



Correlation between the VITEK2 system and cefoxitin disk diffusion for the daily detection of oxacillin resistance in a large number of clinical isolates

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Abstract: The aim of the present study was to compare the performance of the new VITEK2 AST-P551 card with the cefoxitin disk diffusion method for the daily detection of methicillin resistance with a high number of *Staphylococcus aureus* clinical isolates. Detection of the PBP2a protein or *mecA* gene was performed for each discordant case. Seventy (3.3%) isolates out of 2107 clinical strains showed discordant results, 2 very major errors, 4 major errors, and 64 minor errors. Fifty-nine (84%) discordant results were resolved with a final overall agreement of 99.5%. Eleven strains (0.5%) remained discordant (mE). Four of 370 MRSA strains were misclassified as susceptible in daily practice by the cefoxitin disk diffusion method. All these strains were resistant to aminoglycosides and/or fluoroquinolones. VITEK2 system is highly reliable for methicillin resistance detection at the routine level. Oxacillin susceptible classified clinical strains with associated resistances pattern required attention.

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1/Response to Editor-in-Chief

I revised my manuscript as a brief report, as required.

2/Response to Reviewers 1' comments:

- the sensitivity-specificity-NPV-PPV were removed from the revised manuscript as the results were similar to the other studies.
- the Tables 1 and 2 were kept with three typewritten pages in length, as recommended.
- Our simple core message was underlined at the end of discussion.

3/Response to Reviewer 2' comments:

- We tried to make a clear comparison between both systems.
- The end of discussion underlined the importance of aminoglycosides and /or fluoroquinolones associated resistances.

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Key-words: detection, *mecA*, oxacillin, cefoxitin, resistance, VITEK2 system

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Abstract. The aim of the present study was to compare the performance of the new VITEK2 AST-P551 card with the cefoxitin disk diffusion method for the daily detection of methicillin resistance with a high number of *Staphylococcus aureus* clinical isolates. Detection of the PBP2a protein or *mecA* gene was performed for each discordant case. Seventy (3.3%) isolates out of 2107 clinical strains showed discordant results, 2 very major errors, 4 major errors, and 64 minor errors. Fifty-nine (84%) discordant results were resolved with a final overall agreement of 99.5%. Eleven strains (0.5%) remained discordant (mE). Four of 370 MRSA strains were misclassified as susceptible in daily practice by the cefoxitin disk diffusion method. All these strains were resistant to aminoglycosides and/or fluoroquinolones. VITEK2 system is highly reliable for methicillin resistance detection at the routine level. Oxacillin susceptible classified clinical strains with associated resistances pattern required attention.

The detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is still a challenge as some heteroresistant isolates may be classified as oxacillin susceptible based on phenotypic methods. In recent years, the ceftioxin disk diffusion method was recommended by many National Committees and the Clinical and Laboratory Standards Institute (CLSI) [1]. The automated VITEK2 system (bioMérieux, Marcy l'Etoile, France) provides *S. aureus* susceptibility testing categorisation within about 8 hours. In the previous published studies comparing several tests for MRSA detection, only a limited number of strains (less than 500) were included. The purpose of this study was to compare the performance of the new VITEK2 system AST-P551 card with the ceftioxin disk diffusion method for the daily detection of SARM with a high number of *S. aureus* clinical isolates.

A total of 2,107 non-duplicate clinical *S. aureus* isolates were routinely collected at Nantes University Hospital. All of the isolates were identified to the species level by standard biochemical methods or with the VITEK2 system. VITEK2 AST-P551 cards were inoculated according to the manufacturer's instructions. The ceftioxin disk diffusion method was performed with an inoculum of approximately 10^6 CFU/mL on Mueller-Hinton agar plates, according to the AntibioGram Committee of the French Microbiology Society (CA-SFM) recommendations [7]. Zone diameters were interpreted according to CA-SFM [7]. In the VITEK2 system, MIC interpretative standards for oxacillin followed the recommendations of CLSI and CA-SFM [1, 7]. Susceptible or resistant results by both methods were considered as concordant. Interpretative category errors were assessed: (i) very major error (VME, susceptible with the VITEK2 system, but resistant by diffusion); (ii) major error (ME, resistant with the VITEK2 system, but susceptible by diffusion); (iii) minor error (mE, susceptible or resistant by one system and intermediate results by the other system). For each discordant strain, the VITEK2 AST-P551 card and the ceftioxin disk diffusion test

were repeated and detection of *mecA* gene by PCR (8) and/or penicillin-binding protein 2a (PBP2a) (Mast Diagnostics, Merseyside, U.K.) was performed, with *mecA* PCR as the gold standard. Overall agreement based on previous category agreement was 96.7%. (Table 1). Seventy (3.3 %) *S. aureus* isolates yielded discordant results: 2 VME (0.1%), 4 ME (0.2%), and 64 mE (3.0%). Forty-four isolates susceptible by VITEK2 and 20 isolates resistant by VITEK2 showed cefoxitin inhibition zone diameters of 25 and 26 mm (intermediate breakpoints). Of the preliminary 70 discordances, 59 (84%) were resolved, giving final overall agreement of 99.5% (Table 2). Eleven MRSA strains (0.5%) remained discordant (mE). All these 11 MRSA harboured associated resistance to fluoroquinolones and/or aminoglycosides and/or macrolide-lincosamide-streptogramin. The cefoxitin inhibition zone diameter was 2 mm larger or smaller than the CA-SFM recommended criteria for 467 concordant strains (401 MSSA and 66 MRSA).

