

Is the Cefoxitin Disk Test Reliable Enough To Detect Oxacillin Resistance in Coagulase-Negative Staphylococci?

Coagulase-negative staphylococci (CoNS) represent an important etiology of nosocomial bloodstream infections (2, 4, 6). The introduction of empirical treatment has been a crucial step in reducing morbidity and mortality caused by such infections and in controlling the spread of resistant strains (1, 6). It has been advocated that the cefoxitin disk would be more sensitive than the 1- μ g oxacillin disk to predict the oxacillin heteroresistance phenotype among staphylococci (1, 2, 4, 7, 8). Thus, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended its use for detection of oxacillin resistance (7). A total of 5 of 241 (2.2%) CoNS displaying cefoxitin inhibition zones of ≥ 25 mm and resistance to oxacillin by disk diffusion test were isolated by the clinical laboratory of the Hospital São Paulo, a tertiary university hospital located in São Paulo, Brazil, during September and October 2004. The bacterial strains were isolated from diverse body sites and referred to the Laboratório Especial de Microbiologia Clínica of Universidade Federal de São Paulo for further characterization. The identification of the CoNS was confirmed based on the morphology of bacterial colonies on blood agar, Gram stain, 3% H₂O₂ catalase and by agglutination tests (Staphytest; PROBAC, São Paulo, Brazil). The strains were tested for coagulase activity by slide tests with rabbit plasma (Difco Laboratories, Detroit, MI). The final identification was carried out using the GPI card Vitek (BioMerieux, Hazelwood, MI). The antimicrobial susceptibility testing of six antimicrobial agents was performed using Etest (AB Biodisk, Solna, Sweden). The results were interpreted according to the NCCLS guidelines (7). *Staphylococcus aureus* ATCC 25923 and ATCC 29213 were used as quality control strains. The *mecA* gene was detected by PCR technique according to the method described previously (6). The genetic relatedness of the five CoNS was evaluated by automated ribotyping using the RiboPrinter microbial characterization system (Qualicon, Wilmington, DE). Isolates were considered to belong to the same ribogroup if their similarity coefficients were ≥ 0.93 . According to the Vitek system, all five isolates were identified as *Staphylococcus epidermidis*. They were susceptible to linezolid, vancomycin, and teicoplanin. These isolates were confirmed to be resistant to oxacillin and exhibited cefoxitin inhibition zones that were ≥ 25 mm, as shown in Table 1. The presence of the *mecA* gene was detected in all five CoNS, which displayed distinct ribotyping patterns.

Oxacillin resistance among staphylococci is caused by ex-

pression of penicillin-binding protein 2a (PBP2a), encoded by the *mecA* gene, which has low binding affinity to all beta-lactam antibiotics available in the clinical practice (1, 2, 3, 4, 5, 9). Detection of oxacillin resistance is complicated because distinct populations of staphylococci express different levels of resistance. Cefoxitin is considered to be a better predictor than oxacillin for the detection of oxacillin heteroresistance because it is a stronger inducer of PBP2a. In addition, it has high affinity for staphylococcal PBP4, and previous experiments have shown a relationship between PBP2, PBP4, and methicillin resistance (2, 4). Many studies have reported that cefoxitin disks had high sensitivities (97.0 to 100.0%) and specificities (99.0 to 100.0%) in detecting heterogeneous populations of oxacillin-resistant staphylococci (2, 4, 9). However, we observed discrepant results between oxacillin and cefoxitin disks when testing CoNS by disk diffusion. Initially, it was thought these CoNS did not belong to *S. epidermidis* species and did not carry the *mecA* gene since they had low oxacillin MICs. However, the Vitek system identified these isolates as *S. epidermidis*, and the presence of the *mecA* gene was confirmed by PCR in all isolates. The chance of clonality was also discarded, since no genetic relatedness was encountered among the CoNS, refuting the possibility of an outbreak caused by a strain exhibiting an unusual phenotype. In the absence of a national guideline, most of the Brazilian laboratories follow the NCCLS recommendations. In addition, dilution tests are not available at most Brazilian laboratories, and disk diffusion has been the most performed susceptibility technique. If the cefoxitin results were considered instead of the oxacillin results, these CoNS would have been falsely reported as susceptible to oxacillin, with direct implications for the choice of drugs for treatment. Although the number of isolates tested was low in this study, our results show that the detection of low-level methicillin resistance by the cefoxitin disk among CoNS can be problematic. Thus, our results highlight the importance of testing the oxacillin disk or using molecular techniques for detection of the *mecA* gene (1, 3, 6, 9). Moreover, changes in the NCCLS cefoxitin disk diffusion breakpoints could be useful for screening such isolates, since all CoNS tested had cefoxitin zones of inhibition between 26 and 29 mm. To increase the sensitivity and specificity of the cefoxitin disk to detect oxacillin heteroresistance among *S. aureus*, other authors have also suggested changes in the interpretative zone diameters of cefoxitin (4, 9).

