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Short Communication

Is it possible to perform bacterial identification and antimicrobial susceptibility testing with a positive blood culture bottle for quick diagnosis of bloodstream infections?

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Abstract

Introduction: Bloodstream infections can be fatal, and timely identification of the etiologic agent is important for treatment. **Methodology:** An alternative method, consisting of direct identification and susceptibility testing of blood culture bottles using the automated VITEK 2® system, was assessed. **Results:** All 37 of the Gram-negative bacilli (GNB) identifications and 57.1% of the 28 Gram-positive cocci (GPC) identifications matched those obtained with standard methods. In susceptibility testing, the agreement was greater than 90%. **Conclusions:** This alternative methodology may assist in the early identification and susceptibility testing of GNB. Further research is necessary to develop appropriate methods for GPC.

Keywords: Blood culture. Sepsis. Rapid diagnosis.

Bloodstream infections (BSI) in general hospitals are serious and life-threatening and are ranked as the third leading cause of health care-related infections. It is vital to prevent BSI from progressing to sepsis, which is a life-threatening organ dysfunction caused by a deregulated immune response to infection, and consequent septic shock (sepsis followed by profound circulatory and cellular/metabolic abnormalities that substantially increase mortality). Rapid identification of the etiologic agent allows for timely administration of appropriate antibiotic therapy[1].

The Surviving Sepsis Campaign (SSC) recommends that, after identifying sepsis or septic shock, the initial empirical antimicrobial treatment include one or more antimicrobial agents that act against all likely pathogens (bacterial, fungal and/or viral). These agents must be administered within 1 hour of identifying septic shock. A study in the United States and Canada of patients diagnosed with septic shock showed that of those who received effective antimicrobial therapy within the first hour, 79.9% survived and were discharged[1,2].

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The blood culture method is still considered the gold standard for the identification of bacteria in Bloodstream. However, even automated identification methods require time for growth in broth, subculture on solid medium, and bacterial identification by manual or automated methods[3].

With BSI, prevention of progression to sepsis or septic shock is directly dependent on the timing of proper antibiotic therapy. Therefore, molecular techniques have been developed to accelerate one or more steps of the diagnostic workflow based on pathogen detection by fluorescence, hybridization probes, polymerase chain reaction (PCR), or mass spectrometry. These methods are used for rapid identification of the bacteria, providing results within a few hours after the first sign of growth in automated blood cultures[3,4]. However, these methods depend require costly equipment for microbiological laboratories. Thus, this study was aimed to reduce the time for identification of bacterial agents and characterization of the resistance profile through direct use of blood culture broth using automated phenotypic methods, which are available in most major laboratories.

A qualitative prospective study of 65 patients who presented positive blood culture samples was performed in a large university hospital in the City of Curitiba, PR from June to November 2015. After positive detection by using the automated

BACTEC FX® (Becton Dickinson, Sparks, MD, USA), a Gram stain test was used for morphological identification, as well as standard and alternative methodologies for bacterial identification and assessment of antimicrobial susceptibility.

The standard methodology consisted of subculture of the blood culture on chocolate agar incubated at 35°C +/- 2°C for 18-24 hours and identification and antimicrobial agent susceptibility testing using the VITEK® 2 Compact system (bioMérieux, Durham, NC, USA). Samples that presented growth of more than one bacterial species were excluded from the analysis, since pure cultures are required to obtain reliable identification and susceptibility testing results using this automated method. For the proposed alternative method, a 5 ml sample was taken from the positive blood culture bottle and transferred to a tube under aseptic conditions. The sample was centrifuged for 5 minutes at 500g. The supernatant was removed and centrifuged for 10 minutes at 1,000g (time and speed were optimized by the authors). Then, the supernatant was discarded, and the pellet was used to make a McFarland suspension (in 0.45% NaCl), which was subjected to identification and antimicrobial susceptibility testing using the VITEK 2® Compact system, according to the manufacturer's instructions.

When analyzing the susceptibility testing results, errors were considered minor when the alternative test method yielded an intermediate result and the standard method results were *susceptible* or *resistant* and vice versa. Errors were considered major when the alternative method result was resistant and the standard method result was susceptible. Errors were considered very major when the alternative method result was susceptible, and the standard method result was resistant.

