Evaluation of the Automated System Vitek2 for Identification and Antimicrobial Susceptibility Testing of Brazilian Gram-Positive Cocci Strains

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Automated instruments offer many advantages for clinical laboratories. Nevertheless, they can have problems identifying and determining susceptibilities of some pathogens. Vitek® 2 (bioMérieux) is an automated system that was recently introduced to Brazil. We evaluated the performance of this equipment for Brazilian isolates that had been characterized using reference identification and antimicrobial susceptibility testing methods. Ninety-nine strains of Gram-positive cocci from a local reference center collection were analyzed, consisting of 50 coagulase-negative *Staphylococcus* (CoNS) and 49 *Enterococcus* and related species. Vitek® 2 correctly identified 79.8% (79/99) of the isolates. Oxacillin resistance was detected in 76% (19/25) of resistant *S. epidermidis* strains and in 88% (22/25) of other resistant CoNS species strains. Vancomycin resistance was detected in 100% (20/20) of resistant *Enterococcus* and related species strains. Vitek® 2 performed very well for the identification of *S. epidermidis* and non-epidermidis staphylococci, and for the detection of vancomycin resistance in *Enterococcus* and related species. However, the system needs improvement in order to provide reliable results for the characterization of some CoNS species, identification of *Enterococcus* and related species and for detecting oxacillin resistance in CoNS.

Key-Words: Automated system, Vitek2, Gram-positive cocci, identification, antimicrobial susceptibility testing.

Automated instruments offer many advantages in clinical laboratories, especially in hospitals. Such instruments improve workflow and provide faster results than conventional methods. Nevertheless, they can have problems identifying and determining susceptibilities of some pathogens. Coagulase-negative staphylococci (CoNS) are the most common microorganisms isolated from blood cultures; however, 85% of the isolates are contaminants, usually from skin contamination at the time of collection [1]. Nevertheless, CoNS are recognized as important nosocomial pathogens; in many institutions, they are among the main agents of nosocomial bacteremias [2-4]. Many clinical laboratories do not routinely identify CoNS to the species level when these microorganisms are detected in blood or other body fluids. However, the significance of CoNS as pathogens has increased. Blood culture isolates should therefore be identified to the species level to determine their clinical relevance and monitor their epidemiology [5]. Resistance to methicillin among these microorganisms is a matter of concern, because of the increasingly high levels that have been detected. In a multicenter study in Brazil, methicillin resistance was observed in 87.7% of CoNS isolated from bloodstream infections [6]. Accurate detection of methicillin resistance among CoNS isolates in the clinical laboratory is important to guide therapy and to promote the correct use of glycopeptides [3,7].

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The importance of the enterococci as nosocomial pathogens has been widely documented in recent years; vancomycin-resistant enterococci (VRE) are particularly relevant due to their increasing prevalence and their capability to acquire antimicrobial resistance. *Enterococcus faecalis* is the species most frequently associated with human infections, followed by *Enterococcus faecium* [8].

In general, these two species are responsible for about 90 to 95% of enterococcal infections in humans. The remaining 5 to 10% are caused by other members of the genus.

Automated systems, such as Vitek® legacy (bioMérieux) and MicroScan® (DadeBehring), are also frequently used for rapid identification and antimicrobial susceptibility testing of Gram-positive cocci in clinical laboratories around the world [9-13]. Vitek®2 (bioMérieux) is an automated system recently introduced to Brazil. We evaluated the performance of this system with Brazilian isolates that had been characterized using reference methods of identification (ID) and antimicrobial susceptibility testing (AST).

Material and Methods

Isolates

Ninety-nine isolates of Gram-positive cocci from a local reference center collection were analyzed: 50 Coagulase-negative *Staphylococcus* (CoNS) and 49 *Enterococcus* spp. and related species. They were obtained from a culture collection maintained in the Microbiology department, Universidade Federal de Ciências da Saúde de Porto Alegre. The isolates were kept at -20ºC in skim milk (Difco) plus 20% glycerol.

Quality control of the tests was done using *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* SS 1274, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus saprophyticcus* CCM 883, *Staphylococcus hominis* ATCC 27844 and *Staphylococcus haemolyticcus* CCM 2737.

The isolates were identified by conventional biochemical tests [8,14].

