Revista da Sociedade Brasileira de Medicina Tropical 47(3):377-381, May-Jun, 2014
http://dx.doi.org/10.1590/0037-8682-0123-2013

Short Communication

Performance of the Vitek 2 system software version 5.03 in the bacterial identification and antimicrobial susceptibility test: evaluation study of clinical and reference strains of Gram-positive cocci

Thiago Galvão da Silva Paim[1], *Vlademir Vicente Cantarelli*[1] *and Pedro Alves d'Azevedo*[1]

[1]. Laboratório de Cocos Gram-Positivos, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS.

ABSTRACT

Introduction. The genera *Enterococcus*, *Staphylococcus* and *Streptococcus* are recognized as important Gram-positive human pathogens. The aim of this study was to evaluate the performance of Vitek 2 in identifying Gram-positive cocci and their antimicrobial susceptibilities. **Methods.** One hundred four isolates were analyzed to determine the accuracy of the automated system for identifying the bacteria and their susceptibility to oxacillin and vancomycin. **Results.** The system correctly identified 77.9% and 97.1% of the isolates at the species and genus levels, respectively. Additionally, 81.8% of the Vitek 2 results agreed with the known antimicrobial susceptibility profiles. **Conclusion.** Vitek 2 correctly identified the commonly isolated strains; however, the limitations of the method may lead to ambiguous findings.

Keywords: Gram-positive cocci. Vitek 2 automated system.

Gram-positive cocci are widely distributed as part of the normal flora in humans; however, some species are recognized as major human pathogens and cause a large variety of infections worldwide. These microorganisms are frequently isolated from bloodstream infections, skin and soft tissue infections, sepsis, urinary tract infections and lower respiratory tract infections[1].

Automated bacterial identification in the clinical laboratory provides a rapid and reliable diagnosis for most pathogens involved in infectious diseases. A previous study demonstrated the satisfactory performances of the automated methodologies, resulting in their use in routine practice with a highly acceptable level of identification accuracy; additionally, automated identification enabled the interpretation of antimicrobial susceptibility tests for the correct treatment of patients[2].

The aim of the present study was to evaluate the performance of the Vitek 2 automated system in the identification of bacteria and antimicrobial susceptibilities of Gram-positive cocci isolates recovered from clinical samples and reference strains.

The study was performed at *Laboratório de Cocos Gram-Positivos* (LCGP) of the *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA) and *Laboratório Qualità*, Rio Grande do Sul, Brazil. The isolates included in the present study were selected from the strain collection belonging LCGP. A total of 104 isolates of Gram-positive cocci were analyzed, including 29 reference strains selected from the American Type Culture Collection (ATCC) and 75 clinical strains isolated from different patients; these strains included *Staphylococcus* coagulase-negative (n=36), *Enterococcus* spp. (n=33) and *Staphylococcus aureus* (n=6). All of these strains have been previously characterized by the LCGP with regard to their virulence factors using molecular methods and susceptibility profiles and were identified at the species level using conventional reference methods[3,4]. For the identification of staphylococci, the following characteristics were tested: catalase; colony morphology and pigmentation; Gram stain; hemolysis; susceptibility to novobiocin; polymyxin B; fosfomycin and deferoxamine; enzyme activity of arginine arylamidase; ornithine decarboxylase and urease; and acid production from trehalose, mannitol, mannose, xylose, cellobiose, arabinose, maltose, lactose, sucrose and raffinose. For the enterococcal isolates, the following phenotypic characteristics were evaluated: catalase; colony morphology and pigmentation; esculin hydrolysis in the presence of 40% bile; growth in 6.5% NaCl; motility; and acid production from mannitol, sorbose, arginine, arabinose, sorbitol, raffinose, sucrose, pyruvate and methyl-glucopyranoside (MGP). Susceptibility to oxacillin and vancomycin was evaluated using the broth microdilution and disk-diffusion reference methods according the Clinical and Laboratory Standards Institute (CLSI) documents M7-A6[5] and M100-S21[6], respectively. Vitek 2 (bioMérieux, Marcy L'E`toile, France) bacterial identification and antimicrobial susceptibility testing (AST) methods were evaluated according to the manufacturer's instructions.

Address to: Ms. Thiago Galvão da Silva Paim. Universidade Federal de Ciências da Saúde de Porto Alegre. Rua Sarmento Leite 245, 90050-170 Porto Alegre, RS, Brasil.
Phone: 55 51 3303-8742; **Fax:** 55 51 3303-8810
e-mail: thiagopucrs@gmail.com
Received 12 June 2013
Accepted 19 November 2013

The comparative analysis of the bacterial identification by Vitek 2 and the conventional methods was performed, and the accuracy (percentage of matched identification) was characterized. The AST results evaluated using the Vitek 2 automated system were compared to those obtained using the reference method, and the agreement rates were analyzed. The type of error was evaluated using the following criteria: I) very major error (VME) defined as a resistant bacterial isolate appearing susceptible by Vitek 2; II) major error (ME) defined as a susceptible isolate displaying a resistant profile by Vitek 2; or III) minor error (MiE) defined as microorganisms for which the AST reference indicated intermediate resistance and susceptibility or resistance by an automated AST test.