Of the 65 samples analyzed in this study, 37 were gram-negative bacilli (GNB) and 28 were gram-positive cocci (GPC). A total of 53 (81.5%) isolates were correctly identified by the alternative method when compared to the standard method identification, and the remaining 12 (18.5%) were not identified or were incorrectly identified by the alternative method.

Several studies have assessed the performance of automated blood culture testing directly from bottles in an attempt to decrease the time required to diagnose sepsis and promote quicker treatment[5,6].

We obtained 100% agreement in the identification of GNB with the alternative automated method using blood culture broth without subculture. Other authors have also obtained satisfactory results, ranging from 86% to 96% agreement, for the correct identification of isolates[6]. Only one study performed in Spain obtained an inferior correlation, with only 62% agreement between the two methods[7].

In the identification of GPC, similar identification results were obtained for 16 (57.1%) samples using the two methods, while 12 (42.9%) yielded different results or were not identified (**Table 1**). Discrepancies were observed for three *Staphylococcus aureus* isolates that were identified as *Staphylococcus intermedius* and for one *Staphylococcus aureus* isolate that was identified as *Gemella sanguinis*.

Staphylococcus intermedius is a coagulase-positive *Staphylococcus* species that is part of the normal skin microbiota of dogs, pigeons, horses, and goats, but is rarely isolated from humans. Its similarity with *S. aureus* might have been the cause of the incorrect identification[8].

Gemella sanguinis is an opportunistic bacterium that causes infective endocarditis. It is a gram-positive, catalase-negative, facultatively anaerobic coccus that is commonly misidentified as *Streptococcus viridans* due to similarities in colony morphology and biochemical properties[9]. It is believed that discrepancies in the direct identification of GPC might occur due to either insufficient inoculum or the presence of mixed cultures[8]. However, the latter hypothesis should be discarded, as this study only used samples containing a single type of bacterium. Similar data were reported in a previous study in which five *S. aureus*

TABLE 1: Results of direct bacterial identification using positive blood culture.

Bacterial species	Total	Identification agreement*
Gram-negative bacilli	**37**	**37**
Acinetobacter baumannii complex	3	3
Enterobacter aerogenes	2	2
Enterobacter cloacae complex	4	4
Escherichia coli	11	11
Klebsiella oxytoca	1	1
Klebsiella pneumoniae	10	10
Pseudomonas aeruginosa	4	4
Serratia marcescens	2	2
Gram-positive cocci	**28**	**16**
Staphylococcus aureus	12	8
Staphylococcus coagulase negative	6	3
Streptococcus anginosus group	1	0
Streptococcus pneumoniae	1	1
Streptococcus viridans group	2	0
Enterococcus faecium	2	2
Enterococcus faecalis	4	2

*Number of samples in which the identification results obtained using the alternative and standard methods agreed.

isolates were obtained, and there was only 43.7% agreement in sample identification[10].

For *Enterococcu*s spp., the identification was correct for 66.6% of the tested isolates. Such data are consistent with results in a study conducted in Brazil, which showed 77.3% correlation for *Enterococcus* spp. and 88.4% correlation for *Staphylococcus* spp.[11].

Previous data on the use of direct methods for the identification of GPC are very conflicting. Some researchers have suggested that this technique is not trustworthy[8,10], whereas others presented more reliable results[12,13]. A recent study revealed that the use of saponin might be useful in the direct identification of these pathogens. The reason for this is that the presence of blood cells in the samples seems to impair the direct identification of this bacterial class, and saponin acts as a detergent that lyses these blood cells, thus facilitating identification[4].

We performed susceptibility testing of 37 isolates of GNB. The results showed 95% agreement between the alternative and standard methods. All isolates were tested for susceptibility to 12 antimicrobial agents, for a total of 444 isolate-antimicrobial agent combinations (or 444 susceptibility determinations). Of the analyzed samples, 16 (43.3%) were multidrug resistant (MDR). The percent errors for each tested drug compared to the standard method are shown in **Table 2**.

