Journal of Medical Microbiology (2004), **53**, 1195–1199 DOI 10.1099/jmm.0.45697-0

Short Communication

Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS)

Juliana Caierão,[1] Maiara Musskopf,[1] Silvana Superti,[2] Eliane Roesch,[3] Cícero G. Dias[1,2] and Pedro A. d'Azevedo[1]

Correspondence
Pedro A. d'Azevedo
pedroaze@fffcmpa.tche.br

[1]Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Rua Sarmento Leite 245/211, Porto Alegre, RS 90050-170, Brazil

[2]Hospital Mãe de Deus, Porto Alegre, Brazil

[3]Laboratório Central de Análises Clínicas da Irmandade Santa Casa de Misericórdia de Porto Alegre, RS, Brazil

Received 9 April 2004
Accepted 5 August 2004

Coagulase-negative staphylococci (CNS) are the major cause of nosocomial infections. Methicillin-resistant strains are particularly important because they narrow therapeutic options. Detecting methicillin resistance among CNS has been a challenge for years. The objective of this study was to determine the accuracy of an agar screening test (0·6 and 4 μg oxacillin ml^{-1}), disc diffusion and the automated MicroScan system to characterize methicillin resistance among CNS. One hundred and seventy five strains were analysed: 41·1 % *Staphylococcus epidermidis* and 59·9 % other species; 69·1 % were *mecA*-positive. The results showed that the methods have optimal correlation with the detection of *mecA* gene for *S. epidermidis*, *Staphylococcus hominis* and *Staphylococcus haemolyticus*. However, accuracy of the tests is impaired when less common species are analysed. The only 100 % accurate test was agar screening with 4 μg oxacillin ml^{-1}.

Introduction

Coagulase-negative staphylococci (CNS) have been the major cause of nosocomial infections (Marshall *et al.*, 1998; Yamazumi *et al.*, 2001). Methicillin resistance among CNS is particularly important due to cross-resistance to virtually all β-lactam agents and other antimicrobial classes. As a result, therapeutic approaches are restricted to glycopeptide and new antimicrobial agents as linezolid (Woods *et al.*, 2002). Therefore, an accurate analysis of methicillin resistance may allow the provision of better antimicrobial therapy and avoid the selection of vancomycin-resistant strains.

The rates of methicillin resistance have increased in the last two decades, according to the National Nosocomial Infectious Surveillance System (NNIS, 2001). In Brazil, a multicentre study showed that 87·7 % of CNS isolated from blood cultures were resistant to methicillin (Sader *et al.*, 1999). The most common mechanism of methicillin resistance is the production of a penicillin-binding protein (PBP) with low affinity for β-lactams, PBP2a. PBP2a is coded by the *mecA* gene and can be detected by PCR, which is considered the gold-standard test to identify methicillin resistance (Chambers, 1997; Swenson *et al.*, 2001).

Abbreviations: CNS, coagulase-negative staphylococci; PBP, penicillin-binding protein.

The detection of methicillin resistance among CNS isolates has been a challenge for years (Gradelski *et al.*, 2001) because these micro-organisms present an hetero-resistant pattern (Chambers, 1997). Although recent breakpoints (NCCLS, 1999) have shown an acceptable correlation with the presence or absence of the *mecA* gene in *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus haemolyticus* isolates, species with borderline MICs (Hussain *et al.*, 2000; Gradelski *et al.*, 2001; Ferreira *et al.*, 2003) and less common species impair test accuracy. Thus, the aim of this study was to verify the accuracy of the agar screening test (0·6 and 4 mg oxacillin ml^{-1}), disc diffusion and the automated MicroScan system in the detection of methicillin resistance among CNS isolates.

Methods

This study included 175 consecutive CNS strains collected from hospitalized patients in southern Brazil. The samples were identified through MicroScan, panel Positive Combo 13 (Dade Behring). Methicillin resistance was characterized through an agar screening test, disc diffusion (NCCLS, 2003) and automated MicroScan system. For the agar screening test, strains were plated on tryptic soy agar with 5 % sheep blood and a 0·5 McFarland standard suspension was prepared for each sample. Mueller–Hinton agar plates (Oxoid) supplemented with 4 % (w/v) NaCl were used and two oxacillin concentrations were tested: 0·6 μg ml^{-1} (Kohner *et al.*, 1999) and 4 μg ml^{-1} (Ferreira *et al.*, 2003).

The plates were inoculated using Steer's replicator, incubated at 35 °C and screened after 24 and 48 h incubation. The growth of more than one colony on the plates indicated oxacillin-resistant strains.

Disc-diffusion test was performed according to NCCLS (2003) guidelines. Methicillin susceptibility testing with MicroScan was performed according to the manufacturers' instructions. The NCCLS (2003) breakpoints and MICs for methicillin susceptibility were used to analyse resistance through disc diffusion and MicroScan.