The Vitek 2 system correctly identified 81 (77.9%) and 101 (97.1%) of the 104 bacterial isolates at the species and genus levels, respectively. Additionally, *Staphylococcus aureus* was identified more quickly than the other staphylococcal isolates ($p<0.05$) (**Table 1**).

The Vitek 2 automated method was accurate for species-level identification of the commonly isolated Gram-positive cocci. All of the clinical isolates of *S. aureus* and *Enterococcus faecalis* were correctly identified. The bacterial isolates were misidentified or showed low-level discrimination in the clinical samples of *Staphylococcus epidermidis* (25%), *Staphylococcus haemolyticus* (66.7%), *Staphylococcus hominis* (16.7%), *Staphylococcus saprophyticus* and *Staphylococcus warneri* (both 20%). In the *Enterococcus* strains, *Enterococcus avium* and *Enterococcus durans* were misidentified as other enterococcal isolates, and the discordant results were found 50% of *Enterococcus casseliflavus*, 75% of *Enterococcus faecium* and 25% of *Enterococcus gallinarum* isolates. Among the 29 reference strains, a definitive species identification was provided by the automated system for 22 (75.9%) strains. The incorrect identification or low-level discrimination results are listed in **Table 2**. The minimal inhibitory concentrations (MICs) of oxacillin and vancomycin generated by the automated system were compared according to the error type, and the results are shown in the **Table 3**. In total, 45 of the 55 (81.8%) bacterial isolates agreed with the identifications made using the conventional antimicrobial susceptibility profile.

At the species level, the automated Vitek 2 system was able to identify the commonly isolated staphylococci and enterococci strains, such as *S. aureus* and *E. faecalis*, respectively, and all of these isolates, as well as the streptococci reference strains, were correctly identified. These results were in agreement with those reported by Chatzigeorgiou[7] et al., who evaluated the performance of the Vitek 2 system in comparison with other automated systems[7]. However, in the present study, the Vitek 2 version 5.03 software was not able to correctly identify all of the *S. epidermidis* isolates, with only 71.4% of the isolates matched at the species level.

S. haemolyticus is frequently isolated from blood cultures and has a tendency to develop resistance to multiple antimicrobial drugs[8], similar to *S. warneri*, which is another CoNS that can cause catheter-related bacteremia and native and prosthetic valve endocarditis[9]. Two isolates of this bacterial species were misclassified as *S. warneri* by the Vitek 2 system.

Two *S. saprophyticus* were misidentified as *S. warneri* and *S. cohnii* subs. *urealyticus* by the Vitek 2 automated system. According to the biochemical profile of these isolates, major error in the bacterial identification can be evaluated by novobiocin susceptibility, as *S. saprophyticus* and *S. cohnii* are resistant, while *S. warneri* is susceptible[3]. Moreover, *S. saprophyticus* and *S. cohnii* subs. *urealyticus* have similar

TABLE 1 - Descriptive measures from the automated system Vitek 2 compact.

Bacterial isolates (n)	Identification (%)		Time to identification (h)[a]
	genus level	species level	
Staphylococcus spp. (59)	96.7	78.0	6.03 (1.20)[b]
Staphylococcus aureus (11)	100.0	100.0	4.82 (0.68)[c]
Staphylococcus epidermidis (7)	100.0	71.4	6.18 (1.40)[c]
Others CoNS (41)	95.2	71.4	6.31 (1.14)[c]
Enterococcus sp. (36)	92.1	77.8	5.84 (1.12)[b]
Enterococcus faecalis (19)	100.0	100.0	5.53 (0.79)[d]
Enterococcus faecium (5)	100.0	40.0	5.60 (1.34)[d]
Others (12)	91.7	58.3	6.46 (1.30)[d]
Streptococcus spp. (9)	88.9	77.8	5.81 (1.49)[b]
Micrococacceae (59)	96.7	76.7	6.03 (1.21)[e]
Streptococcaceae (45)	95.5	77.8	5.84 (1.18)[e]

[a]mean (SD - standard deviation); analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test. The data were tested for normal distributions using the Kolmogorov-Smirnov test. [b]$p=0.71$(among the genus group). [c]$p<0.05$ (among the staphylococcal species). [d]$p=0.06$ (among the enterococcal species). [e]$p=0.41$ (between the Gram-positive cocci families). **CoNS:** coagulase-negative *Staphylococcus* non-*epidermidis*.

TABLE 2 - Bacterial species reported by the Vitek 2 System that were misidentified or showed low discrimination.