In the susceptibility testing of the 28 GPC, results were only available for the 16 (57.1%) bacterial isolates that were identified, because the VITEK system does not show the results of susceptibility testing when the species identification is not conclusive. Of the 16 samples, 96.1% showed agreement between the alternative and standard methods. The isolates were tested for 11 antimicrobial agents, for a total of 156 isolate-antimicrobial agent combinations. The percent errors for each tested drug compared to the standard method are shown in **Table 2**. Notably, for *Enterococcus* spp. isolates, the susceptibility testing showed agreement in all samples.

In the antimicrobial susceptibility testing, we observed that the global agreement for Gram-negative bacteria was well correlated with other studies[13]. In total, we found a minor error rate of 2.3%, a major error rate of 1.1%, and very major error rate of 1.6%. Studies conducted in Buenos Aires reported 5.5-7.6% for minor errors, 1.3-2.4% for major errors, and 0.1-0.4% for very major errors. However, the rates of very major errors (<3%) as well as the sum of major and minor errors (<7%) were below the limits proposed as acceptable[14].

TABLE 2: Antimicrobial susceptibility testing results using positive blood cultures.

Antimicrobial tests	Total tests	Agreement	Minor errors	Major errors	Very major errors
	n	n (%)	n (%)	n (%)	n (%)
Gram-negative bacilli	**444**	**422 (95.0)**	**10 (2.3)**	**5 (1.1)**	**7 (1.6)**
ampicillin/sulbactam	37	34 (91.9)	2 (5.4)	-	1 (2.7)
piperacillin/tazobactam	37	34 (91.9)	2 (5.4)	1 (2.8)	-
ceftazidime	37	34 (91.9)	-	2 (5.4)	1 (2.7)
ceftriaxone	37	35 (94.6)	-	-	2 (5.4)
cefepime	37	35 (94.6)	1 (2.7)	-	1 (2.7)
imipenem	37	36 (97.3)	-	1 (2.7)	-
meropenem	37	35 (94.6)	2 (5.4)	-	-
amikacin	37	37 (100.0)	-	-	-
gentamicin	37	37 (100.0)	-	-	-
ciprofloxacin	37	37 (100.0)	-	-	-
tigecycline	37	31 (83.8)	3 (8.1)	1 (2.7)	2 (5.4)
colistin	37	37 (100.0)	-	-	-
Gram-positive cocci	**156**	**150 (96.1)**	**3 (1.9)**	**-**	**3 (1.9)**
benzylpenicillin	16	15 (93.7)	1(6.2)	-	-
oxacillin	11	11 (100.0)	-	-	-
gentamicin	11	9 (81.8)	2 (18.1)	-	-
ciprofloxacin	16	16 (100.0)	-	-	-
erythromycin	16	16 (100.0)	-	-	-
clindamycin	16	16 (100.0)	-	-	-
teicoplanin	16	16 (100.0)	-	-	-
vancomycin	16	16 (100.0)	-	-	-
tigecycline	16	16 (100.0)	-	-	-
rifampicin	11	11 (100.0)	-	-	-
trimethoprim/sulfamethoxazole	11	8 (72.7)	-	-	3 (27.2)

For GPC, susceptibility testing was obtained for only 57.1% of the samples because one of the limitations of using the VITEK automated system is that when there is no bacterial identification, susceptibility testing is not allowed. One suggestion to improve the test methodology is to simultaneously conduct the coagulase test directly with the blood culture broth and manually enter the *Staphylococcus* identification results into the VITEK system[14]. In this testing, there was a minor error rate of 1.9% and a very major error rate of 1.9%.

The results obtained in this study agreed with previous research[8,12]. In addition, we observed 100% agreement for oxacillin, ciprofloxacin, erythromycin, clindamycin, teicoplanin, vancomycin, tigecycline, and rifampicin susceptibility testing. Two of these antibiotics, vancomycin and teicoplanin, are the most commonly used antibiotics for the treatment of systemic infections caused by *Enterococcus* spp. and *Staphylococcus* spp.

