Evaluation of the Vitek 2 AST-P559 Card for Detection of Oxacillin Resistance in *Staphylococcus aureus*[▽]

Staphylococcus aureus strains with resistance to methicillin or oxacillin (MRSA) represent one of the main nosocomial pathogens at present. MRSA infections are clearly associated with higher mortality and economic cost than those caused by methicillin-susceptible *S. aureus* (1). In Spain, the prevalence of methicillin resistance among *S. aureus* isolates has increased since the 1990s and in some cases has now reached levels higher than 30% (2).

Rapid and accurate detection of MRSA strains in hospital as well as community settings is imperative.

The performance of cefoxitin either as a disk or as a supplement in agar medium for the detection of MRSA has been confirmed extensively (6, 7). Also, cefoxitin-based screening broth proved to be more sensitive and rapid than oxacillin-based broth (3). Recently, a new Vitek card (AST P549) for determining susceptibility of *S. aureus* to oxacillin has been reported (5). The Vitek 2 AST-P559 card (bioMérieux) incorporates specific antimicrobial agents to test susceptibility and also includes the determination of oxacillin MICs as well as screening for cefoxitin (6 µg/ml). The main advantage of the card is its promptness in detecting methicillin resistance, as it is possible to interpret the results of a cefoxitin screen after 4 h of card inoculation. However, final confirmation is done after 12 h upon reading the oxacillin MICs as well as the cefoxitin screen well and according to the manufacturer's instructions. The Vitek 2 AST-P559 card cannot be manually read, so interpretive information must be derived from the software interpretation.

A total of 301 *S. aureus* strains were evaluated (51 *mecA* negative and 250 *mecA* positive as determined by PCR). *S. aureus* ATCC 29213 was used as a negative control. Molecular typing of the X region of the *spa* gene was done with the 250 *mecA*-positive isolates (4), and these were then grouped into a *spa* clonal complex (BURST.Ridom StaphType software), with 4 types more prevalent than the others (t02, 23.6%; t18, 22%; t67, 17.6%; and t12, 16.3%).

Overall results are shown in Table 1. Three out of 250 isolates yielded a negative result by the cefoxitin screening methods as well as yielding an oxacillin MIC of ≤2. However, when these isolates were preincubated for 24 h in BBL CHROMagar MRSA medium (BD Diagnostics, Sparks, MD), which includes 6 µg/ml of cefoxitin, and the assay was repeated, they yielded a positive result with both antibiotics, therefore suggesting that the existence of subpopulations heteroresistant to oxacillin may cause a false-negative result with the Vitek 2 card. All *mecA*-positive isolates yielded a positive result with the cefoxitin screening (and also yielded an oxacillin MIC of ≥4 µg/ml) and showed positive PBP 2a agglutination with the Slidex MRSA detection kit (bioMérieux). Regarding specificity, all *mecA*-negative *S. aureus* strains were negative by the cefoxitin screening method and also yielded an oxacillin MIC of ≤2 µg/ml. However, PBP 2a agglutination yielded three false-positive results (96% specificity).

Considering the results from these three isolates at preincubation time with the cefoxitin agar plate (false negative), the overall results of this study with the AST-P559 card showed sensitivity and specificity of 98.8% and 100%, respectively. The positive and negative predictive values were 100% and 94%,

TABLE 1. Number of isolates yielding results corresponding to the different tests with the Vitek 2 AST-P559 card

<i>S. aureus</i> isolates (n = 301)	No. of isolates with result by:					
	Cefoxitin screening		Oxacillin MIC		PBP 2a agglutination	
	Negative	Positive	≤2 µg/ml	≥4 µg/ml	Negative	Positive
<i>mecA</i> positive (n = 250)	3 ^a	247	3 ^a	247	0	250
<i>mecA</i> negative (n = 51)	51	0	51	0	48	3

^a Became positive after preincubation with agar containing 6 µg/ml of cefoxitin.

respectively. However, the values for all parameters increased to 100% when the three strains were analyzed after induction of the *mecA* gene product with cefoxitin.

The main advantage of the AST-P559 card is the incorporation of two tests to provide simultaneous determination of oxacillin MICs as well as cefoxitin screening. Several studies (6, 7) have shown that cefoxitin is a better predictor of the presence of *mecA* than oxacillin, and, indeed, Velasco et al. (7) reported an increase of 2% in sensitivity to detect *mecA* when the cefoxitin screening test was used in addition to the oxacillin MIC. For these reasons, the simultaneous presence of two tests (oxacillin MIC and cefoxitin screening) in the same card may offer a noticeable advantage for detection of some *mecA*-negative *S. aureus* isolates which are identified by cefoxitin screening rather than oxacillin MIC. In addition, information about susceptibility to antibiotics against gram-positive microorganisms is provided by this card, which offers an undoubted advantage with respect to chromogenic agars, agglutination latex, or molecular techniques, since they only detect the marker of resistance to oxacillin. In addition to that, the possibility of diagnosis of an MRSA infection in 4 h (by the software interpretation of the cefoxitin screening) will make the use of this automated method very attractive for clinical microbiology laboratories worldwide.

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