Evaluation of the Automated System Vitek2 for Identification and Antimicrobial Susceptibility Testing of Brazilian Gram-Positive Cocio 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 cocio, identification, antimicrobial susceptibility testing.

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 -20°C 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 saprophyticus CCM 883, Staphylococcus hominis ATCC 27844 and Staphylococcus haemolyticus 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 inocula 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. cohni subsp. cohni, 2 S. capitis subsp. capitis, 2 S. hominis subsp. hominis, 2 S. hominis subsp. novobiosepticus, 2 S. cohni subsp. urealyticus, 1 S. caprae, 1 S. auricularis, 1 S. equorum, 1 S. lugdunensis, 1 S. saprophyticus, 1 S. simulans and 1 S. silius. 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 garviae, 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. cohni 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 μg/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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References
