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Evaluation of VITEK 2 Compact and VITEK MS in the identification of coagulase-negative staphylococci isolated from blood cultures

Avaliação do VITEK 2 Compact e VITEK MS na identificação de estafilococos coagulase-negativos isolados de hemoculturas

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ABSTRACT

Introduction: In most clinical laboratories, coagulase-negative staphylococci (CNS) are classified through their biochemical characteristics using traditional and/or automated phenotypic methods. However, identification by phenotypic method is a great challenge despite the improvement in identification by automated systems in recent years. **Objective:** The main objective was to compare the identification of CNS between two automated methods: phenotypic (VITEK® 2 Compact, bioMérieux) and mass spectrometry (MALDI-TOF VITEK® MS, bioMérieux). **Materials and method:** 133 CNS isolates from whole blood samples from September 2015 to May 2016 were analyzed. **Results:** All the 133 isolates were identified by VITEK® 2 Compact as CNS, whereas VITEK MS did not identify seven, and there was uncertainty among three species in one isolate. The two devices had a concordance of 92.8% (117/126) of which: 56 *Staphylococcus epidermidis*, 34 *Staphylococcus hominis* ssp. *hominis*, 15 *Staphylococcus haemolyticus*, 10 *Staphylococcus capitis*, one *Staphylococcus cohnii* ssp. *urealyticus* and one *Staphylococcus sciuri*. There was disagreement in identification of *S. lugdunensis* and *S. aureus*. **Conclusion:** Through the analysis between the two devices and based on scientific articles comparing the methods involved in the study, it was possible to observe the great biochemical similarity among the coagulase-negative staphylococci species, this being the main limitation of the phenotypic method. The VITEK MS equipment showed high discrimination between species when compared to the gold standard method, besides being rapid and easy to use.

Key words: mass spectrometry; *Staphylococcus*; staphylococcal infections.

INTRODUCTION

In 2014, 47 species and 23 subspecies of the *Staphylococcus* genus were described, with the *S. aureus* being the best known, and frequently associated with a series of infections and severe intoxications in human beings[1, 2]. Most species belong to the group of coagulase-negative staphylococci (CNS), which for many years were considered skin-colonizing or rarely pathogenic bacteria[2].

CNS are among the most common microorganisms isolated from blood stream infections. They are, in general, connected with foreign body infections, being classified as healthcare-associated infections[3]. However, they are some of the most common blood culture contaminants, thus the use of different clinical and laboratory criteria is necessary to distinguish between contamination and clinically significant bacteremia[4].

In most clinical laboratories, CNS are classified through their biochemical characteristics using traditional and/or automated phenotypic methods. Nevertheless, identification by a phenotypic method is a great challenge, despite the improvement in identification by automated systems in the latest years. Besides taxonomy of the *Staphylococcus* genus has been evolving over the years, the use of commercial kits based on biochemical reactions does not produce reliable results for CNS identification in clinical samples[5-7]. Several automated methods based on phenotypic characteristics have been developed to improve identification,

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but there are limitations because they depend on the expression of metabolic activities and/or morphologic characteristics, and, principally, due to phenotypic differences between isolates of the same species[1, 2, 8].

There have been new technologies to devise more rapid precise and sensitive methods aimed at enhancing assistance to patient and therapy, as, for instance, genetic sequencing, which has a highly discriminatory power of diagnosis, uncovering numerous errors in phenotypic identification and becoming, as a result, the gold standard method for microorganism identification[9]. However, such methods are employed mainly by reference laboratories and require specialized instrumentation, dedicated laboratory space and highly trained staff. Such restrictions are unfeasible for most laboratories, and because of that, the use of automated systems based on phenotypic analysis still predominates, despite imperfections in precision, robustness and the need for confirmatory testing[9].

The Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) method comes as an alternative for biochemical analysis and identification based on gene sequencing[10]. The main advantage of MALDI-TOF technology over other laboratory techniques for microorganism identification is that it is rapid to obtain results. From inoculum preparation to final reading, an isolated result can be obtained in less than 30 minutes[11]. Nowadays two instruments like these are available in the market and registered at the Ministry of Health: Microflex LT™ (Bruker Daltonics/BD Diagnostics) and VITEK MS™ (bioMérieux)[12].

Therefore, this study aims principally to evaluate the identification of CNS isolated in the laboratory routine by means of an automated phenotypic method and mass spectrometry, so as to compare both methods.

