



HAL
open science

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

P. Bemer, M. E. Juvin, G. Gargasson, H. Drugeon, A. Reynaud, S. Corvec

► To cite this version:

P. Bemer, M. E. Juvin, G. Gargasson, H. Drugeon, A. Reynaud, et al.. Correlation between the VITEK2 system and cefoxitin disk diffusion for the daily detection of oxacillin resistance in a large number of clinical isolates. *European Journal of Clinical Microbiology and Infectious Diseases*, 2010, 29 (6), pp.745-747. 10.1007/s10096-010-0920-4 . hal-00583575

HAL Id: hal-00583575

<https://hal.science/hal-00583575>

Submitted on 6 Apr 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Editorial Manager(tm) for European Journal of Clinical Microbiology & Infectious
Diseases
Manuscript Draft

Manuscript Number: EJCMIID-D-09-00494R1

Title: Correlation between VITEK2 system and ceftoxitin disk diffusion for the daily detection of oxacillin resistance in a large number of clinical *Staphylococcus aureus* isolates.

Article Type: Brief Report

Keywords: detection; *mecA*; oxacillin; ceftoxitin; resistance; VITEK2 system

Corresponding Author: Dr pascale berner, M.D.

Corresponding Author's Institution:

First Author: pascale berner, M.D.

Order of Authors: pascale berner, M.D.; marie-emmanuelle juvin; guislaine le gargasson; henri drugeon; alain reynaud; stéphanie corvec

Abstract: The aim of the present study was to compare the performance of the new VITEK2 AST-P551 card with the ceftoxitin 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 ceftoxitin 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.

Ref.: Ms. No. EJCMIID-D-09-00494

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

European Journal of Clinical Microbiology & Infectious Diseases

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.

EJCMID-D-09-00494

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2

4

6

Correlation between VITEK2 system and ceftazidime disk diffusion

8

for the daily detection of oxacillin resistance

in a large number of clinical *Staphylococcus aureus* isolates.

10

P. Bemer, ME Juvin, G. Le Gargasson, H. Drugeon, A. Reynaud

and S. Corvec .

12

CHU de Nantes, Service de Bactériologie-Hygiène, Nantes, France

14

16

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

18

20

Corresponding author: P. Bemer,

Department of Microbiology, CHU Hôtel-Dieu,

22

9, quai Moncousu, 44093 Nantes cedex 01, France

Email: pascale.bemer@chu-nantes.fr

24

Phone: 33 2 40 08 39 79

Fax: 33 2 40 08 38 29

26 **Abstract.** The aim of the present study was to compare the performance of
1 the new VITEK2 AST-P551 card with the cefoxitin disk diffusion method for the daily
2
3
4 28 detection of methicillin resistance with a high number of *Staphylococcus aureus*
5
6 clinical isolates. Detection of the PBP2a protein or *mecA* gene was performed for
7
8
9 30 each discordant case. Seventy (3.3%) isolates out of 2107 clinical strains showed
10
11 discordant results, 2 very major errors, 4 major errors, and 64 minor errors. Fifty-nine
12
13 (84%) discordant results were resolved with a final overall agreement of 99.5%.
14 32
15
16 Eleven strains (0.5%) remained discordant (mE). Four of 370 MRSA strains were
17
18 misclassified as susceptible in daily practice by the cefoxitin disk diffusion method. All
19 34
20 these strains were resistant to aminoglycosides and/or fluoroquinolones. VITEK2
21
22 system is highly reliable for methicillin resistance detection at the routine level.
23 36
24
25 Oxacillin susceptible classified clinical strains with associated resistances pattern
26
27
28 38 required attention.

30
31
32
33
34
35 40
36
37
38
39
40
41
42
43 42
44
45
46
47
48
49
50 44
51
52
53
54
55
56
57 46
58
59
60
61
62
63
64
65

1
2 48 The detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is still a
3
4 challenge as some heteroresistant isolates may be classified as oxacillin susceptible
5
6 based on phenotypic methods. In recent years, the cefoxitin disk diffusion method
7
8 was recommended by many National Committees and the Clinical and Laboratory
9
10 Standards Institute (CLSI) [1]. The automated VITEK2 system (bioMérieux, Marcy
11
12 l'Etoile, France) provides *S. aureus* susceptibility testing categorisation within about 8
13
14 hours. In the previous published studies comparing several tests for MRSA detection,
15
16 54 only a limited number of strains (less than 500) were included. The purpose of this
17
18 study was to compare the performance of the new VITEK2 system AST-P551 card
19
20 with the cefoxitin disk diffusion method for the daily detection of SARM with a high
21
22 56 number of *S. aureus* clinical isolates.
23
24
25
26

