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Comparison of Rapid and Routine Methods of Identification and Antibiotic Susceptibility Testing of Microorganisms from Blood Culture Bottles

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Abstract

Reporting of the results of routine laboratory blood culture tests to clinicians is vital to the patients' early treatment. This study aimed to perform identification and antibiotic susceptibility tests of the blood cultures showing positive signals of microbial growth in the first 12 hours of incubation by using centrifugation and Gram staining of 5 ml of liquid from the vial, thus achieving faster results. This study included 152 consecutively incubated blood culture samples showing positive microbial growth signals in the first 12 hours. The samples were centrifuged and then categorized into two groups (Gram-positive and Gram-negative) using Gram staining. Identification and antibiotic susceptibility tests were performed using an automated culture antibiogram device. For routine processing, media inoculated with positive blood culture were kept in the incubator for at least 24 hours. To compare the two methods in terms of the bacteria identification, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of the growing colony was studied. By Gram staining, the same bacterial strains were obtained for 138 (92%) of the 152 samples, similar to the results of the procedures mentioned earlier. With the samples tested with both methods, the antibiotic susceptibility profiles were compared using the antibiogram results for 1,984 samples that underwent the antibiotic testing. A 97.4% (for 1,934 antibiotic susceptibility assays) agreement was observed between the two methods. Comparing the results of the post-centrifugation Gram staining to those obtained for the specimens using routine procedures, the clinicians reported a high success rate (approximately 97%).

Key words: blood culture, Gram staining, antibiotic susceptibility test, rapid reporting, MALDI-TOF MS

Introduction

Bloodstream infections, especially in intensive care units, are among the most important causes of morbidity and mortality despite the antimicrobial treatment. The early diagnosis and treatment of these infections is clinically vital (Durmaz et al. 2003; Ferreira et al. 2011). For the proper identification and antibiotic susceptibility testing, the bacteria grown in blood culture bottles are sent to laboratories; the detection of bloodstream infections under the appropriate growth conditions, at the appropriate periods, and using the appropriate conventional methods constitute a significant portion of microbiological testing. The recently developed automated systems have been shown to be suitable for bacterial culture, and upon detection of a growth signal, clinicians can remove the bottles from the device and inoculate the sample into media. These media generally include resins or coal particles that absorb antimicrobial agents or other substances that inhibit bacterial growth and may be present in the patient's blood sample (Morrell et al. 2005; Kirn et al. 2014; Nataraj et al. 2016; Jacobs et al. 2017). Early and correct treatment of patients is essential for reducing morbidity and mortality.

For this reason, rapid identification of microorganisms and antibiotic susceptibility testing is crucial. Due to Gram staining of microorganisms in blood cultures, their identification and antibiotic sensitivity results are obtained early, clinicians are informed quickly, and the patient is given the opportunity to start early antibiotic treatment. Continuous monitoring of automated blood

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culture systems allows a positive blood culture to be detected within 6 to 24 hours of incubation (Morrell et al. 2005; Behera et al. 2010; Ferreira et al. 2011; Kirn et al. 2014; Nataraj et al. 2016).

In recent years, advances in the methods involving polymerase chain reaction and DNA sequencing have changed the nature of clinical microbiology laboratories. However, the lack of diagnostic tests that are fast, reliable, easy to use, and inexpensive, as well as applicable for use as diagnostic checkpoints is a significant limitation (Tuite et al. 2014; Yis 2015; Caliendo and Hodinka 2017). Although several molecular or proteomic methods (e.g., Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry [MALDI-TOF MS]) exist that can be used to direct evaluation of specimens or colonies, the gold-standard methods with the highest sensitivity and safety still remain blood culture-based methods (Mamishi et al. 2005; Behera et al. 2010; Tuite et al. 2014; Yis 2015; Nataraj et al. 2016; Caliendo and Hodinka 2017; Jacobs et al. 2017).

This study aimed to use a different, faster-than-usual method, which involves centrifugation at 12 hours of bacterial culture to allow clinicians to rapidly (in less than 12 hours) complete the antibiotic susceptibility testing. It may be due to more accurate information about the Gram-staining characteristics of growing microorganisms via a positive signal of microbial growth in the incubator.

Experimental

Materials and Methods

Clinical samples. The study included 152 bottles of blood culture (aerobic, pediatric blood culture vials, BD, USA), which gave positive signals in the first 12 hours in the incubator (Bactec FX, BD, USA) out of 2,455 blood culture samples sent from different clinics of our hospital to the Medical Microbiology Laboratory between August 2018 and July 2019. Of these 152 blood culture bottles, 102 were sent from intensive care units, 31 from internal sciences clinics, and 19 from surgical sciences clinics. Of these, 130 belonged to adult patients (≥ 18 years old), and 22 belonged to pediatric patients (< 18 years old).

The rapid method. Centrifugation. Five ml of the inoculating fluid was taken from the positive blood culture vials, and, unlike in the routine procedure, the centrifugation was conducted in a standard blood collection tube (BD Vacutainer 5.0 ml, BD, USA) at 2,000 rpm for 10 minutes. After centrifugation, the blood components (erythrocytes, etc.) were placed under a gel, and the bacteria in the serum adhered to the gel to provide a gelatinous coating that formed a film layer. Then, the supernatant liquid remaining on the gel in the collection tube was slowly discharged. A small number of the bacteria collected in the form of a layer on the gel in the collection tube was removed with a cotton swab and spread on a sterile microscope slide for Gram staining.