MATERIALS AND METHOD

The microorganisms came from whole blood samples collected between September 2015 and May 2016. Because this is a comparative study, there were not clinical criteria for sample selection. Samples of a same patient were rejected. All microorganisms that had an index greater than or equal to 90% confidence level were included in the study. In the VITEK® 2 Compact device, identification was made by the Advanced Colorimetry™ technology, while VITEK MS used MALDI-TOF technology.

Blood culture vials were incubated in the BacT/ALERT® (bioMérieux) instrument for a maximum period of five days. Positive samples in this period were streaked onto growth media sheep-blood agar and chocolate agar (bioMérieux). The plates were incubated in bacteriological incubator at 35ºC ± 1ºC for 18-24 hours.

The identification of all CNS isolated in the procedure was firstly made by VITEK 2 Compact according to the manufacturer's manual. GP TEST KIT VTK 2 card was used for identification of all Gram-positive cocci.

The isolates already identified were stored in 20% Skim Milk growth medium and frozen at -20ºC for later identification by VITEK MS. Following, the stored samples were thawed, activated in brain heart infusion (BHI) broth, and streaked in sheep-blood agar (bioMérieux). Microorganisms were then identified by VITEK MS. Later, the results yielded by identification with the automated phenotypic method were compared to those from MALDI-TOF MS, so as to compare both methods.

RESULTS

A total of non-repeated 133 isolates from blood cultures were analyzed. CNS identification was made by an automated phenotypic method and proteomic analysis with VITEK 2 Compact and VITEK MS devices, respectively.

Both instruments had a 92.8% (117/126) agreement, of which 56 were *Staphylococcus epidermidis*, 34 *Staphylococcus hominis* ssp. *hominis*, 15 *Staphylococcus haemolyticus*, 10 *Staphylococcus capitis*, one *Staphylococcus cohnii* ssp. *urealyticus*, and one *Staphylococcus sciuri*. All the 133 isolates were identified by VITEK® 2 Compact as CNS, while VITEK MS did not identify seven samples; there was uncertainty between three species in an isolate.

The isolates not identified by MALDI-TOF technology were reassessed in duplicate, and still their characterization was not possible. There was an inconclusive result in the identification of a microorganism by MALDI-TOF, with uncertainty between *Staphylococcus xylosus*, *Staphylococcus saprophyticus* and *Staphylococcus cohnii* ssp. *urealyticus*. This same microorganism was identified by another method as *Staphylococcus xylosus*, with 95% confidence level.

The isolates with the wider disagreement in identification were *Staphylococcus hominis* ssp. *hominis* and *Staphylococcus epidermidis*. The microorganism *Staphylococcus lugdunensis*, identified by the phenotypic method, was identified by MALDI-TOF as *Staphylococcus aureus*, this being a coagulase-positive staphylococcus. All conflicting results were found at the species level (**Table**).

TABLE – Disagreeing results in CNS identification by VITEK® 2 Compact and VITEK MS (bioMérieux, France)

VITEK® 2 COMPACT	%	VITEK MS	%
Staphylococcus hominis ssp. *hominis*	93	*Staphylococcus epidermidis*	99.9
Staphylococcus lugdunensis	95	*Staphylococcus aureus*	99.9
Staphylococcus hominis ssp. *hominis*	97	*Staphylococcus saprophyticus*	99.9
Staphylococcus hominis ssp. *hominis*	93	*Staphylococcus capitis*	99.9
Staphylococcus hominis ssp. *hominis*	93	*Staphylococcus haemolyticus*	99.9
Staphylococcus hominis ssp. *hominis*	98	*Staphylococcus epidermidis*	99.9
Staphylococcus xylosus	95	*S. xylosus/S. saprophyticus/ S. cohnii* ssp. *urealyticus*	-
Staphylococcus hominis ssp. *hominis*	99	*Staphylococcus epidermidis*	99.9
Staphylococcus epidermidis	99	*Staphylococcus hominis* ssp. *hominis*	99.9

CNS: coagulase-negative staphylococci.

DISCUSSION

In the latest years, CNS identification was commonly carried out by phenotypic methods through automated commercial systems that many times could not differentiate the variable expression of some staphylococcal phenotypic characteristics. The *S. epidermidis* group is characterized by the pronounced genomic diversity, while other species such as *S. haemolyticus*, *S. lugdunensis* and *S. schleiferi* present less diversity as pulsed-field gel electrophoresis shows[1].