The results were compared to PCR for the detection of the *mecA* gene (Santos *et al.*, 1999). *Staphylococcus aureus* ATCC 33591 (methicillin-resistant) and *S. aureus* ATCC 25923 (methicillin-susceptible) were included as control organisms.

Results and Discussion

Table 1 shows the CNS species distribution and the methicillin susceptibility profile, considering the presence or absence of *mecA* gene. According to MicroScan identification 72 (41·1 %) were *S. epidermidis*, 36 (20·6 %) *S. hominis* subspecies *hominis*, 6 (3·4 %) *S. hominis* subspecies *novobiosepticus*, 41 (23·4 %) *S. haemolyticus*, 5 (2·9 %) *Staphylococcus warneri*, 5 (2·9 %) *Staphylococcus simulans*, 3 (1·7 %) *Staphylococcus capitis* subspecies *urealyticus*, 2 (1·1 %) *Staphylococcus auricularis*, 2 (1·1 %) *Staphylococcus saprophyticus*, 1 (0·6 %) *Staphylococcus sciuri*, 1 (0·6 %) *Staphylococcus cohnii* subspecies *cohnii* and 1 micro-organism not identified by MicroScan. The presence of the *mecA* gene was detected in 69·1 % of strains (121/175).

The results of phenotypic methods are shown in Table 2. The disc-diffusion test showed that 112 out of 121 strains were *mecA*-positive (92·5 % sensitive). On the other hand, 49 out of 54 *mecA*-negative strains were susceptible to methicillin (90·7 % specific) by disc-diffusion test.

The agar screening test containing 0·6 μg oxacillin ml^{-1} showed that 119 out of 121 *mecA*-positive strains were resistant (98·3 % sensitive). Only one *S. saprophyticus* *mecA*-negative strain was methicillin-resistant (98·1 % specific). The agar screening test containing 4 μg oxacillin ml^{-1} was totally accurate.

MicroScan characterized 120 out of 121 strains as methicillin-resistant. The system classified 46 out of 54 *mecA*-negative strains as susceptible (85·1 % specific). All strains but one *S. epidermidis*, described as false-positive, presented borderline MICs (Table 3).

As a result of the difficulty of characterizing methicillin resistance among CNS, phenotypic methods have been constantly studied. Except for the agar screening test containing 4 μg oxacillin ml^{-1}, all the other methods misclassified resistant and susceptible strains. The false-negative results may have occurred due to an extremely heterogeneous expression of resistance (Gerberding *et al.*, 1991; Chambers, 1997).

On the other hand, the false-positive results may have been the result of overproduction of penicillinase, or super-expression/alterations of constitutive PBPs. It is known that penicillinase-resistant penicillins may show some degree of lysis when such enzymes are present. The super-expression and alterations of constitutive PBPs generate a higher concentration of free transpeptidase which will synthesize the bacteria's cell wall (Chambers, 1997).

Even though researchers have shown that an agar screening test containing 6 μg oxacillin ml^{-1} is not efficient towards CNS (Tenover *et al.*, 1999), and it is no longer recommended for CNS (NCCLS, 1999), comparative studies to assess the

Table 1. Identification of CNS isolates and the methicillin susceptibility profile based on the presence or absence of *mecA* gene

Species	% (no./total)	*mecA*	
		Positive (%)	Negative (%)
S. epidermidis	41·1 (72/175)	51 (70·8)	21 (29·2)
S. hominis subsp. *hominis*	20·6 (36/175)	18 (50·0)	18 (50·0)
S. hominis subsp. *nov.*	3·4 (6/175)	6 (100)	–
S. haemolyticus	23·4 (41/175)	37 (90·2)	4 (9·8)
S. warneri	2·9 (5/175)	1 (20·0)	4 (80·0)
S. simulans	2·9 (5/175)	3 (60·0)	2 (40·0)
S. capitis subsp. *urealyticus*	1·7 (3/175)	1 (33·3)	2 (66·6)
S. auricularis	1·1 (2/175)	2 (100)	–
S. saprophyticus	1·1 (2/175)	–	2 (100)
S. sciuri	0·6 (1/175)	1 (33·3)	–
S. cohnii subsp. *cohnii*	0·6 (1/175)	–	1 (100)
Unidentified organism	0·6 (1/175)	1 (100)	–
Total	100 (175/175)	121 (69·1)	54 (30·9)

Table 2. Phenotypic test results relative to PCR for *mecA* gene to determine methicillin resistance

R, Resistant; S, susceptible; Sens., sensitivity; Sp., specificity.