Gram staining. After the serum fraction was removed, a few bacteria were taken from the bacterial film layer just above the gel. Then, a small volume of this layer was spread and air-dried on a slide. The air-dried preparations were subjected to Gram staining (Becerra et al. 2016) using a Gram-staining set (Moslab, Ankara, Turkey). For this, crystal violet was poured onto the slide, left for 2 minutes, and washed with water. Then, the Lugol solution was poured, left for 2 minutes, washed with water, then decolorized with alcohol and washed with water. Finally, diluted fuchsin was poured onto the slide, and after 30 seconds, the preparation was washed with water and allowed to air-dry. The preparations were then examined with a microscope with a 100× objective. Based on the results of the study, the strains were defined as Gram-positive or Gram-negative (Figs. 1–4).

Identification and antibiotic susceptibility testing using the rapid method. After Gram staining, the strains of Gram-positive and Gram-negative bacteria or yeast were picked from the gel with a cotton-tipped extruder. Bacteria of a concentration equivalent to a McFarland standard of 0.5–0.63 (for yeasts, 1.8–2.2) were diluted with the manufacturer's identification solution (ID broth, BD, USA). Then, 0.5 µl of the dilution was transferred to the antibiotic susceptibility test solution (AST broth, BD, USA), and identification and antibiotic susceptibility tests were conducted using an automated identification and susceptibility testing system (Phoenix 100, BD, USA) and the appropriate kits (GP ID/AST, GN ID/AST or YEAST ID, BD, USA). The tests run for an average of 8–12 hours, depending on the type of microorganism. The results were reported quickly within 24 hours by the clinician involved.

The routine procedures. For comparison, the microorganisms from bottled blood samples were also cultured on sheep blood agar (SBA), eosin methylene blue (EMB) agar, and some other media without centrifugation and routinely incubated at 37°C for 18–24 hours. The next day, when sufficient growth was observed, routine Gram-staining and antibiotic susceptibility testing were performed. With the help of a sterile loop, a small volume of the colonies grown on the medium was taken and mixed in the identification broth (ID broth, BD, USA). Then, 0.5 µl of the dilution was transferred to the solution for antibiotic susceptibility testing (AST broth, BD, USA). Both solutions were transferred to the appropriate kits. Identification and antibiotic susceptibility tests were conducted using the automated identification and susceptibility testing system (Phoenix 100, BD, USA), and the appropriate

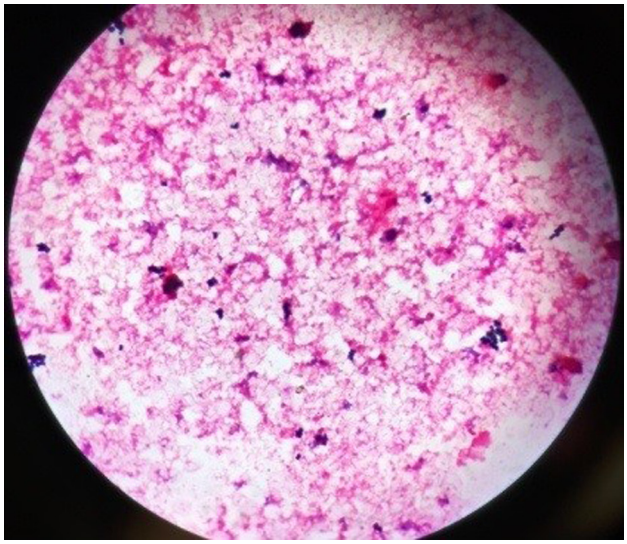

Fig. 1. Microscopic appearance of Gram-positive bacteria after Gram staining with the routine method at a magnification of 1000×.

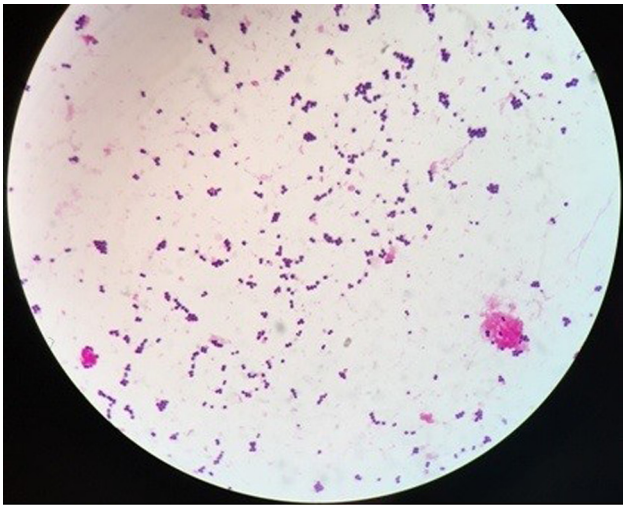

Fig. 2. Microscopic appearance of Gram-positive bacteria after Gram staining with the rapid method at a magnification of 1000×.