Staphylococcus epidermidis is the most common species found in infections, followed by *S. hominis*, *S. haemolyticus* and *S. capitis*[1]. The group *S. epidermidis* comprises a common pathogen, needing a rapid decision each time it is found in clinical samples, because it can represent a true infection or just colonization.

Layer *et al*. (2006)[2] conducted a study assessing CNS identification with different methods, which showed 95.24% agreement with the gold standard method (VITEK 2 Compact, bioMérieux) and 76.19% (Phoenix®, BD Diagnostics, Sparks) for *S. epidermidis*. Another study, conducted by Ligozzi *et al*. (2002)[13], demonstrated that 86 out of the 100 CNS isolates were identified at the species level, of which *S. epidermidis* was identified with 92.7% of accuracy.

Spanu *et al.* (2003)[14] demonstrated that VITEK® 2 Compact identified correctly 90.5% of CNS and clearly indicated there is still necessity of improvement in the identification of some species by the phenotypic method, especially in *S. hominis.* isolates. This fact can explain discrepancies observed in our study, in which *S. hominis* appears in seven out of the nine disagreeing isolates.

MALDI-TOF was between three species in an isolate identified by VITEK® 2 Compact as *Staphylococcus xylosus*. The identified species were *S. xylosus*, *S. saprophyticus* and *S. cohnii* ssp. *urealyticus.* A study carried out by Delport *et al.* (2015)[15] compared the CNS identification with MALDI-TOF, VITEK® 2 Compact and gas chromatography and used strains previously identified by the Kloos and Schleifer[16, 17] classification scheme. A *Staphylococcus saprophyticus* was identified as *Staphylococcus xylosus* by MALDI-TOF and gas chromatography; it was confirmed by rRNA16S sequencing as *Staphylococcus xylosus.* Another study, performed by Lee *et al.* (2013)[7], compared identification of CNS from urine isolates among several methods and verified that two isolates of *Staphylococcus cohnii* were identified as *Staphylococcus xylosus* by VITEK® 2 Compact with 95% confidence level.

In general, novobiocin-resistant staphylococci are reported as *Staphylococcus saprophyticus*, but nowadays other CNS species that are also novobiocin-resistant have been recognized, such as *S. saprophyticus*, *S. cohnii* subsp. *cohnii*, *S. cohnii* subsp. *urealyticus*, *S. sciuri* and *S. xylosus*[18, 19]. According to McTaggart (1989)[20], *S. saprophyticus* presumptively identified just by Gram stain, catalase, coagulase, novobiocin resistance and DNAse reaction had biochemical profiles similar to species *S. cohnii*, *S. chrornogenes*, *S. intermedius*, *S. xylosus* and *S. simulans*, which share phenotypic similarities with *S. saprophyticus*. This finding illustrates heterogeneity of CNS resistant to novobiocin. Such resistance is generally encoded by plasmids and thus, inter-species transfer of novobiocin-resistant genes is possible.

An isolate of *Staphylococcus lugdunensis* identified by VITEK® 2 Compact with 95% confidence level was identified by VITEK MS as *Staphylococcus aureus*. Identification of *S. lugdunensis* by phenotypic method may reveal the presence of specific surface proteins, leading to positive slide coagulase and clumping factor tests, suggesting *S. aureus.* Although classified as CNS, *S. lugdunensis* has pathogenic features similar to those of *S. aureus*, therefore it requires the same antibiogram interpretative criteria as those recommended for *S. aureus* and different from other CNS[21]. For this reason, the correct species discrimination is important on laboratory routine for characterization of susceptibility testing.

Studies show the efficiency of mass spectrometry technology, relating it to phenotypic methods with the same or better accuracy in identification. Dupont *et al.* (2010)[22] compared CNS identification between systems VITEK® 2 and Phoenix® (Becton, Dickinson) – 234 CNS (20 species) were isolated –. The study used as an identification reference the *sodA* gene sequencing. On the whole, VITEK MS, VITEK® 2 and Phoenix® correctly identified 93.2%, 75.6% and 75.2% of the isolates, respectively. Another study, now using BioTyper 2.0 software instead of the in-house database, compared the results of VITEK MS with the sequencing of the *tuf* gene for identification of 62 CNS. All the isolates in this study were identified at species level, demonstrating a 100% agreement with

the reference method for CNS identification[23]. Therefore, VITEK MS showed high precision in identification of CNS at species level, in a simple and rapid form.