Species	Disc diffusion				MicroScan				Agar screening 0·6 μg ml^{-1}				Agar screening 4 μg ml^{-1}			
	R/*mecA*$^+$	S/*mecA*$^-$	Sens. (%)	Sp. (%)	R/*mecA*$^+$	S/*mecA*$^-$	Sens. (%)	Sp. (%)	R/*mecA*$^+$	S/*mecA*$^-$	Sens. (%)	Sp. (%)	R/*mecA*$^+$	S/*mecA*$^-$	Sens. (%)	Sp. (%)
S. epidermidis	51/51	21/21	100	100	51/51	20/21	100	95·2	51/51	21/21	100	100	51/51	21/21	100	100
S. haemolyticus	37/37	3/4	100	75	37/37	3/4	100	75	37/37	4/4	100	100	37/37	4/4	100	100
S. hominis	23/24	18/18	95·8	100	24/24	17/18	100	94·4	24/24	18/18	100	100	24/24	18/18	100	100
Other species	1/9	7/11	11	63·6	8/9	6/11	88·8	54·5	7/9	10/11	77·7	90·9	9/9	11/11	100	100
Total	112/121	49/54	92·5	90·7	120/121	46/54	99·1	85·1	119/121	53/54	98·3	98·1	121/121	54/54	100	100

agar screening test and disc-diffusion have pointed out that agar screening is more sensitive for identifying methicillin-resistant strains (York *et al.*, 1996; Hussain *et al.*, 1998; Frebourg *et al.*, 1998; Kohner *et al.*, 1999). For the agar screening test we used Mueller–Hinton agar plates supplemented with 4 % NaCl and incubated for 48 h because, under these conditions, methicillin resistance coded by the *mecA* gene is easily characterized (York *et al.*, 1996).

The oxacillin concentration of 0·6 μg ml^{-1} proposed by Kohner *et al.* (1999) is based on the tenfold decrease in resistance breakpoints for CNS isolates. Rowe *et al.* (2002) showed that this concentration is 100 % accurate for the detection of the *mecA* gene in *S. epidermidis* but it is less accurate for all other species. We believe it is important to have accurate methods to detect methicillin resistance among less frequently found species, considering their increasing importance as opportunistic pathogens.

The agar screening test containing 4 μg oxacillin ml^{-1} was used based on results reached in a recent study (Ferreira *et al.*, 2003) which analysed different antimicrobial concentrations (1, 2, 4 and 6 μg ml^{-1}). In our study, agar screening with 4 μg oxacillin ml^{-1} did not present false-negative results, which makes its specificity superior to 0·6 μg oxacillin ml^{-1}. Both concentrations correctly characterized all *mecA*-negative isolates, even considering less common species.

MicroScan was very specific but had low sensitivity, impairing its accuracy. The disc-diffusion test was the least accurate method, in agreement with previously reported data. Kolbert *et al.* (1995) showed that disc diffusion failed to characterize two *mecA*-positive and 13 *mecA*-negative strains. Three out of the false-negative isolates were β-lactam hyper-producers. Ferreira *et al.* (2003) presented sensitivity and specificity comparable to the results we have obtained for disc diffusion: 94·2 and 91·8 %, respectively. In another study (Hussain *et al.*, 1998), the researchers proved that the disc-diffusion test had low sensitivity, characterizing only 66 out of 99 *mecA*-positive CNS as resistant.

Finally, our results show that all phenotypic tests had excellent correlation with the genotypic characterization of resistance if the most common species, *S. epidermidis*, *S. hominis* and *S. haemolyticus*, are analysed. The performance of the methods was impaired when less common species were analysed, as demonstrated previously by Hussain *et al.* (2000) and Gradelski *et al.* (2001). However, even though when species other than the most frequent ones are taken into consideration, the agar screening test with 4 μg oxacillin ml^{-1} was totally accurate. Based on these results, and considering just the techniques we evaluated (which did not include detection of PBP2a or the recent indication of the NCCLS to use cefoxitin discs for the disc-diffusion test), we recommend this phenotypic method as the most reliable to characterize methicillin resistance among CNS isolates. Besides, it is a technically simple option for routine clinical laboratories.

Table 3. Results of MICs obtained by the automated MicroScan system for false-positive strains compared to PCR for the *mecA* gene

Strain no.	Species	MIC (μg ml^{-1})	Disc diffusion	Agar screening 0·6 μg ml^{-1}
101	*S. epidermidis*	>2	–	–
180	*S. cohnii* subsp. *cohnii*	0·5	False-positive	–
172	*S. haemolyticus*	1	False-positive	–
87	*S. saprophyticus*	0·5	False-positive	False-positive
74	*S. saprophyticus*	0·5	–	–
195	*S. warneri*	0·5	False-positive	–
198	*S. warneri*	0·5	False-positive	–
93	*S. hominis* subsp. *hominis*	1	–	–

Acknowledgements

The authors wish to thank Dr Lúcia Martins Teixeira, Instituto de Microbiologia Professor Paulo Góes, Universidade Federal do Rio de Janeiro, Brazil, for her cooperation. This study was supported in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Faculdade Federal de Ciências Médicas de Porto Alegre (FFFCMPA).