27 58 A total of 2,107 non-duplicate clinical *S. aureus* isolates were routinely
28
29 collected at Nantes University Hospital. All of the isolates were identified to the
30
31 species level by standard biochemical methods or with the VITEK2 system. VITEK2
32
33 60 AST-P551 cards were inoculated according to the manufacturer's instructions. The
34
35 cefoxitin disk diffusion method was performed with an inoculum of approximately 10^6
36
37 62 CFU/mL on Mueller-Hinton agar plates, according to the Antibiogram Committee of
38
39 the French Microbiology Society (CA-SFM) recommendations [7]. Zone diameters
40
41 64 were interpreted according to CA-SFM [7]. In the VITEK2 system, MIC interpretative
42
43 standards for oxacillin followed the recommendations of CLSI and CA-SFM [1, 7].
44
45 Susceptible or resistant results by both methods were considered as concordant.
46
47 66 Interpretative category errors were assessed: (i) very major error (VME, susceptible
48
49 with the VITEK2 system, but resistant by diffusion); (ii) major error (ME, resistant with
50
51 68 the VITEK2 system, but susceptible by diffusion); (iii) minor error (mE, susceptible or
52
53 resistant by one system and intermediate results by the other system). For each
54
55 70 discordant strain, the VITEK2 AST-P551 card and the cefoxitin disk diffusion test
56
57
58
59
60
61 72
62
63
64
65

1 were repeated and detection of *mecA* gene by PCR (8) and/or penicillin-binding
2 74 protein 2a (PBP2a) (Mast Diagnostics, Merseyside, U.K.) was performed, with *mecA*
3
4 PCR as the gold standard. Overall agreement based on previous category
5
6 76 agreement was 96.7%. (Table 1). Seventy (3.3 %) *S. aureus* isolates yielded
7
8 discordant results: 2 VME (0.1%), 4 ME (0.2%), and 64 mE (3.0%). Forty-four
9
10
11 78 isolates susceptible by VITEK2 and 20 isolates resistant by VITEK2 showed cefoxitin
12
13 inhibition zone diameters of 25 and 26 mm (intermediate breakpoints). Of the
14
15 preliminary 70 discordances, 59 (84%) were resolved, giving final overall agreement
16 80 of 99.5% (Table 2). Eleven MRSA strains (0.5%) remained discordant (mE). All these
17
18
19
20
21 82 11 MRSA harboured associated resistance to fluoroquinolones and/or
22
23 aminoglycosides and/or macrolide-lincosamide-streptogramin. The cefoxitin inhibition
24
25 zone diameter was 2 mm larger or smaller than the CA-SFM recommended criteria
26 84 for 467 concordant strains (401 MSSA and 66 MRSA).
27
28
29
30

31
32 86 Since 2001, the detection of oxacillin resistance by the cefoxitin disk diffusion
33
34 method was evaluated using both CLSI [1, 5, 9] and non-CLSI recommendations [3,
35
36 88 10]. The standard conditions differed by the use of media (MH agar, Iso-Sensitest
37
38 agar), a different inoculum size (semi-confluent with 10⁶ CFU/mL or confluent with
39
40 90 10⁸ CFU/mL), and several incubation temperatures. Felten *et al.* reported the
41
42 excellent performance of cefoxitin 30 µg disk on MHA with semi-confluent and
43
44 confluent inocula [3]. Among 152 *S. aureus* strains, one MRSA isolate and one
45
46 92 MSSA isolate gave a 26-mm cefoxitin inhibition zone diameter [3]. Thus, the cefoxitin
47
48 disk diffusion method using a semi-confluent inoculum on MHA was recommended
49
50
51 94 by CA-SFM [7]. Each strain with a cefoxitin zone diameter of 25 or 26 mm should be
52
53 examined by a *mecA*-specific test [7]. In our daily practice, the gap between the
54
55 measured inhibition zone diameters and the breakpoints was very narrow for 467
56 96 (22%) concordant strains. The 3% of intermediate cefoxitin diffusion results mostly
57
58
59
60
61
62
63
64
65

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.

