Evaluation of the Vitek Card GPS105 and VTK-RO7.01 Software for Detection of Oxacillin Resistance in Clinically Relevant Coagulase-Negative Staphylococci

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The performance of Vitek cards GPS105 with software version VTK-R07.01 for detection of oxacillin resistance in coagulase-negative staphylococci (CoNS) was compared to disk diffusion and PCR detection for *mecA*. The sensitivity and specificity of the Vitek GPS105 method were 97.6 and 85.5%, respectively.

Coagulase-negative staphylococci (CoNS) are important causes of nosocomial bacteremia, especially in patients with intravenous catheters or indwelling medical devices (1, 3, 7, 14). In some institutions, 70 to 75% of CoNS are resistant to oxacillin; consequently, vancomycin is the therapy of choice. If the isolate is oxacillin susceptible, treatment with a penicillinase-resistant penicillin is preferred to minimize selection of vancomycin-resistant organisms. A susceptibility test method that provides accurate oxacillin results is critical.

The National Committee for Clinical Laboratory Standards (NCCLS) changed the breakpoints for oxacillin testing of CoNS in January 1999, because of discrepancies between results of *mecA* genetic assays, which are considered the reference method for detecting oxacillin resistance in staphylococci, and the former NCCLS breakpoints of ≥ 4 $\mu\text{g/ml}$ for resistance and ≤ 2 $\mu\text{g/ml}$ for susceptibility. Currently, for microtiter dilution and disk diffusion testing, respectively, CoNS isolates with an MIC of ≥ 0.5 $\mu\text{g/ml}$ or a zone diameter of ≤ 17 mm are considered resistant, and those with an MIC of ≤ 0.25 $\mu\text{g/ml}$ or a zone diameter of ≥ 18 mm are considered susceptible (9). To comply with these guidelines, it was necessary for laboratories using the Vitek (bioMérieux Vitek, Inc.) to use an alternate method when testing CoNS. Vitek updated its software and increased the number of oxacillin concentrations tested to accommodate the revised oxacillin breakpoints for CoNS in September 1999. The purpose of the present study was to evaluate the performance of the Vitek card GPS105 with software version VTK-R07.01. The results were compared to disk diffusion and detection of the *mecA* gene by PCR.

Isolates of CoNS ($n = 202$) from cultures of clinical specimens of blood and urine were collected prospectively and stored at -70°C . Prior to testing, isolates were subcultured twice on sheep blood agar. Isolates were identified as CoNS using Gram stain, catalase, and latex agglutination tests (Staphaurex; Murex Biotech Ltd., Dartford, England). CoNS were identified to species with the API Staph-Ident system (bioMérieux Vitek, Inc.). Disk diffusion testing was performed according to NCCLS guidelines (10) by using Mueller-Hinton

agar without additional NaCl (BD Biosciences, Sparks, Md.) and 1- μg oxacillin disks (BD Biosciences). Zone diameters were measured after plates were incubated in ambient air at 35°C for 24 h. Vitek cards GPS105 were inoculated and incubated according to manufacturer's recommendations. Results were interpreted by Vitek software version VTK-R07.01. If disk diffusion and Vitek results disagreed, both tests were repeated. Multiplex PCR for detection of the *mecA* gene and the *Staphylococcus* IS431 sequence was performed as previously described (13); if isolates did not contain the *mecA* gene or an IS431 sequence, amplification of the *Staphylococcus* 16S rRNA sequence was performed as described previously (6).

The 202 CoNS isolates tested (141 from urine, 61 from blood) in this study belonged to 11 species (Table 1). The *mecA* gene was detected in 126 isolates (62.4%), of five species: *Staphylococcus epidermidis*, *S. haemolyticus*, *S. hominis*, *S. warneri*, and *S. xylosum*. *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. saprophyticus*, *S. sciuri*, and *S. simulans* were consistently *mecA* negative.

Initial Vitek and disk diffusion results are summarized by source in Table 1. The majority of the CoNS from blood were *S. epidermidis*. The *mecA* gene was detected in 44 (72%) of all blood isolates; all were determined to be oxacillin resistant by both disk diffusion and Vitek. All 115 *mecA*-negative isolates were determined to be susceptible by Vitek and disk diffusion (sensitivity and specificity of 100%).

Of the urine isolates ($n = 141$), 80 were *mecA* positive and 61 were *mecA* negative. Both Vitek and disk diffusion methods did not perform as well on isolates of CoNS from urine: 6 of the *mecA* positive isolates were determined to be susceptible by Vitek, and 11 of the *mecA* negative isolates were determined to be resistant (sensitivity, 92.5%; specificity, 82%). The 11 isolates that were *mecA* negative but oxacillin resistant as determined by Vitek and disk diffusion included six *S. saprophyticus* isolates, two *S. lugdunensis* isolates, and one isolate each of *S. capitis*, *S. cohnii*, and *S. sciuri*. Three species (*S. epidermidis*, *S. hominis*, and *S. haemolyticus*) comprised the majority of the isolates (122 of 141; 86.5%). For these three species, the sensitivity and specificity of Vitek were high (92.4 and 97.6%, respectively). After repeat testing, Vitek correctly identified 76 of the 79 *mecA*-positive isolates as resistant and 100% of the *mecA*-negative isolates as susceptible (sensitivity, 96.2; specificity, 100%).