References

Chambers, H. F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* **10**, 781–791.

Ferreira, R. B. R., Ioro, N. L. P., Malvar, K. L., Nunes, A. P. F., Fonseca, L. S., Bastos, C. C. R. & Santos, K. R. N. (2003). Coagulase-negative staphylococci: comparison of phenotypic and genotypic oxacillin susceptibility tests and evaluation of the agar screening test by using different concentrations of oxacillin. *J Clin Microbiol* **41**, 3609–3614.

Frebourg, N. B., Nouet, D., Lemée, L., Martin, E. & Lemeland, J. (1998). Comparison of ATB staph, rapid ATB staph, Vitek and E-test methods for detection of oxacillin heteroresistance in staphylococci possessing *mecA*. *J Clin Microbiol* **36**, 52–57.

Gerberding, J. L., Miick, C., Liu, H. H. & Chambers, H. F. (1991). Comparison of conventional susceptibility tests with direct detection of penicillin-binding protein 2a in borderline oxacillin-resistant strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **35**, 2574–2579.

Gradelski, E., Valera, L., Aleksunes, L., Bonner, D. & Fung-Tomc, J. (2001). Correlation between genotype and phenotypic categorization of staphylococci based on methicillin susceptibility and resistance. *J Clin Microbiol* **39**, 2961–2963.

Hussain, Z., Stoakes, L., Lannigan, R., Longo, S. & Nancekivell, B. (1998). Evaluation of screening and commercial methods for detection of methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* **36**, 273–274.

Hussain, Z., Stoakes, L., Massey, V., Diagre, D., Fitzgerald, V., El Sayed, S. & Lannigan, R. (2000). Correlation of oxacillin MIC with *mecA* gene carriage in coagulase-negative staphylococci. *J Clin Microbiol* **38**, 752–754.

Kohner, P., Uhl, J., Kolbert, C., Persing, D. & Cockerill, F., III (1999). 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. *J Clin Microbiol* **37**, 2952–2961.

Kolbert, C. P., Connolly, J. E., Lee, M. J. & Persing, D. H. (1995). Detection of staphylococcal *mecA* gene by chemiluminescent DNA hybridization. *J Clin Microbiol* **33**, 2179–2182.

Marshall, S. A., Wilke, W. W., Pfaller, M. A. & Jones, R. N. (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.

NCCLS (1999). *Performance standards for antimicrobial susceptibility testing*. 9th information supplement, NCCLS document M100-S9. Wayne, PA: National Committee for Clinical Laboratory Standards.

NCCLS (2003). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved standard, 6th edition, NCCLS document M7-A6. Wayne, PA: National Committee for Clinical Laboratory Standards.

NNIS (2001). National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992–June 2001, issued August 2001. *Am J Infect Control* **29**, 404–421.

Rowe, F., Superti, S. V., Scheibe, R. M. & Dias, C. G. (2002). Agar diffusion, agar dilution, Etest , and agar screening test in the detection of methicillin resistance in staphylococci. *Diagn Microbiol Infect Dis* **43**, 45–48.

Sader, H., Sampaio, J. L., Zoccoli, C. & Jones, R. N. (1999). Results of the 1997 SENTRY Antimicrobial Surveillance Program in three Brazilian medical centers. *Braz J Infect Dis* **3**, 63–79.

Santos, K. R. N., Teixeira, L. M., Leal, G. S., Fonseca, L. S. & Gontijo Filho, P. P. (1999). DNA typing of methicillin-resistant *Staphylococcus aureus*: isolates and factors associated with nosocomial acquisition in two Brazilian university hospitals. *J Med Microbiol* **48**, 17–23.

Swenson, J. M., Williams, P. P., Killgore, G., O'Hara, C. M. & Tenover, F. C. (2001). Performance of eight methods, including two new rapid methods for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organisms. *J Clin Microbiol* **39**, 3785–3788.

Tenover, F. C., Jones, R. N., Swenson, J. M., Zimmer, B., McAllister, S. & Jorgensen, J. H. (1999). Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. *J Clin Microbiol* **37**, 4051–4058.

Woods, W., Ramotar, K., Lem, P. & Toye, B. (2002). Oxacillin

susceptibility testing of coagulase-negative staphylococci using the disk diffusion method and the Vitek GPS-105 Card. *Diagn Microbiol Infect Dis* **42**, 291–294.

Yamazumi, T., Furuta, I., Diekema, D. J., Pfaller, M. A. & Jones, R. N. (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.

York, M. K., Gibbs, L., Chehab, F. & Brooks, G. F. (1996). Comparison of PCR detection of *mecA* with standard susceptibility tests methods to determine methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* **34**, 249–253.