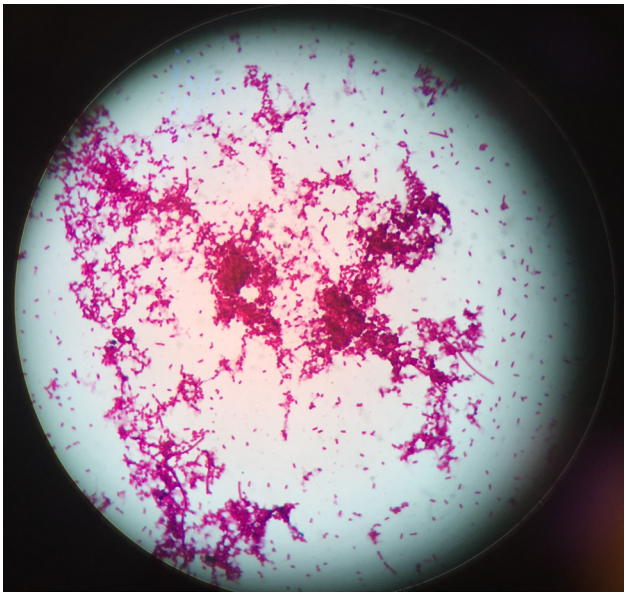

Fig. 3. Microscopic appearance of Gram-negative bacteria after Gram staining with the routine method at a magnification of 1000×.

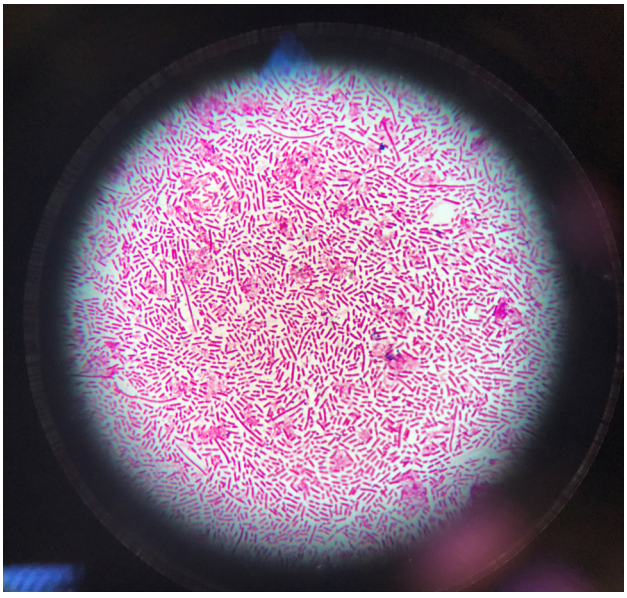

Fig. 4. Microscopic appearance of Gram-negative bacteria after Gram staining with the rapid method at a magnification of 1000×.

kits (GP ID/AST or GN ID/AST, BD, USA). The tests were run for an average of 8–12 hours, depending on the type of microorganism. The results were reported quickly, within 24 hours, by the clinician involved.

MALDI-TOF MS Analysis. The samples were collected with the aid of a sterile loop from colonies of microorganisms grown on the appropriate medium using a routine method, and each bacterial or yeast isolate was transferred to a separate well on a distinct plate in the MALDI-TOF system. One microliter of 70% formic acid (FA) (FA extraction solution, Bruker Daltonik GmbH, Germany) was added to the plate, and after drying at room temperature, 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution (HCCA) (IVD Matrix HCCA, Bruker Daltonik GmbH, Germany) was again dried at room temperature (20–25°C). The dried plates were placed in the instrument (MSP 96 target polished steel, Bruker Daltonik GmbH, Germany) and loaded onto the MALDI-TOF MS (Bruker, Germany) for analysis. At the end of the analysis, the reported microorganisms were recorded using a particular software system (IVD MALDI Biotyper, Bruker Daltonik GmbH, Germany) (Schulthess et al. 2014). For the scoring used in the identification, values were in the range from 0 to 3. Microorganisms with a score ≥ 2 were evaluated as correctly identified at the genus and

species level. A score of 1.7–2 given to organisms indicated a correct identification at the genus level. Organisms with a score of < 1.7 were identified as those that required testing again.

Data analysis. In terms of identification, in the Phoenix 100 Expert System's interpretation, organisms with a reliability > 90 were accepted as being correctly identified. Those with a value of < 90 were considered incorrectly identified. In terms of susceptibility, the minimum inhibitory concentration (MIC) values of the bacteria against the antimicrobials evaluated by both the rapid and routine method were translated into clinical categories (susceptible [S], intermediate [I], or resistant [R]) according to the Phoenix 100 Expert System's interpretive criteria.

For comparison of the results of both methods, the Clinical and Laboratory Standards Institute (CLSI) M52 Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing System (Campigotto et al. 2018) was used. According to this methodology, the identification results were divided into three categories: not identified, misidentified (organism was incorrectly identified at the genus or species level [discrepancy]), and full consistency (accurate identification at the same genus and species level by both methods). For the antibiotic susceptibility results, the discrepancies were classified as minor errors (mEs: susceptible/resistant versus intermediate susceptibility), major errors (MEs: false resistant, considered as resistant susceptibility by one method but susceptible by the other method), and very major errors (VMEs: false susceptibility).

Statistical analysis. The statistical analyses were performed using software (SPSS 15.0, IBM, USA). The results of the continuous data analyses were given as minimum, maximum, median, and mean values, and the results of some categorical variables were given as frequencies and percentages.

Results

A positive signal, which was a bacterial growth marker, was recorded for 152 bottles of blood culture included in the study. They were sent from 102 (67.1%) intensive care units (84 internal intensive care, nine coronary intensive care, and nine pediatric intensive care), 31 (20.4%) from internal clinics (15 internal medicine clinics, ten oncology/hematology clinics, and six pediatric clinics), and 19 (12.5%) from surgical clinics (10 general surgery clinics, seven pediatric surgery clinics, and two gynecology clinics). Of the 152 samples, 130 (85.5%) came from adult patients and 22 (14.5%) from pediatric patients. Of the patients, 82 (54%) were female, 70 (46%) were male, and the youngest patient was one year old, while the oldest was 89 years old (their mean age was 57.5). Because of the lack of blood-based elements in the bacterial layer and the presence of a more significant number of microorganisms than usual, the images of the microorganisms obtained by microscopic examination following Gram staining after centrifugation were more satisfactory in terms of their quality and quantity than those obtained following Gram staining without centrifugation.