For the GNB and GPC, we obtained mean final release times of 30 hours and 36 hours, respectively, using the alternative method. This means that the results were obtained 18 hours earlier than with the standard methods. Other authors reported times that were 12-24h faster results using direct inoculation of blood culture with a BD Phoenix and/or VITEK 2 system using similar methods[4,10,13]. Time reduction has recently become one of the most discussed topics, as it aims to improve empiric antimicrobial therapy by minimizing the time of hospitalization, therapeutic error, and the selection of resistant strains, thus reducing both adverse effects in patients and hospitalization costs. In fact, this approach has reduced the turnaround time for blood culture results and has had a positive impact on patient care[1-3].

In this study, we observed that the proposed alternative methodology may assist in the rapid identification and early susceptibility testing of GNB. However, further studies are needed for GPC. It is important to note that the obtained data could inform appropriate therapy and rational use of broad-spectrum antimicrobials, thus helping to improve hospitalization time and reduce mortality in patients with sepsis.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for sepsis for the Third International Consensus Definitions for Sepsis and Septic Shock. JAMA. 2016;315(8):762-74.
2. Lagu T, Rothberg MB, Shieh MS, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. Crit Care Med. 2012;40(3):754-61.
3. Kothari A, Morgan M, Haake DA. Emerging technologies for rapid identification of bloodstream pathogens. Clin Infect Dis. 2014;59(2):272-8.
4. Lupetti A, Barnini S, Castagna B, Nibbering PH, Campa M. Rapid identification and antimicrobial susceptibility testing of Gram-positive cocci in blood cultures by direct inoculation in to the BD Phoenix system. Clin Microbiol Infect. 2010;16(7):986-91.
5. Bruins MJ, Bloembergen P, Ruijs GJHM, Wolfhagen MJHM. Identification and susceptibility testing of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by direct inoculation from positive BACTEC blood culture bottles into Vitek 2. J Clin Microbiol. 2004;42(1):7-11.
6. Putnam LR, Howard WJ, Pfaller MA, Koontz FP, Jones RN. Accuracy of the Vitek system for antimicrobial susceptibility testing *Enterobacteriaceae* bloodstream infection isolates: use of "direct" inoculation from Bactec 9240 blood culture bottles. Diag Microbiol Infect Dis. 1997;28(2):101-4.
7. Quesada MD, Giménez M, Molinos S, Fernández G, Sánchez MD, Rivelo R, et al. Performance of VITEK-2 Compact and overnight Micro Scan panels for direct identification and susceptibility testing of Gram-negative bacilli from positive FAN BacT/ALERT blood culture bottles. Clin Microbiol Infect. 2010;16(2):137-40.
8. de Cueto M, Ceballos E, Martinez-Martinez L, Perea JE, Pascual A. Use of positive blood cultures for direct identification and susceptibility testing with the vitek 2 system. J Clin Microbiol. 2004;42(8):3734-8.
9. Leung DT, Davis EM, Qian Q, Gold HS. First Report of prosthetic joint infection by *Gemella sanguinis* and associated "pseudosatelliting" phenomenon on culture. J Clin Microbiol. 2011;49(9):3395-7.
10. 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 culture of Gram-negative and Gram-positive isolates. Diagn Microbiol Infect Dis. 2012;72(1):20-31.
11. Paim TGS, Cantarelli VV, d' Azevedo PA. Performance of the Vitek2 system software version 5.03 in the bacterial identification and antimicrobial susceptibility test: evaluation study of clinical and reference strains of Gram-positive cocci. Rev Soc Bras Med Trop. 2014;47(3):377-81.
12. Spanu T, Sanguinetti M, Ciccaglione D, D'Inzeo T, Romano L, Leone F, et al. Use of the VITEK 2 System for rapid identification of clinical isolates of Staphylococci from bloodstream infections. J Clin Microbiol. 2003;41(9):4259-63.
13. Ling KWT, Liu ZK, Cheng AFB. Evaluation of the VITEK 2 System for rapid direct identification and susceptibility testing of Gram-negative bacilli from positive blood cultures. J Clin Microbiol. 2003;41(10):4705-7.
14. Jorgensen JH. Selection criteria for an antimicrobial susceptibility testing system. J Clin Microbiol. 1993;31(11):2841-44.