Reference bacterial identification (no. isolates)	Vitek 2 identification (% probability)
Staphylococcus caprae ATCC 35538 (1)	*Staphylococcus cohnii subs. urealyticus* (99%)
Staphylococcus epidermidis (1)	*Staphylococcus hominis subs. hominis* (51%)
	Staphylococcus hominis subs. novobiosepticus (49%)
Staphylococcus epidermidis ATCC 12228 (1)	*Staphylococcus epidermidis* (50%)
	Staphylococcus hominis subs. hominis (50%)
Staphylococcus haemolyticus (1)	*Staphylococcus warneri* (50%)
	Staphylococcus hominis subs. hominis (50%)
Staphylococcus haemolyticus (2)	*Aerococcus viridans* (50%)
	Staphylococcus hominis subs. hominis (50%)
Staphylococcus haemolyticus (1)	*Aerococcus viridans* (33%)
	Staphylococcus haemolyticus (33%)
	Staphylococcus hominis subs. hominis (33%)
Staphylococcus haemolyticus ATCC 29970 (1)	*Staphylococcus warneri* (95%)
Staphylococcus hominis subs. hominis (1)	*Staphylococcus auricularis* (50%)
	Staphylococcus hominis subs. hominis (50%)
Staphylococcus intermedius ATCC 29663 (1)	*Staphylococcus chromogenes* (89%)
Staphylococcus saprophyticus (1)	*Staphylococcus warneri* (93%)
Staphylococcus saprophyticus ATCC 15305 (1)	*Staphylococcus cohnii subs.urealyticus* (89%)
Staphylococcus warneri (1)	*Staphylococcus hominis subs. hominis* (50%)
	Staphylococcus warneri (50%)
Enterococcus avium (1)	*Enterococcus faecalis* (99%)
Enterococcus casseliflavus (1)	*Enterococcus casseliflavus* (50%)
	Enterococcus gallinarum (50%)
Enterococcus durans (1)	*Enterococcus hirae* (99%)
Enterococcus durans (1)	*Pediococcus acidilactici* (91%)
Enterococcus faecium (1)	*Enterococcus durans* (50%)
	Enterococcus faecium (50%)
Enterococcus faecium (1)	*Enterococcus gallinarum* (99%)
Enterococcus faecium (1)	*Enterococcus faecalis* (98%)
Enterococcus gallinarum (1)	*Enterococcus faecium* (51%)
	Enterococcus gallinarum (49%)
Streptococcus equi subs. equi ATCC 9528 (1)	*Enterococcus faecalis* (98%)
Streptococcus salivarius ATCC 7073 (1)	*Staphylococcus equinus* (98%)

Subs: subspecie.

TABLE 3 - Agreement of the antimicrobial susceptibility tests and the type of error among the staphylococci and enterococci isolates.[a]

	Agreements		Errors		Type of error (n)		
Isolates (n)	n	%	n	%	VME	ME	MiE
Staphylococci (33)[b]	28	84.8	5	15.2	3	2	0
Enterococci (22)[c]	17	77.3	5	22.7	1	4	0

[a]Streptococci reference strains were not tested. [b]Susceptibility to oxacillin. [c]Susceptibility to vancomycin. **VME:** very major error; **ME:** major error; **MiE:** minor error.

phenotypic profiles and exhibit low discriminatory power in bacterial identification; both species are urease positive and novobiocin resistant. The clinical laboratory exams should be able to differentiate these strains, as *S. cohnii* subs. *urealyticus* is recognized as a pathogen in infectious diseases, such as endocarditis, septicemia and urinary tract infections; however, previous study have reported difficulty in routinely identifying this microorganism[10].

In the present study, the agreement identification rate of the enterococci isolates was similar to a previous study, and all of the *E. faecalis* isolates were correctly identified[11]. However, 3 of the 5 *E. faecium* strains were misclassified or showed low-level discrimination. The bacterial identification of the *Enterococcus* isolates is important because this genus includes some of the most important multidrug-resistant organisms in healthcare-associated infections. These isolates usually affect patients who are debilitated by other concurrent illnesses or are undergoing prolonged hospitalization. Including the *E. faecium* strains, these pathogens have the ability to succeed in the hospital environment[12].

The automated system was able to identify 11 *Enterococcus*, 26 *Staphylococcus* and 37 *Streptococcus* species and subspecies using the Gram-Positive Card (GP-Card - bioMeriéux). New Gram-positive cocci species have been identified from clinical specimens in recent years, and these species exhibit phenotypic profiles similar to other staphylococcal strains[13]. Although these species were not included in our study, the tested strains allowed us to evaluate the accuracy of the automated system in identifying the common species isolated in the clinical setting.