According to the results obtained, of the 152 samples included in the study, 150 (98.7%) contained only one type of bacteria. While 116 (77.3%) of these 150 strains stained as Gram-positive (107 cocci and nine bacilli), and 30 (20%) stained as Gram-negative bacilli. The remaining four strains (2.7%) stained as Gram-positive, but they were placed in a separate category because they were yeast. In the other two (1.3%) samples, two bacterial species (*Escherichia coli* and *Staphylococcus aureus*), one Gram-positive and one Gram-negative were detected. In the microscopic examination of the stained cells, these two specimens, that contained more than one type of microorganism, were not evaluated because these bacteria could not be separately tested for antibiotic susceptibility and because the bacteria must be inoculated onto appropriate media. In other words, these two samples were passaged and terminated 18–24 hours after incubation according to routine procedures.

For the identification and antibiotic susceptibility testing, 116 (77.3%) of the 150 microbial strains were analyzed using GP ID/AST kits because these strains were detected as being Gram-positive by Gram staining, and 30 (20%) strains evaluated using GN ID/AST kits because these strains were detected as being Gram-negative by Gram staining. Furthermore, four samples were determined using a YEAST/ID kit because they were observed as yeast in the Gram staining (Table I).

According to the results obtained the following morning, of the 116 Gram-positive strains studied by MALDI-TOF MS analysis, 71 (61.2%) were identified as coagulase-negative *Staphylococcus* (CoNS), and seven (6.0%) were identified as *Staphylococcus aureus*

Table I
Distribution of microorganisms detected by Gram staining after centrifugation according to the rapid method procedure.

Gram-staining result	n (%)
Gram-positive cocci	107 (70.4)
Gram-negative bacilli	30 (19.7)
Gram-positive bacilli	9 (5.3)
Gram-positive cocci + Gram-negative bacilli	2 (1.3)
Yeast (*Candida* spp.)	4 (2.6)
Total number of samples	152 (100)

n – number

Table II
Distribution of bacteria identified by both methods at the genus and species level.

Rapid method	n (%)	Routine method	n (%)
Gram-positive bacteria (total)	**116**	**Gram-positive bacteria (total)**	**116**
Staphylococcus aureus	7 (6.0)	***Staphylococcus aureus***	8 (6.9)
MRSA: 2, MSSA: 5		MRSA: 3, MSSA: 5	
Coagulase-negative Staphylococci	**71 (61.2)**	***Coagulase-negative Staphylococci***	
Staphylococcus epidermidis: 45		*Staphylococcus epidermidis: 48*	**76 (65.5)**
Staphylococcus hominis: 16		*Staphylococcus hominis: 18*	
Staphylococcusschleiferi: 4		*Staphylococcusschleiferi: 4*	
Staphylococcus haemolyticus: 2		*Staphylococcus haemolyticus: 2*	
Staphylococcus capitis: 2		*Staphylococcus capitis: 2*	
Staphylococcus warneri: 2		*Staphylococcus warneri: 2*	
Enterococci	**20 (17.2)**	***Enterococci***	**20 (17.2)**
Enterococcus faecalis: 18		*Enterococcus faecalis: 18*	
Enterococcus faecium: 2	3 (2.6)	*Enterococcus faecium:* 2	2 (1.8)
Arcanobacterium haemolyticum	3 (2.6)	*Arcanobacterium haemolyticum*	2 (1.8)
Bacillus cereus/subtilis	2 (1.8)	*Bacillus subtilis*	2 (1.8)
Dermacoccus nishinomiyaensis	4 (3.5)	*Dermacoccus nishinomiyaensis*	2 (1.8)
Micrococcus luteus/lylae	3 (2.6)	*Micrococcus luteus/lylae*	2 (1.8)
Corynebacterium amycolatum	3 (2.6)	*Corynebacterium amycolatum*	2 (1.8)
Corynebacterium jeikeium		*Corynebacterium jeikeium*	
Gram-negative bacteria (total)	**30**	**Gram-negative bacteria (total)**	**30**
Escherichia coli	15 (50)	*Escherichia coli*	15 (50)
Pseudomonas aeruginosa	5 (16.7)	*Pseudomonas aeruginosa*	5 (16.7)
Klebsiella pneumoniae	5 (16.7)	*Klebsiella pneumoniae*	5 (16.7)
Stenotrophomonas maltophilia	3 (10.0)	*Stenotrophomonas maltophilia*	3 (10.0)
Acinetobacter baumannii	2 (6.7)	*Acinetobacter baumannii*	2 (6.7)
Yeasts	**4**	**Yeasts**	**4**
Candida parapsilosis	2 (50)	*Candida parapsilosis*	2 (50)
Candida tropicalis	2 (50)	*Candida tropicalis*	2 (50)

MRSA – methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*; n – number

(one isolate, identified as *S. epidermidis*, was, in fact, methicillin-resistant *S. aureus* [MRSA] as demonstrated with both the routine method and the MALDI-TOF system), while 20 (17.2%) were *Enterococcus* species. These values, when studied by the routine method, were as follows: 76 (65.5%), 8 (6.9%), and 20 (17.2%), respectively. No isolates of vancomycin-resistant enterococci (VRE) were found in this study. In addition, 15 (50%) of Gram-negative isolates were identified as *E. coli*, five as *P. aeruginosa*, five as *Klebsiella pneumoniae,* three as *Stenotrophomonas maltophilia,* and two as *Acinetobacter baumannii*. When the yeast isolates were analyzed using the appropriate kit (YEAST/ID, BD, USA) and the appropriate McFarland (1.8–2.2) standard (BD PhoenixSpec, BD, USA), the results obtained by both methods were found to be fully compatible (two of the four specimens were identified as *Candida parapsilosis* and the other two as *Candida tropicalis*) (Table II).