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TABLE 1. Vitek and disk diffusion oxacillin results after initial testing

Source ^a and species	<i>mecA</i> result	Total no.	Disk diffusion (no. of isolates)		No. of isolates with Vitek result (MIC [μg/ml]) of:				
			Sensitive	Resistant	≤0.25	0.5	2	4	≥8
Blood									
<i>S. epidermidis</i>	—	11	11	0	11	0	0	0	0
<i>S. epidermidis</i>	+	39	0	39	0	1	4	7	27
<i>S. hominis</i>	—	2	2	0	2	0	0	0	0
<i>S. hominis</i>	+	3	0	3	0	0	1	0	2
<i>S. haemolyticus</i>	—	1	1	0	1	0	0	0	0
<i>S. haemolyticus</i>	+	2	0	2	0	1	0	0	1
<i>S. lugdunensis</i>	—	1	1	0	1	0	0	0	0
<i>S. warneri</i>	+	2	0	2	0	1	0	1	0
Urine									
<i>S. epidermidis</i>	—	34	34	0	33	0	0	0	1
<i>S. epidermidis</i>	+	71	2	69	2	7	6	9	47
<i>S. hominis</i>	—	5	5	0	5	0	0	0	0
<i>S. hominis</i>	+	5	1	4	3	1	0	0	1
<i>S. haemolyticus</i>	—	4	4	0	4	0	0	0	0
<i>S. haemolyticus</i>	+	3	0	3	1	1	1	0	0
<i>S. capitis</i>	—	4	3	1	3	1	0	0	0
<i>S. cohnii</i>	—	1	0	1	1	0	0	0	0
<i>S. lugdunensis</i>	—	2	1	1	0	2	0	0	0
<i>S. saprophyticus</i>	—	6	1	5	0	6	0	0	0
<i>S. sciuri</i>	—	2	1	1	1	1	0	0	0
<i>S. simulans</i>	—	2	2	0	2	0	0	0	0
<i>S. warneri</i>	—	1	1	0	1	0	0	0	0
<i>S. xylosus</i>	+	1	0	1	0	0	0	0	1

^a A total of 61 CoNS isolates from blood and 141 from urine specimens were tested.

Discrepancies between results of *mecA* PCR assays when CoNS are tested and the NCCLS microtiter dilution and disk diffusion breakpoints for oxacillin against *S. aureus* have been recognized for several years. For this reason, various investigators recommended lowering the oxacillin MIC breakpoint for CoNS: York et al. (15) suggested ≤ 1 μ g/ml for susceptibility, Cormican et al. (2) and McDonald et al. (8) suggested ≤ 0.5 μ g/ml, and Marshall et al. (7) suggested ≤ 0.25 μ g/ml.

Tenover et al. (12) concurred with the breakpoints of ≤ 0.25 μ g/ml for susceptibility and ≥ 0.5 μ g/ml for resistance because these values allowed maximum sensitivity for detecting *mecA*-positive isolates of *S. epidermidis* without drastically compromising specificity. These authors acknowledged, however, that these breakpoints were considerably less specific for *mecA* in species of CoNS other than *S. epidermidis*. Tenover et al. (12) also suggested modified breakpoints for disk diffusion: ≤ 17 mm for resistance and ≥ 18 mm for susceptibility. The NCCLS considered data from all studies and adopted the oxacillin MIC and disk diffusion breakpoints for CoNS advocated by Tenover et al. Our results show that the new Vitek revised cards and software system accurately predicts the presence or absence of *mecA* in the three most commonly encountered species of CoNS.

For species of CoNS other than *S. epidermidis*, *S. hominis*, and *S. haemolyticus*, we found poor correlation between *mecA* PCR and oxacillin testing by Vitek or disk diffusion. In particular, isolates of *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. saprophyticus*, and *S. sciuri* that had an oxacillin MIC of 0.5 μ g/ml as determined by Vitek (and were resistant by disk diffusion) were consistently *mecA* negative. Similar findings have been reported by other investigators (4, 5, 12). Hussain et al. (4)

tested 493 isolates of 11 species of CoNS (no *S. sciuri*) and found *mecA*-negative isolates of *S. capitis*, *S. cohnii*, *S. lugdunensis*, and *S. saprophyticus* with oxacillin MICs of 0.5 to 2.0 μ g/ml by agar dilution. In a second study, Hussain et al. (5) tested 463 isolates of 13 species of CoNS (no *S. sciuri*) and found *mecA*-negative *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. saprophyticus*, *S. caprae*, *S. warneri*, and *S. xylosus* with oxacillin MICs of ≥ 0.5 μ g/ml. In the latter study, isolates were tested by a latex agglutination test that detects penicillin-binding protein (PBP) 2a. Compared to *mecA* PCR, the sensitivity of that latex test was 100% and the specificity was 99.5%, whereas the specificity of the new NCCLS breakpoint was 60.8%. The mechanism(s) of decreased susceptibility to oxacillin in *mecA*-negative CoNS with an oxacillin MIC of 0.5 to 2.0 μ g/ml is unknown, but alterations in PBPs other than PBP2a have been documented in some strains of *S. haemolyticus* and *S. saprophyticus* (11) and, therefore, suggested as one possibility (12). It is not known whether penicillinase-resistant penicillins can eradicate clinical isolates of *mecA*-negative CoNS with an oxacillin MIC of 0.5 to 2.0 μ g/ml. Clinical trials designed to answer this question are needed.

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