Our data indicated that the Vitek 2 system provided inaccurate susceptibility test results for oxacillin and vancomycin, as the agreement rate with the reference method was very low, and the error rates, mainly VMEs and MEs, were higher compared to other studies[14]. Although our study has evaluated the antimicrobial susceptibility of commonly isolated strains, such as *S. aureus* and *E. faecalis*, most of the isolates were microorganisms that belong to coagulase-negative staphylococci (CoNS) and non-faecalis enterococci. A previous meta-analysis reported that the discordant results in bacterial identification could be explained by the metabolic rate of the bacterial isolates, as slow metabolism can lead to ambiguous reactions during the short incubation times used by the automated instruments[15]. These characteristics of the samples could interfere with the AST automated method, as the staphylococci and enterococci isolates exhibited very major errors.

In conclusion, the Vitek 2 Compact system software version 5.03 correctly identified the commonly isolated Gram-positive cocci; however, the limitations of the method may lead to ambiguous findings and the inability to identify uncommon microorganisms. Therefore, additional phenotypic tests may be necessary to identify some strains at the species level. Additionally, critical inquiry of the AST results reported by the automated method showed discrepancies in the antimicrobial susceptibilities that might occur in uncommon isolated pathogens.

ACKNOWLEDGMENTS

We are thankful to bioMeriéux for the opportunity to work with the Vitek 2 Compact system technology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

This work was supported by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS) (*Edital* Programa de Apoio a Núcleos Emergentes - PRONEM 03/2011 number 11/2058-5).

REFERENCES

1. Landrum ML, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, et al. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005-2010. JAMA 2012; 308:50-59.

2. Wallet F, Loïez C, Renaux E, Lemaitre N, Courcol RJ. Performances of VITEK 2 colorimetric cards for identification of gram-positive and gram-negative bacteria. J Clin Microbiol 2005; 43:4402-4406.

3. Bannerman TL, Peacock SJ. *Staphylococcus*, *Micrococcus* and other catalase-positive cocci. *In:* Murray PR, Baron EJ, Jorgensen J, Pfaller M, Landry ML, editors. Manual of Clinical Microbiology. Washington, D.C: ASM; 2007.

4. Teixeira LM, Carvalho MGS, Facklam RR. *Enterococcus*. *In*: Murray PR, Baron EJ, Jorgensen J, Pfaller M, Landry ML, editors. Manual of Clinical Microbiology, Washington, DC: ASM; 2007.

5. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Sixth edition. document M07-A6. Wayne, PA: CLSI; 2003.
6. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing - Twenty-first informational supplement. document M100-S21 Wayne, PA: CLSI; 2011.
7. Chatzigeorgiou KS, Siafakas N, Petinaki E, Argyropoulou A, Tarpatzi A, Bobola M, et al. Identification of staphylococci by Phoenix: validation of a new protocol and comparison with Vitek 2. Diagn Microbiol Infect Dis 2010; 68:375-381.
8. Barros EM, Ceotto H, Bastos MC, Santos KR, Giambiagi-Demarval M. *Staphylococcus haemolyticus* as an important hospital pathogen and carrier of methicillin resistance genes. J Clin Microbiol 2012; 50:166-168.
9. Arslan F, Saltoglu N, Mete B, Mert A. Recurrent *Staphylococcus warnerii* prosthetic valve endocarditis: a case report and review. Ann Clin Microbiol Antimicrob 2011; 23:14.
10. d'Azevedo PA, Antunes AL, Martino MD, Pignatari AC. *Staphylococcus cohnii* spp *urealyticus*: case report on an uncommon pathogen. Rev Soc Bras Med Trop 2008; 41:197-199.
11. d'Azevedo PA, Siquiera I, Gugel J, Antunes AL, Secchi C, Pasternak J, et al. Evaluation of the automated system Vitek 2 for identification and antimicrobial susceptibility testing of Brazilian Gram-positive cocci strains. Braz J Infect Dis 2009; 13:107-110.
12. Arias CA, Murray BE. The rise of the *Enterococcus*: beyond vancomycin resistance. Nat Rev Microbiol 2012; 16:266-178.
13. d'Azevedo PA. Comin G, Cantarelli V. Characterization of a new coagulase-negative *Staphylococcus* species (*Staphylococcus pettenkoferi*) isolated from blood cultures from a hospitalized patient in Porto Alegre, Brazil. Rev Soc Bras Med Trop 2010; 43:331-332.
14. Gherardi G, Angeletti S, Panitti M, Pompilio A, Di Bonaventura G, Crea F, et al. Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. Diagn Microbiol Infect Dis 2012; 72:20-31.
15. Chatzigeorgiou KS, Sergentanis TN, Tsiodras S, Hamodrakas SJ, Bagos PG. Phoenix 100 versus Vitek 2 in the identification of gram-positive and gram-negative bacteria: a comprehensive meta-analysis. J Clin Microbiol 2011; 49:3284-3291.