Two of the three strains identified as *S. epidermidis* were identified by MALDI-TOF as *Micrococcus luteus/lylae,* and one was identified as *Arcanobacterium haemolyticum.* One isolate was identified as *Corynebacterium amycolatum,* which should have been identified as *S. aureus.* One of the two isolates, which should have been identified as *Staphylococcus hominis*, was identified as *Bacillus cereus/subtilis*, and the other was identified as *Corynebacterium jeikeium*. As a result, 138 (92%) out of 150 isolates were correctly identified, while the number misidentified was only 12. In fact, all false identifications at the genus or species level were also associated with Gram-positive bacteria (Table III).

These values were compared with the identification and antibiotic susceptibility results obtained by the routine procedures. According to this comparison, for 138 (92%) of the 150 strains studied, the same results for the identification testing were obtained using

Table III
Distribution of the identified species according to MALDI-TOF MS results when working with both methods.

Organisms	MALDI-TOF Biotyper scores < 1.7; 1.7–2.0; ≥ 2 n n n Total	Rapid method n	Routine method n
Gram-positive bacteria	**116**	**116**	**116**
Staphylococcus aureus	0 0 8 8	7	8
Staphylococcus epidermidis	0 12 36 48	45	48
Staphylococcus hominis	0 2 16 18	16	18
Staphylococcusschleiferi	0 0 4 4	4	4
Staphylococcus haemolyticus	0 0 2 2	2	2
Staphylococcus capitis	0 0 2 2	2	2
Staphylococcus warneri	0 0 2 2	2	2
Enterococcus faecalis	0 0 18 18	18	18
Enterococcus faecium	0 0 2 2	2	2
Arcanobacterium haemolyticum	0 1 1 2	3	2
Bacillus cereus/subtilis	0 0 0 0	1	0
Bacillus subtilis	0 0 2 2	2	2
Dermacoccus nishinomiyaensis	0 0 2 2	2	2
Micrococcus luteus/lylae	0 1 1 2	4	2
Corynebacterium amycolatum	0 1 1 2	3	2
Corynebacterium jeikeium	0 1 1 2	3	2
Gram-negative bacteria	**30**	**30**	**30**
Escherichia coli	0 0 15 15	15	15
Pseudomonas aeruginosa	0 1 4 5	5	5
Klebsiella pneumoniae	0 0 5 5	5	5
Stenotrophomonas maltophilia	0 0 3 3	3	3
Acinetobacter baumannii	0 0 2 2	2	2
Yeasts	**4**	**4**	**4**
Candida parapsilosis	0 0 2 2	2	2
Candida tropicalis	0 0 2 2	2	
Total	0 19 131 150	150	150

MALDI-TOF MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; n – number

both methods, demonstrating complete consistency between the two methods. However, a discrepancy was detected between the two methods for 12 (8%) strains, with small differences observed in only Gram-positive bacteria. Conversely, for the 30 Gram-negative strains, 100% consistency was observed between the rapid method and the MALDI-TOF MS system. Two samples were terminated without processing because they contained more than one type of microorganism (polymicrobial) and were not evaluated. Therefore, when the identification and antibiotic susceptibility tests of all the Gram-negative bacteria were compared, no discrepancy occurred between the results from either method (of the 150 strains, 30 [20%] Gram-negative bacilli continued to be studied; the same identification and antibiotic susceptibility results were obtained with both methods). No difference was observed between the results obtained by both methods for all strains of enterococci belonging to Gram-positive bacteria (20 [13.3%] of the 150 strains). However, when other Gram-positive bacteria were evaluated, a small inconsistency (identification of different microorganisms at the genus or species level using each method) equal to 8% was observed between these methods. In addition, this inconsistency was also observed in the results obtained for the Gram-positive strains, which can be regarded as indicative of contamination (Table IV).

The agreement for the AST results performed by both methods are the same in terms of the MIC values (100% of similarity) for most of the antimicrobials (e.g.,

Table IV
Analysis of the compatibility between the identification results of the rapid method when compared with the MALDI-TOF MS.

Bacteria	Full consistency n (%)	Misidentified n (%)	Not identified n (%)
Gram-positive bacteria	104 (89.7)	12 (10.3)	0
Gram-negative bacteria	30 (100)	0	0
Yeasts	4 (100)	0	0
Total	138 (92)	12 (8)	0

MALDI-TOF MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; n – number

vancomycin, teicoplanin, ampicillin, and linezolid), whereas the lowest agreement value was obtained for the piperacillin/tazobactam combination (Table V). However, when all the antibiotic susceptibility results between the two methods were examined, the ratio of the full agreement was 97.4%, the VME ratio (0.8%) was slightly higher than the ME ratio (0.1%), and the mE value was found as expected: 33 (1.7%) (Table VI).

Table V
Distribution of the resistance profiles for Gram-positive and Gram-negative bacteria as a result of the antibiotic susceptibility testing when both methods were applied.