TABLE 1. Species identifications, antimicrobial susceptibility profiles, ribotyping patterns, and detection of *mecA* gene among CoNS recently isolated at Hospital São Paulo, São Paulo, Brazil

Bacterial strain	Vitek identity	Disk diffusion zones (mm)		MIC (μ g/ml) ^a						Ribogroup	<i>mecA</i> gene
		OXA ^b	CFX	OXA	VAN	TP	LZ	LEV	TMP-SMX		
438308	<i>S. epidermidis</i>	10	26	2.0	1.5	2.0	0.38	1.5	0.50	145-5	Positive
413321	<i>S. epidermidis</i>	10	26	2.0	2.0	3.0	0.50	16.0	3.0	154-6	Positive
370018	<i>S. epidermidis</i>	12	29	1.0	3.0	2.0	0.38	0.094	0.19	154-3	Positive
420689	<i>S. epidermidis</i>	12	27	1.5	1.5	4.0	0.75	0.125	>32.0	154-4	Positive
420387	<i>S. epidermidis</i>	10	26	1.5	1.5	3.0	0.50	2.0	0.064	154-5	Positive

^a Determined by Etest (Solna, Sweden).

^b CFX, cefoxitin; LEV, levofloxacin; LZ, linezolid; OXA, oxacillin; TP, teicoplanin; VAN, vancomycin; TMP-SMX, trimethoprim-sulfamethoxazole.

REFERENCES

1. **Boubaker, I. B. B., R. B. Abbes, H. B. Abdallah, K. Mamlouk, F. Mahjoubi, A. Kammoun, A. Hammami, and S. B. Redjeb.** 2004. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **10**:762–765.
2. **Cauwelier, B., B. Gordts, P. Descheemaeker, and H. Van Lanuyt.** 2004. Evaluation of a disk diffusion method with cefoxitin (30 µg) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur. J. Clin. Microbiol. Infect. Dis.* **23**:389–392.
3. **Cavassini, M., A. Wenger, K. Jaton, D. S. Blanc, and J. Bille.** 1990. Evaluation of MRSA-Screen, a simple anti-PBP-2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. *J. Clin. Microbiol.* **37**:1591–1594.
4. **Felten, A., B. Grandy, P. H. Lagrange, and I. Casin.** 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. *J. Clin. Microbiol.* **40**:2766–2771.
5. **Louie, L., S. O. Matsumur, E. Choi, M. Louie, and A. E. Simor.** 2000. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. *J. Clin. Microbiol.* **38**:2170–2173.
6. **Murakami, K., W. Minamide, K. Wada, E. Nakamura, H. Teraoka, and S. Watanabe.** 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J. Clin. Microbiol.* **29**:2240–2244.
7. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing, 14th information supplement. M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Okonogi, K., Y. Noji, M. Kondo, A. Imada, and T. Yokota.** 1989. Emergence of methicillin-resistant clones from cephamycin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **24**:637–645.
9. **Skov, R., R. Smyth, M. Clausen, A. R. Larsen, N. Frimodt-Moller, B. Olsson-Liljequist, and G. Kahlmeter.** 2003. Evaluation of a cefoxitin 30 µg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **52**:204–207.

Eliete A. M. Frigatto
Antonia M. O. Machado
Laboratório Central
Division of Clinical Pathology
Hospital São Paulo
Universidade Federal de São Paulo
São Paulo, Brazil

Antônio C. C. Pignatari
Ana C. Gales*
Laboratório Especial de Microbiologia Clínica
Division of Infectious Diseases
Universidade Federal de São Paulo
Rua Leandro Duprét, 188
São Paulo SP, Brazil 04025-010

*Phone: 55-11-50812819
 Fax: 55-11-5571 5180
 E-mail: galesac@aol.com