

Detection of Oxacillin Resistance in Coagulase Negative Staphylococci with Phoenix

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INTRODUCTION

■ CNS belong to the normal flora of the human skin and mucous membrane and are amongst the 5 most frequent causes of nosocomial infections. Despite the low virulence, the past years have shown a dramatic increase of incidence of nosocomial septicemia and foreign body associated infections due to CNS. Antibiotic therapy of CNS is increasingly problematic. The majority of clinically relevant CNS strains carry the *mecA* gene which codes for an additional penicillin binding protein, the PBP2a. PBP2a is crucial for the phenotypic expression of the methicillin resistance due to its low affinity for all β -lactam antibiotics. It's well known that phenotypic detection of methicillin resistance in CNS is difficult due to the heterogenic *mecA* expression and due to the detection system's focus for *S. aureus*. Tests like the oxacillin agar screen, agar diffusion or MIC by micro-dilution are based on modified culture conditions in order to increase expression of resistance. Disadvantage of phenotypic methods is the long TTR, low sensitivity or a high workload. PCR detection of *mecA* is highly sensitive and seen as the gold standard. However, except for reference centers this technique is very laborous, expensive and not feasible. The Phoenix, a new automated system for ID and AST, was evaluated for its performance of the detection of this important resistance mechanism.

MATERIALS AND METHODS

The results of the *mecA* PCR as gold standard for detection of methicillin resistance were compared with the oxacillin MIC of the Phoenix Gram positive combo panels (≤ 0.25 thru 4 $\mu\text{g/mL}$). In addition we analyzed the time to detection for the oxacillin MIC. We tested 200 CNS, mainly isolated from blood cultures and infected catheters. *S. aureus* ATCC 43300 (*mecA*-positive) and ATCC 29213 (*mecA*-negative) was used as QC for each run. ID was done with ID 32 Staph and conventional methods. The *mecA* PCR was done with the primers. *S. epidermidis* 1457 served as negative control and *S. epidermidis* 1057 and RP62A as positive control. PBP2a was done with a latex agglutination test (Denka). The oxacillin screen agar test (6 μg oxacillin, 2% NaCl) was incubated at 30°C for 48 hours.

RESULTS

■ This strain collection contained 13 different species (see table 1). *MecA* was detected in 124 strains (99 *S.epidermidis* and 25 non-*S.epidermidis*). Using the DIN oxacillin breakpoint of 2 µg/ml, 122/124 *mecA*-positive isolates were detected as resistant, and 70/76 *mecA*-negative strains as susceptible (table 3). False positive results were obtained with 4 *mecA*-negative strains at an oxacillin MIC of exactly 2 µg/ml (table 2, 3 and 4). There was only 1 false negative result with a *mecA*-positive strain at an oxacillin MIC of ≤ 0.25 µg/ml (table 2, 3 and 4). This resulted in a sensitivity and specificity of 99.2% and 94.6%, respectively. (table 3). The current NCCLS oxacillin MIC breakpoint of ≥ 0.5 µg/ml resulted in a sensitivity of 99.2%, however with a relatively low specificity of 64.9%, caused by false positive results of 26 *mecA*-negative strains, 15 of those being non-*S.epidermidis* (table 3). In contrast to that, the NCCLS breakpoint of 4 µg/ml valid before 1999 resulted in a sensitivity and specificity of 96% and 100%, respectively. False negative results were obtained in 4 *mecA*-positive strains with an oxacillin MIC of 2 up to ≤ 2.5 µg/ml (table 3). Three strains didn't show sufficient growth in the test system (table 2). Fifty one percent of the results for PCR concordant data were obtained in 9 hours, after 17 hours results for all strains were available (table 5).

Table 1
Species of CNS Strains

140	<i>Staphylococcus epidermidis</i>
16	<i>S. haemolyticus</i>
10	<i>S. hominis</i>
9	<i>S. saprophyticus</i>
6	<i>S. capitis</i>
4	<i>S. lugdunensis</i>
4	<i>S. wamery</i>
4	<i>S. xylosus</i>
2	<i>S. schleiferi</i> and <i>S. cohnii</i>
1	<i>S. kloosii</i> , <i>S. chromogenes</i> and <i>S. simulans</i>

Table 2
Results of the Oxacillin MIC (using the DIN standard)

Concordant with PCR <i>mecA</i>	192/200	(96%)
Discordant results	5/200	(2.5%)
No growth	3/200	(1.5%)

Table 3
Phoenix Oxacillin Results with Different Breakpoints versus PCR Result for *mecA*

	Resistant	Sensitive	Sensitivity	Specificity
2 g/ml (DIN) <i>mecA</i> -positive <i>mecA</i> -negative	122	1 70	99.2%	94.6%
0.5 g/ml (NCCLS, Actual) <i>mecA</i> -positive <i>mecA</i> -negative	122	1 48	99.2%	64.9%
4 g/ml (NCCLS, or 1999) <i>mecA</i> -positive <i>mecA</i> -negative	119	4 74	96.7%	100%

Table 4
CNS with Discrepant Results

Identification	Oxacillin MIC	PCR for <i>mecA</i>	OAS ^A	PBP2a
<i>S. epidermidis</i>	2 ^a	negative	negative	negative
<i>S. epidermidis</i>	2	negative	negative	negative
<i>S. cohnii</i>	2	negative	negative	negative
<i>S. epidermidis</i>	2	negative	negative	negative
<i>S. epidermidis</i>	0.25	positive	positive (48h)	positive

^A Oxacillin-Agar-Screen ^a g/ml

Table 5
TTR for Oxacillin MIC

4 h	5 h	6 h	7 h	8 h	9 h	16 h
0*	2	8	23	46	102	200
0 %	1 %	4 %	11,5 %	23 %	51 %	100 %

* Number of detected CNS isolates

CONCLUSION

■ Phoenix is an excellent phenotypic procedure for the detection of methicillin resistance in CNS.

We recommend additional test methods for strains with a critical oxacillin MIC of 2 µg/ml in order to increase detection of methicillin resistance.

The DIN oxacillin MIC breakpoint proves to be good with a sensitivity of 99.2% and a specificity of 94.6%.

Validation of oxacillin MIC results is very much dependent on the selected breakpoint standard.

False negative results based on the current NCCLS standard were seen heaped with *mecA*-negative non-*S. epidermidis* strains (15 out of 26).

Results were available for half the strains within 9 hours.

When using the current NCCLS breakpoint standard, 26 false positive results were seen. Fifteen of these strains were non-*S. epidermidis*.