Antibiotics	The rapid method, n (%)			The routine method, n (%)		
	S	I	R	S	I	R
Amikacin	28 (93.3)	–	2 (6.7)	28 (93.3)	–	2 (6.7)
Ampicillin	18 (19.1)	–	76 (80.9)	18 (19.1)	–	76 (80.9)
Amoxicillin/Clavulanic acid	18 (15.8)	–	96 (84.2)	18 (15.8)	–	96 (84.2)
Aztreonam	18 (60)	1 (3.3)	11 (36.7)	15 (50)	–	15 (50)
Cefepime	20 (66.7)	–	10 (33.3)	14 (46.7)	–	16 (53.3)
Cefoxitin	–	36 (41.9)	50 (58.1)	–	35 (40.7)	51 (59.3)
Ceftazidime	10 (33.3)	10 (33.3)	10 (33.3)	10 (33.3)	10 (33.3)	10 (33.3)
Ceftriaxone	18 (60)	–	12 (40)	18 (60)	–	12 (40)
Ciprofloxacin	82 (66.1)	–	42 (33.9)	66 (53.2)	10 (8.1)	48 (38.7)
Colistin	30 (100)	–	–	30 (100)	–	–
Clindamycin	50 (53.2)	1 (1.1)	43 (45.7)	51 (554.3)	–	43 (45.7)
Daptomycin	71 (75.5)	23 (24.5)	–	65 (69.1)	29 (30.9)	–
Ertapenem	28 (93.3)	–	2 (6.7)	28 (93.3)	–	2 (6.7)
Erythromycin	14 (14.9)	–	80 (85.1)	14 (14.9)	–	80 (85.1)
Fusidic acid	35 (40.7)	–	51 (59.3)	36 (41.9)	–	50 (58.1)
Gentamicin	66 (53.2)	–	58 (46.8)	66 (53.2)	1 (0.8)	57 (50)
Imipenem	28 (93.3)	–	2 (6.7)	28 (93.3)	–	2 (6.7)
Meropenem	28 (93.3)	–	2 (6.7)	28 (93.3)	–	2 (6.7)
Linezolid	94 (100)	–	–	94 (100)	–	–
Oxacillin	12 (12.8)	–	82 (87.2)	12 (12.8)	–	82 (87.2)
Piperacillin/Tazobactam	10 (33.3)	10 (33.3)	10 (33.3)	16 (53.3)	2 (6.7)	12 (40)
Rifampin	62 (66)	–	32 (34)	62 (66)	–	32 (34)
Teicoplanin	94 (100)	–	–	94 (100)	–	–
Tetracycline	35 (40.7)	–	51 (59.3)	35 (40.7)	–	51 (59.3)
Tigecycline	84 (89.4)	8 (8.5)	2 (2.1)	86 (91.5)	6 (6.4)	2 (2.1)
TMP/SX	55 (44.4)	–	69 (55.6)	51 (41.1)	3 (2.4)	70 (56.5)
Vancomycin	94(100)	–	–	94 (100)	–	–
Total	1102 (55.5)	89 (4.5)	793 (40)	1077 (54.3)	96 (4.8)	811 (40.9)

n – number; I – intermediate; R – resistant; S – susceptible; TMP/SX – Trimethoprim/Sulfamethoxazole

Table VI
Categorical distribution of discrepancies in the antibiotic susceptibility results of the rapid method compared to the routine method.

Antibiotics	Agreement n (%)	mEs n (%)	MEs n (%)	VMEs n (%)	Total n
Amikacin	30 (100)				30
Ampicillin	94 (100)				94
Amoxicillin/Clavulanic acid	114 (100)				114
Aztreonam	26 (86.7)	1 (3.3)		3 (10)	30
Cefepime	24 (80)			6 (20)	30
Cefoxitin	85 (98.8)	1 (1.2)			86
Ceftazidime	30 (100)				30
Ceftriaxone	30 (100)				30
Ciprofloxacin	108 (87.1)	10 (8.1)		6 (4.8)	124
Colistin	30 (100)				30
Clindamycin	93 (98.9)	1 (1.1)			94
Daptomycin	88 (93.6)	6 (6.4)			94
Ertapenem	30 (100)				30
Erythromycin	94 (100)				94
Fusidic acid	85 (98.8)		1 (1.2)		86
Gentamicin	123 (99.2)	1 (0.8)			124
Imipenem	30 (100)				30
Meropenem	30 (100)				30
Linezolid	94 (100)				94
Oxacillin	94 (100)				94
Piperacillin/Tazobactam	22 (73.3)	8 (26.7)			30
Rifampin	94 (100)				94
Teicoplanin	94 (100)				94
Tetracycline	86 (100)				86
Tigecycline	92 (97.9)	2 (2.1)			94
TMP/SX	120 (96.8)	3 (2.4)		1 (0.8)	124
Vancomycin	94 (100)				94
Total	1934 (97.4)	33 (1.7)	1 (0.1)	16 (0.8)	1984

n – number; mEs – minor errors; MEs – major errors; TMP/SX – Trimethoprim/Sulfamethoxazole; VMEs – very major errors

Discussion

To date, a limited number of studies have been conducted to use rapid methods to determine the type of organisms that grow in blood cultures and evaluate their antibiotic susceptibility. However, the rapid identification of infectious agents circulating in patients' blood and the determination of the species and the correct antibiotic to be used will shorten the treatment time by 12 hours, and reduce the cost. The early identification of bloodstream infections allows for the early modification of antimicrobial treatment and a diminished need for other diagnostic tests. Thus, patients' hospital stay can be shortened, and expenditures on patients can be reduced (Beekmann et al. 2003).