Most isolates were also identified by analysis of electrophoretic whole-cell protein profiles according to the procedures described by Merquior et al. [15] for enterococci and by molecular (*sodA* sequencing) methods for CoNS [16]. *Conventional antimicrobial susceptibility testing* - Susceptibilities to oxacillin and vancomycin were detected by the disk-diffusion (DD), Etest® (AB Biodisk, Solna, Suécia) and agar dilution (CLSI) [17] procedures. The *mecA* and *vanA* alleles were detected by PCR; this was considered the "gold standard" method [18,19].

Identification and Antimicrobial Susceptibility Testing Using Automated Systems

Species identification and antimicrobial susceptibility were determined using the automated systems Vitek2® (bioMérieux – software 4,03), panel GP card (identification) and GP01 (susceptibility testing). The manufacturer's instructions were followed for the preparation of the inoculua and incubation of the isolates.

Results

Based on the combination of results obtained from the conventional biochemical tests, the 99 isolates of CoNS, enterococci and related genera were identified as follows: CoNS - 25 *S. epidermidis*, 3 *S. haemolyticus*, 3 *S. sciuri*, 2 *S. warneri*, 2 *S. cohnii* subsp. *cohnii*, 2 *S. capitis* subsp. *capitis*, 2 *S. hominis* subsp. *hominis*, 2 *S. hominis* subsp. *novobiosepticus*, 2 *S. cohnii* subsp. *urealyticus*, 1 *S. caprae*, 1 *S. auricularis*, 1 *S. equorum*, 1 *S. lugdunensis*, 1 *S. saprophyticus*, 1 *S. simulans* and 1 *S. xilosus*. Enterococci and related genera - 17 *E. faecium*, 9 *E. faecalis*, 7 *E. gallinarum*, 3 *E. avium*, 2 *E. casseliflavus*, 2 *E. hirae*, 1 *E. durans*, 1 *E. raffinosus*, 1 *Lactococcus garvieae*, 2 *Leuconostoc pseudomesenteroides*, 2 *Streptococcus bovis* and 2 *Pediococcus* sp.

Vitek2 correctly identified 80 of the 99 isolates (accuracy of 80.8%), 43/50 (86%) of CoNS and 37/49 (75.5%) of *Enterococcus* sp. and related species. Among CoNS, the identifications were concordant for *S. epidermidis, S. caprae, S. capitis* subsp. *capitis, S. cohnii* subsp. *urealyticus, S. lugdunensis, S. haemolyticus, S. hominis* subsp. *hominis, S. sciuri,* and *S. warneri*. The system failed to identify the other species and subspecies tested. Among *Enterococcus* sp. and related species the identifications were as follows: *Enterococcus* sp. 71.4% (30/42), *Pediococcus* sp. 0% (0/2), *Leuconostoc pseudomesenteroides* 100% (2/2), *Lactococcus garviae* 100% (1/1), and *Streptococcus bovis* 0% (0/2) (Table 1). Methicillin resistance was associated with the *mecA* gene in 19 of the 25 samples (76.0%) in *S. epidermidis* isolates and in 22/25 (88%) in other CoNS species. Vancomycin resistance was detected in 100% of the *Enterococcus* species and related genera (20/20) (Tables 1 and 2).

Discussion

Automated methods are faster and improve laboratory workflow. Most of the discrepant results in identification involved the less-frequently-isolated species. We need to be cautious when CoNS other than *S. epidermidis* are identified with this system. Nonhoff et al. (2005) [20] found similar limitations when he tested Vitek®2 on methicillin-resistant staphylococci from Belgium. We must also be cautious with the less frequent *Enterococcus* sp. and related species. Vitek®2 performed well and identified these two species; it correctly identified 25/26 (96.2%) of *E. faecalis* and *E. faecium*, which are normally responsible for approximately 90% to 95% of enterococcal infections in humans.

For less-frequently-isolated species, Vitek®2 may need improvement. A major mistake was made with *E. gallinarum*, an important vancomycin-resistant pathogen, which should be correctly identified in a clinical hospital laboratory.