References

- 1
2
3
4 128 1. **Clinical and Laboratory Standards Institute/NCCLS.** 2007. Performance
5
6 Standards for Antimicrobial Susceptibility Testing. 17th informational supplement
7
8
9 130 M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
- 10
11 2. **Barbier-Frebourg, N., D. Nouet, L. Lemee, E. Martin, and J. F. Lemeland.**
12
13 1998. Comparison of ATB Staph, Rapid ATB Staph, Vitek, and E-Test Methods
14 132 for Detection of Oxacillin Heteroresistance in *Staphylococci* Possessing *mecA*. J.
15
16 Clin. Microbiol. **36**:52-57.
17
18 134
- 19
20 3. **Felten, A., B. Grandry, P. H. Lagrange, and I. Casin.** 2002. Evaluation of Three
21
22
23 136 Techniques for Detection of Low-Level Methicillin-Resistant *Staphylococcus*
24
25 *aureus* (MRSA): a Disk Diffusion Method with Cefoxitin and Moxalactam, the
26
27 VITEK2 system, and the MRSA-Screen Latex Agglutination Test. J. Clin.
28 138 Microbiol. **40**:2766-2771.
29
30
31
32
- 33 140 4. **Roisin S., C. Nonhoff, O. Denis, and M. Struelens.** 2008. Evaluation of new
34
35 Vitek 2 card and disk diffusion method for determining susceptibility of
36
37 *Staphylococcus aureus* to oxacillin. J. Clin. Microbiol. **46**:2525-8.
38 142
39
- 40
41 5. **Swenson, J. M., D. Lonsway, S. McAllister, A. Thompson, L. Jevitt, W. Zhu,**
42
43 144 **and J. B. Patel.** 2007. Detection of *mecA*-mediated resistance using reference
44
45 and commercial testing methods in a collection of *Staphylococcus aureus*
46
47 expressing borderline oxacillin MICs. Diagn. Microbiol. Infect. Dis. **58**:33-39.
48 146
49
- 50
51 6. **Torres, E., S. Perez, R. Villanueva, G. Bou.** 2008. Evaluation of the Vitek 2
52
53 148 AST-P559 Card for Detection of Oxacillin Resistance in *Staphylococcus aureus*.
54
55 J. Clin. Microbiol. **46**:4114-4115.
56
- 57 150 7. **Communiqué de l'Antibiogramme de la Société Française de Microbiologie.**
58
59 2005. Bull. Soc. Fr. Microbiol. <http://www.sfm.asso.fr>.
60
61
62
63
64
65

- 152 8. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien,**
1 **G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito,**
2
3
4 154 **and K. Hiramatsu.** 2002. Dissemination of New Methicillin-Resistant
5
6 *Staphylococcus aureus* Clones in the Community. J. Clin. Microbiol. November
7
8
9 156 **40:** 4289-4294.
- 10
11 9. **Pottumarthy, S., T. R. Fritsche, and R. Jones.** 2005. Evaluation of alternative
12
13 disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an
14 158 international collection of *Staphylococci*: validation report from the SENTRY
15
16 Antimicrobial Surveillance Program. Diagn. Microbiol. Infect. Dis. **51:**57-62.
17
18 160
19
20
21 10. **Skov, R., R. Smyth, M. Clausen, A. R. Larsen, N. Frimodt-Moller, B. Olsson-**
22
23 162 **Liljequit and G. Kahlmeter.** 2003. Evaluation of a 30 µg disc on Iso-Sensitest
24
25 agar for detection of methicillin-resistant *Staphylococcus aureus*. J Antimicrob.
26
27 Chemother. **52:**204-207.
28 164
29
30
31
32
33 166
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 168
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26 170
27
28
29
30
31 172
32
33
34
35
36 174
37
38
39
40 176
41
42
43
44
45 178
46
47
48
49 180
50
51
52
53 182
54
55
56
57
58 184
59
60
61
62
63
64
65

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 I by Dif	R by VK2 I by Dif
2 VME	1		1 ^d	
4 ME		4		
64 mE	41	13		10 ^d
Total	42	17	1 ^d	10 ^d

190 VME, very major error, S with the VITEK2 system and R with the disk diffusion
 192 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.

194 ^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;

196 ^d associated resistances (fluoroquinolones, aminoglycosides).