In a study conducted by a group of researchers, 501 microorganisms from blood-circulating infections were detected in the patients with bacteremia or candidiasis with MALDI-TOF with the Antimicrobial Stewardship Team test. MALDI-TOF analysis of 245 patients in the intervention group and 256 patients in the preintervention group showed reductions in the identification times (84 hours to 56 hours), effective antibiotic treatment times (30 to 20 hours), and optimal antibiotic treatment times (90 to 47 hours) for the organism (Huang et al. 2013). Our study reports the identification and antibiotic susceptibility tests that are much shorter (< 24 hours) than conventional tests (routinely > 72 hours). In contrast to routine tests, this study achieved 97% success without having to use any expensive methods.

In a study (Dodemont et al. 2014), nucleic acid-based tests were performed on Gram-positive and Gram-negative bacteria from blood cultures; the test

was designed to rapidly identify multiple bacterial strains and the resistance associated with these strains. This method was compared with the routine method for 117 isolates, and the identification and antibiotic resistance markers were reported as correct and accurate, achieving 97% and 92% rates of detection of pathogens from the blood cultures and reduced laboratory times. In our study, the observed 92% accurate identification and 97% antibiotic susceptibility compliance results were similar.

In another study (Mancini et al. 2014), a total of 102 positive blood cultures were tested with the Verigene BC-GN test, which detected certain Gram-negative bacteria and selected resistance genes. Ninety-eight percent of the isolates were correctly identified, and 29% were identified as those carrying the resistance genes (CTX-M, KPC, VIM, and OXA genes). The other researchers (Hill et al. 2014) included 54 blood culture samples that gave positive signals between 7 and 23 hours using the Verigene BC-GN test, and correctly identified 51 of these samples as Gram-negative bacilli, whereas Mancini et al. (2014)) reported that they correctly identified the full-length carbapenemase enzyme. In a test run, the identification of the organisms was reported to be faster than the conventional method by an average of 24 hours. The other group (Sothoron et al. 2015) studied the blood cultures of 126 patients with Gram-negative bacteremia using an antimicrobial stewardship program test. In a study evaluating the VITEK 2 system with the Verigene BC-GN test, the mean survival rate in the optimal treatment was shorter in the post-intervention group than in the pre-intervention group (38 to 49 hours, respectively). The rapid method in our study does not require any additional costs, systems, or devices, and it is 24 hours shorter than the method in the abovementioned study. We believe our method can be used for all bacteria instead of only certain bacteria.

The MALDI-TOF and a VITEK 2 system were used together in a study by Machen and coworkers (Machen et al. 2014) performed to the same-day identification and the full panel antimicrobial susceptibility testing of bacteria from a total of 100 positive blood culture bottles. Compared to conventional methods, the direct results were 94% correctly identified with the VITEK system, and the identification and antibiograms were concluded on the same day. In our study, direct results were also reported, with a high success rate of 94% being achieved (without 24 hours of incubation) using 5 ml of liquid from the blood culture fluid and without the need for a device that adds costs to the conventional method.

In a study conducted by another group of researchers (Banerjee et al. 2015), the detection of bacterial and fungal resistance genes in the strains directly identified in 617 positive blood cultures was performed using a direct rapid multiplex PCR (rmPCR). It was followed by the evaluation of the treatment duration and mortality. The shortest period after Gram staining needed to appropriate evaluation of antimicrobial susceptibility was achieved by rmPCR. These results allowed for the reduction of mortality and the use of antibiotics. Because we were working with fewer samples in our study (which would not significantly change the results), the rapid delivery of blood culture results was critical to the patient; in terms of cost, the centrifugation of blood culture bottles with positive signals required no extra cost, and this method was used to obtain faster results (< 24 hours).

In a study by Stevenson et al. (2010), a total of 212 patient samples, 179 of which were positive blood cultures and 33 of which were isolated broth species, were prepared and processed for MALDI-TOF analysis. In this study, 42 strains (12 *Propionibacterium acnes* and seven *S. epidermidis*) with spectral scores < 1.7 could not be identified. Twenty-four strains with scores between 1.7 and 1.9 were correctly identified (six were *S. epidermidis*). The strains with scores of 1.9 were correctly identified at the species level. Similarly, in our study, the species determination and antibiotic susceptibility testing of strains from a single-species culture in the blood cultures were performed using a rapid method. Alternatively, for rapid reporting (97% success) of the antibiotic susceptibility results, we used a direct analysis with a centrifugation process. Notably, the use of mass spectrometry technology can only be established in large laboratories.

The other researchers (Lupetti et al. 2010) studied 57 blood cultures that were collected from different clinics and found to be a single-species culture of Gram-positive cocci by Gram staining. The addition of saponin in the new procedure was compared with the routine method. Discrepancies between the two methods were resolved by ID32 Staph or by Rapid ID32 Strep and E-test. With the new method, 44 (80%) of the Gram-positive cocci were detected as CoNS (34 [62%] *S. epidermidis*). In our study, unlike the one above, not only Gram-positive bacteria but also other bacteria and yeasts were studied. In addition, saponin or a similar substance was not used in this study. Among the identification values obtained by the rapid method, the CoNS rate was slightly lower (62%). Although our antibiotic susceptibility findings were similar, we notably had few VMEs, which maybe because of the presence of gel in the tubes.