Since 2001, the detection of oxacillin resistance by the cefoxitin disk diffusion method was evaluated using both CLSI [1, 5, 9] and non-CLSI recommendations [3, 10]. The standard conditions differed by the use of media (MH agar, Iso-Sensitest agar), a different inoculum size (semi-confluent with 10^6 CFU/mL or confluent with 10^8 CFU/mL), and several incubation temperatures. Felten *et al.* reported the excellent performance of cefoxitin 30 µg disk on MHA with semi-confluent and confluent inocula [3]. Among 152 *S. aureus* strains, one MRSA isolate and one MSSA isolate gave a 26-mm cefoxitin inhibition zone diameter [3]. Thus, the cefoxitin disk diffusion method using a semi-confluent inoculum on MHA was recommended by CA-SFM [7]. Each strain with a cefoxitin zone diameter of 25 or 26 mm should be examined by a *mecA*-specific test [7]. In our daily practice, the gap between the measured inhibition zone diameters and the breakpoints was very narrow for 467 (22%) concordant strains. The 3% of intermediate cefoxitin diffusion results mostly

resolved in concordant MSSA or MRSA strains after repeated tests underlined the difficulty to perform a well-standardized inoculum. Moreover, the existence of an intermediate zone underscore the need for complementary *mecA* specific tests [7].

When the detection of methicillin resistance was only based on oxacillin MIC determination, VITEK2 was shown to be superior to ATB Staph and Rapid ATB Staph with 4.7%, 11% and 23% false-susceptible results respectively [2]. From the years 2000s, an oxacillin screen test comparable to the oxacillin agar screen test was introduced in the VITEK2 system [3, 5]. Swenson *et al.* reported a good sensitivity (91.1%) but a lack of specificity (75%) [5]. New VITEK2 antimicrobial susceptibility testing cards were successively developed with the replacement of oxacillin by cefoxitin in the screen test. The VITEK2 cefoxitin screen test allowed to detect more MRSA strains, as shown by the very high sensitivities obtained in two studies with a higher number of tested MRSA strains as before (97.5% and 98.8% respectively [4, 6].

Our study is one of the most large-scale ones including *S. aureus* clinical strains. The results showed the excellent concordance between VITEK2 and cefoxitin disk diffusion methods, as seen with the very low percentage of discordant results in the daily practice. It will be a real advantage for the VITEK2 system users to detect *S. aureus* oxacillin resistance by the automated system alone in the daily practice. Nevertheless, according to our results, this choice may be dangerous as some MRSA strains were misclassified as susceptible by the VITEK2 system or by the disk diffusion method. These MRSA strains were resistant to aminoglycosides and/or fluoroquinolones. In any case, the microbiologists should be aware of MRSA strains misclassified as oxacillin susceptible with associated resistances and should perform *mecA*-specific tests at the slightest doubt.

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Table 1. Comparison of cefoxitin disk diffusion method and VITEK2 system for detection of methicillin resistance in 2107 isolates of *S. aureus*

	FOX inhibition zone diameter (mm)						
	≤ 22	23	24	25-26	27	28	≥ 29
Initial results							
Concordant results	301	29	23		168	233	1283
Discordant results			2	64	3	1	
After control							
Concordant results	304	36	30		168	233	1325
Discordant results				11			

Broken lines indicated intermediate breakpoints: for those inhibition zone diameters, *mecA* specific tests should be used, according to CA-SFM recommendations [7].

Table 2. Resolution of discordant results by complementary tests.

Initial discordant results	Results after control			
	Concordant results		MRSA ^c discordant strains	
	MSSA ^a	MRSA ^b	S by VK2	R by VK2
			I by Dif	I by Dif
2 VME	1		1 ^d	
4 ME		4		
64 mE	41	13		10 ^d
Total	42	17	1 ^d	10 ^d

VME, very major error, S with the VITEK2 system and R with the disk diffusion method, ME, major error, R with the VITEK2 system and S with the disk diffusion method; mE, minor error, S or R with one system and intermediately-resistant with the other system.

^a MSSA, methicillin-susceptible *S. aureus*, S by VITEK2 System and Dif. ^b MRSA, methicillin-resistant *S. aureus*, R by VITEK2 system and Dif. ^c *mecA* positive strains;

^d associated resistances (fluoroquinolones, aminoglycosides).