The system had good performance in the determination of methicillin resistance, especially for *S.* non-*epidermidis* 22/25 (88%). However, the interpretative criteria of the CLSI (2005) may overestimate resistance of the other species. The discrepancies in the susceptibility tests for CoNS included major errors. The Minimum Inhibitory Concentration (MICs) determination of the strains that showed false resistance were near the established breakpoints to classify them as susceptible. According to the CLSI (2008) [17], strains isolated from serious infections that have MICs varying from 0.5 to 2.0 μL/mL should be tested for the presence of the *mecA* gene or for the protein expressed by this gene, considering that they may present confusing phenotypes. Less frequent species of CoNS have been associated with serious infections in hospital institutions, and these have become increasingly common.

The advantages of automated systems for species identification and antimicrobial susceptibility testing are speed and better workflow. Improvements in their accuracy would help make them practical.

In the analysis of vancomycin resistance in *Enterococcus* sp. and related species the automated system detected 20/20 isolates (100%). Considering that vancomycin-resistant enterococci require implementation of infection control measures, some species identification is needed.

The reporting time for CoNS identification by the VITEK 2 system ranged from 4.25h to 8h, and the mean time to result was 5.79h. Enterococci required from 3.25 h to 8 h to identify; the mean time was 5.78 h.

In conclusion, the automated system Vitek2 needs further improvement in order to provide reliable results for the characterization of the other CoNS and enterococci-related species and for detection of oxacillin resistance.

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Table 1. Comparison of identification of isolates by the Vitek2 automated system with identifications using conventional identification methodology.

Conventional identification	Vitek2 identification
Staphylococcus epidermidis (n = 25)	*S. epidermidis* (n = 25)
S. haemolyticus (n = 3)	*S. haemolyticus* (n = 3)
S. sciuri (n = 3)	*S. sciuri* (n = 3)
S. warneri (n = 2)	*S. warneri* (n = 2)
S. cohnii subsp *cohnii* (n=2)	*S. cohnii* subsp *urealyticus* (n = 2)
S. capitis subsp *capitis* (n=2)	*S. capitis* subsp *capitis* (n=2)
S. hominis subsp *hominis* (n=2)	*S. hominis* subsp *hominis* (n=2)
S. hominis subsp *novobiosepticus* (n=2)	*S. hominis* (n=2)
S. cohnii subsp *urealyticus* (n=2)	*S. cohnii* subsp *urealyticus* (n=2)
S. auricularis (n=1)	*S. warneri* (n = 1)
S. caprae (n=1)	*S. caprae* (n=1)
S. equorum (n=1)	*S. xilosus* (n=1)
S. lugdunensis (n=1)	*S. lugdunensis* (n=1)
S. saprophyticus (n =1)	*S. warneri* (n = 1)
S. simulans (n =1)	*S. haemolyticus* (n=1)
S. xilosus (n=1)	*S. hominis* (n=1)
Enterococcus faecium (n=17)	*E. faecium* (n=16)
	E. gallinarum (n=1)
E. faecalis (n=9)	*E. faecalis* (n=9)
E. gallinarum (n=7)	*E. gallinarum* (n=3)
	E. casseliflavus (n=1)
	E. durans (n=1)
	E. faecalis (n=2)
E. avium (n=3)	*E. avium* (n=2)
	E. faecium (n=1)
E. casseliflavus (n=2)	*E. casseliflavus* (n=1)
E. hirae (n=2)	*E. hirae* (n=1)
	E. durans (n=1)
E. durans (n=1)	*E. hirae* (n=1)
Lactococcus garvieae (n=1)	*Lactococcus garvieae* (n=1)
Leuconostoc pseudomesenteroides (n=2)	*Leuconostoc pseudomesenteroides* (n=2)
Streptococcus bovis (n=2)	*Lactococcus garvieae* (n=1)
	Pediococcus (n=1)
Pediococcus (n=2)	*E. faecalis* (n=2)
Total	**99**

Table 2. Discrepancies in the susceptibility tests and type of error in identifying coagulase-negative *Staphylococcus* based on PCR identification of the *mecA* gene compared with the automatic testing system Vitek2.

Isolates	*mecA*	Vitek2 Phenotype	Error
S. epidermidis (n=1)	Pos.	S	VM
S. epidermidis (n=5)	Neg.	R	M
S. cohnii subsp. *urealyticus* (n=1)	Pos.	S	VM
S. warneri (n=1)	Pos.	S	VM
S. capitis (n=1)	Neg.	R	M

S: sensitive; R: resistant; M: major error; VM: very major error; Pos.: positive; Neg.: negative.

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