In another study (Chen et al. 2015), Gram staining was performed after samples were cultured on blood; this method was performed on 400 positive-signal blood cultures on weekdays between 8:00 am and 3:00 pm. Samples that were a single species culture after the Gram staining were included in the study. While

358 (89.5%) isolates were identified correctly by VITEK MS, 343 (85.8%) were identified correctly at the species level, and 15 (3.7%) were identified correctly at the genus level. Of these definitions, 146 (96.1%) isolates of 152 Gram-negative bacilli (78 *E. coli* and 25 *Klebsiella* species isolates) were correctly identified, while 197 (79.4%) isolates of 248 Gram-positive organisms (76 of 95 CoNS and 56 of 58 *S. aureus*) were correctly identified. In our study, Gram-negative bacilli and yeasts were correctly identified in 100%, and Gram--positive bacteria were correctly identified in approximately 90%. These identifications were obtained without any culturing on media or incubation after centrifugation; instead, the identification occurred by loading directly into the culture antibiogram device. In addition, enterococci were also completely identified when compared with MALDI-TOF MS.

In one study (Tian et al. 2016), 485 positive specimens were evaluated by injecting various body fluids into blood culture bottles. Then, the researchers combined MALDI-TOF MS with a VITEK AST system and conducted rapid microbial identification (RMI), and rapid multiple AST (RMAST). Then, the RMAST results were compared with the standard method results. Discrepancies in the MIC values were resolved by broth dilution, according to CLSI (2015) guidelines. RMI correctly identified Gram-negative and Gram-positive bacteria (98.9%, and 87.2%, respectively), and fungi (75.7%). As a result, the RMI and RMAST were completed 18–36 hours in advance of the report notification. In our study, only blood cultures were evaluated. A longer centrifugation time with a lower speed was utilized. After comparable identification and antibiotic susceptibility testing procedures, similar results were obtained. Our report notifications were made within 24 hours.

Recently, in a study conducted by Campigottoa et al. (2018), MALDI-TOF MS analysis was used for the identification of bacteria directly from blood cultures, followed by antibiotic susceptibility tests and rapid determination of methicillin-resistance and beta-lactam-resistance status. A total of 125 positive blood cultures sent from various intensive care units were included in the study. The VITEK 2 system and appropriate AST cards were used to determine the antibiotic susceptibility, and standard procedures were applied. When compared with the routine method, 91.2% were correctly identified at the genus level, 82.4% had species-level compatibility, and eight unidentified isolates at the genus level were Gram-positive organisms. Gram-negative bacteria were detected at a high species level of 100% and 93%, respectively. All four fungal isolates *C. albicans*, *C. parapsilosis,* and *C. tropicalis* were correctly identified at the species level. In contrast to the above study, in our study, the centrifugation time was longer, the rpm was lower, and the bacterial suspension was prepared and loaded directly onto the Phoenix 100 instrument. Similar error rates (2.6% in total) and agreement (97%) were observed. Furthermore, in our study, the goal of reporting results in 24 hours was successfully achieved.

Conclusions

The search for methods that do not require additional financing and are widely available is on-going. In this study, 5 ml of blood samples were taken directly from blood cultures with positive signals, and the results were obtained within 24 hours. It was achieved with centrifugation, Gram staining, and an appropriate antimicrobial panel (Gram-positive or Gram-negative). The Gram-negative strains were reported with 100% consistency, and the Gram-positive strains were reported with 90% consistency among the new and routine methods. For the Gram-positive bacteria that were inconsistently identified with these methods, we believe that further studies in this area will help to distinguish pathogenic and nonpathogenic strains. In particular, we believe that unnecessary antibiotic treatments can be avoided for patients infected with Gram-positive bacteria, which can be identified as contaminants sooner than Gram-negative bacteria. Due to the rapid culture antimicrobial susceptibility testing used in this study, antibiotic therapies could begin early, since the patients' blood culture results will be reported very quickly (in about 12 hours) without the need for extra costs. In addition, since this can shorten the length of hospital stay of patients, medical and economic benefits will be achieved at the national level. We also believe that the volume of the sample taken directly from the bottles should be increased (to at least 10 ml) and that employees should have sufficient experience; then, the efficiency of this method can be higher. However, the most crucial limitation of this method is the presence of more than one species in the blood cultures (polymicrobial cultures). Finally, the rapid method used in the study allows obtaining the diagnosis of bloodstream infections in a short span of 12 hours and with an accuracy of 97% (100% for Gram-negative bacteria).

List of abbreviations

AST – antibiotic susceptibility test
BC-GN – Gram-negative blood culture
CLSI – Clinical and Laboratory Standards Institute
CoNS – coagulase-negative *Staphylococcus*
EMB – eosin methylene blue
FA – formic acid
GN – Gram-negative
GP – Gram-positive
HCCA – α-cyano-4-hydroxycinnamic acid matrix solution

ID – identification
MALDI-TOF MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
ME – major error
mE – minor error
MIC – minimum inhibitory concentration
MRSA: methicillin-resistant *Staphylococcus aureus*
RMAST – rapid multiple AST
RMI – rapid microbial identification
rmPCR – rapid multiplex PCR
SBA – sheep blood agar
VME – very major error

Authors' contributions

SA and HSS participated in the study design and coordination and conducted the data analyses. SA participated in and performed the measurements, laboratory testing, and data collection. All authors read and approved the final manuscript. All authors contributed to the draft of the manuscript and discussed the results. All authors gave final approval for publication.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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