Maher, and R. J. Fass.** 1995. Revised interpretation of oxacillin MICs for *Staphylococcus epidermidis* based on *mecA* detection. Antimicrob. Agents Chemother. **39:**982–984.

31. **Murray, B. E.** 2000. Vancomycin-resistant enterococcal infections. N. Engl. J. Med. **342:**710–721.
32. **Mylonakis, E., and S. B. Calderwood.** 2001. Infective endocarditis in adults. N. Engl. J. Med. **345:**1318–1330.
33. **National Committee for Clinical Laboratory Standards.** 2003. Performance standards for antimicrobial susceptibility testing; 13th informational supplement. M100-S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.
34. **Ramotar, K., W. Woods, and B. Toye.** 2001. Oxacillin susceptibility testing of *Staphylococcus saprophyticus* using disk diffusion, agar dilution, broth microdilution, and the Vitek GPS-105 card. Diagn. Microbiol. Infect. Dis. **40:**203–205.
35. **Rupp, M. E., and G. L. Archer.** 1994. Coagulase-negative staphylococci: pathogens associated with medical progress. Clin. Infect. Dis. **19:**231–243.
36. **Small, P. M., and H. F. Chambers.** 1990. Vancomycin for *Staphylococcus aureus* endocarditis in intravenous drug users. Antimicrob. Agents Chemother. **34:**1227–1231.
37. **Smith, T. L., M. L. Pearson, K. R. Wilcox, C. Cruz, M. V. Lancaster, B. Robinson-Dunn, F. C. Tenover, M. J. Zervos, J. D. Band, E. White, W. R. Jarvis, et al.** 1999. Emergence of vancomycin resistance in *Staphylococcus aureus*. N. Engl. J. Med. **340:**493–501.
38. **Stürenburg, E., I. Sobottka, H. H. Feucht, D. Mack, and R. Laufs.** 2003. Comparison of BDPhoenix and VITEK2 automated antimicrobial susceptibility test systems for extended-spectrum beta-lactamase detection in *Escherichia coli* and *Klebsiella* species clinical isolates. Diagn. Microbiol. Infect. Dis. **45:**29–34.
39. **Suzuki, E., K. Hiramatsu, and T. Yokota.** 1992. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. Antimicrob. Agents Chemother. **36:**429–434.
40. **Tenover, F. C., R. N. Jones, J. M. Swenson, B. Zimmer, S. McAllister, and J. H. Jorgensen.** 1999. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J. Clin. Microbiol. **37:**4051–4058.
41. **Thylefors, J. D., S. Harbarth, and D. Pittet.** 1998. Increasing bacteremia due to coagulase-negative staphylococci: fiction or reality? Infect. Control Hosp. Epidemiol. **19:**581–589.
42. **Trenholme, G. M., R. L. Kaplan, P. H. Karakusis, T. Stine, J. Fuhrer, W. Landau, and S. Levin.** 1989. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. J. Clin. Microbiol. **27:**1342–1345.
43. **U.S. Department of Health and Human Services.** 2000. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992–April 2000, issued June 2000. Am. J. Infect. Control **28:**429–448.
44. **Udo, E. E., E. M. Mokadas, A. Al Haddad, B. Mathew, L. E. Jacob, and S. C. Sanyal.** 2000. Rapid detection of methicillin resistance in staphylococci using a slide latex agglutination kit. Int. J. Antimicrob. Agents **15:**19–24.
45. **Wilson, W. R., A. W. Karchmer, A. S. Dajani, K. A. Taubert, A. Bayer, D. Kaye, A. L. Bisno, P. Ferrieri, S. T. Shulman, D. T. Durack, et al.** 1995. Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylococci, and HACEK microorganisms. JAMA **274:**1706–1713.
46. **Yamazumi, T., I. Furuta, D. J. Diekema, M. A. Pfaller, and R. N. Jones.** 2001. Comparison of the Vitek gram-positive susceptibility 106 card, the MRSA-Screen latex agglutination test, and *mecA* analysis for detecting oxacillin resistance in a geographically diverse collection of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol. **39:**3633–3636.
47. **York, M. K., L. Gibbs, F. Chehab, and G. F. Brooks.** 1996. Comparison of PCR detection of *mecA* with standard susceptibility testing methods to determine methicillin resistance in coagulase-negative staphylococci. J. Clin. Microbiol. **34:**249–253.
48. **Zbinden, R., M. Ritzler, E. Ritzler, and B. Berger-Bächi.** 2001. Detection of penicillin-binding protein 2a by rapid slide latex agglutination test in coagulase-negative staphylococci. J. Clin. Microbiol. **39:**412.