In the study performed by Loonen *et al.* (2012)[24], five methods were compared to evaluate accuracy in CNS identification. Results showed identification of 85.9%, 92.3%, 70.4%, 93% and 99.3% for ID 32 Staph strip (bioMérieux), VITEK 2 (GP TEST KIT VTK 2), partial sequencing of RNA 16S (MicroSeq; Applied Biosystems), partial sequencing of the *tuf* gene (in-house) and Microflex LT MS (Bruker Daltonics), respectively. MALDI-TOF presented superiority in CNS identification in this study, in which 99.3% of isolates were correctly identified.

All CNS, except *Staphylococcus lugdunensis*, have the same criteria for susceptibility testing recommended by the document M100-S26 of Clinical Laboratory Standards Institute (CLSI) – Performance Standards for Antimicrobial Susceptibility Testing –. In these cases, incorrect identification of CNS affects mainly the emission of epidemiological data to the hospital infection control service and the clinical interpretation in case of two or more different CNS in blood cultures, showing a collection-related contamination. The erroneous identification of *Staphylococcus lugdunensis* as another CNS can result in therapeutic failure, because the criteria of the antibiogram cut-off point are the same as those of *S. aureus* and are rarely considered contaminants in sterile sites.

In this study there was 92.8% agreement in CNS identification between both proposed methods. The non-identification of the seven isolates by VITEK MS can be associated with: 1) an interferent in the slide preparation; 2) contamination in the isolate streaking; or 3) incubation time.

As a limitation of this study, we can refer the fact that it does not have a gold standard method to evaluate identifications between both instruments, as well as to analyze accuracy of each method.

CONCLUSION

After comparison between the automated phenotypic method and proteomic analysis, based on studies comparing both methods with a gold standard method, a phenotypic similarity was observed between isolates of the same species, this being the main limitation of the phenotypic method. The technology of mass spectrometry displayed high discrimination in species identification.

Differentiation between CNS species becomes more crucial in blood cultures, in which the presence of two CNS in different samples can rule out a true bacteremia.

With descriptions of many new species found in the last two decades and improved approaches to identification in diagnosis, other microorganisms of the group became evident. At an era in which proteomics and the whole genome sequencing have been established, such species can be increasingly better identified.

RESUMO

Introdução: *Estafilococos coagulase-negativos (ECN) estão entre os principais colonizantes de pele e mucosas, atuando como comensais. Apesar de apresentarem baixa virulência, podem causar infecções graves, principalmente quando são utilizados dispositivos invasivos, como cateteres. Como têm grande capacidade de formar biofilme, conseguem atingir rapidamente a corrente sanguínea, o que pode levar à septicemia.* ***Objetivo:*** *O objetivo principal foi comparar a identificação dos ECN entre duas metodologias automatizadas: fenotípica (VITEK® 2 Compact, bioMérieux) e espectrometria de massa (MALDI-TOF VITEK® MS, bioMérieux).* ***Materiais e método:*** *Foram analisados 133 isolados de ECN provenientes de amostras de hemoculturas, no período de setembro de 2015 a maio de 2016.* ***Resultados:*** *Todos os 133 isolados foram identificados no VITEK® 2 Compact como ECN, enquanto o VITEK MS não identificou sete amostras; houve incerteza entre três espécies em um isolado. Os dois equipamentos tiveram concordância de 92,8% (117/126), dos quais foram 56* Staphylococcus epidermidis*, 34* Staphylococcus hominis *ssp.* hominis*, 15* Staphylococcus haemolyticus*, 10* Staphylococcus capitis*, um* Staphylococcus cohnii *ssp.* urealyticus *e um* Staphylococcus sciuri*. Houve discordância em uma identificação de* S. lugdunensis *e* S. aureus*.* ***Conclusão:*** *Por meio da análise entre os dois equipamentos e com base em artigos científicos que comparam os métodos envolvidos neste estudo, foi possível observar a grande similaridade bioquímica entre as espécies de ECN, sendo esta a principal limitação do método fenotípico. O equipamento VITEK MS apresentou alta discriminação entre as espécies quando comparado com o método* gold standard*, além da facilidade e da agilidade da técnica.*

Unitermos: espectrometria de massas; Staphylococcus*; infecções estafilocócicas.*

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