

Rapid Detection of Pathogens and Resistance Genes Grown in Blood Cultures with Two Multiplex Tandem Real-Time PCR Kits

Barış Gülhan¹ , Aytekin Çıkman² , Merve Aydın^{1,3} , Mürşit Hasbek⁴ , Tuncer Özekinci⁵ , Sümeyye Akyüz¹ , Faruk Karakeçili⁶ 

¹ Department of Medical Microbiology, Erzincan Binali Yıldırım University School of Medicine, Erzincan, Türkiye

² Rize Özel Gözlem Medical Laboratory, Rize, Türkiye

³ Department of Medical Microbiology, KTO Karatay University School of Medicine, Konya, Türkiye

⁴ Department of Medical Microbiology, Cumhuriyet University School of Medicine, Sivas, Türkiye

⁵ Department of Medical Microbiology, İstanbul Medeniyet University School of Medicine, İstanbul, Türkiye

⁶ Department of Infectious Diseases and Clinical Microbiology, Erzincan Binali Yıldırım University School of Medicine, Erzincan, Türkiye

ABSTRACT

Objective: In this study, two multiplex tandem real-time PCR kits were used to rapidly diagnose common Gram-positive cocci and Gram-negative bacilli, detect their commonly seen antibiotic resistance genes, and evaluate the two kits' performance.

Materials and Methods: Gram-positive 12 (GP-12) kit (AusDiagnostics, Australia) and Gram-negative 12 (GN-12) kit (AusDiagnostics, Australia) were used in the study. Seventy-eight Gram-negative bacilli and 54 Gram-positive cocci grown in blood culture vials were applied to GN-12 and GP-12 panels. At the same time, the passages of the samples were made and incubated. After that, identification and antibiograms were made in the Phoenix™ automated system (Becton, Dickinson and Company, USA) and VITEK 2 Compact automated system (bioMérieux, France).

Results: Twenty-one *Staphylococcus aureus*, twelve coagulase-negative staphylococci (CoNS), two *Streptococcus pneumoniae*, two *Enterococcus faecium*, and three *Enterococcus faecalis* were found to match the results from the automated GP-12 Kit. Pathogens present in the panel were successfully identified using the GN-12 kit. Both panels were found to be more effective in diagnosing polymicrobial infections.

Conclusion: These evaluated kits were rapid (approximately three hours) and valuable in identifying common sepsis pathogens and resistance genes. Thus, these tests can easily be used in the diagnosis of sepsis.

Keywords: rapid identification, resistance genes, sepsis pathogens

Corresponding Author:
Faruk Karakeçili

E-mail:
drfarukkarakeçili@hotmail.com

Received: September 25, 2024
Accepted: December 18, 2024
Published: March 27, 2025

Suggested citation:
Gülhan B, Çıkman A, Aydın-Terzioğlu M, Hasbek M, Özekinci T, Akyüz S, et al. Rapid detection of pathogens and resistance genes grown in blood cultures with two multiplex tandem real-time PCR kits. *Infect Dis Clin Microbiol.* 2025;1:37-46.

DOI: 10.36519/idcm.2025.465



INTRODUCTION

Rapid diagnosis of pathogens grown in blood cultures reduces mortality and morbidity as appropriate treatment is started early. It notably reduces hospital costs because of less need for intensive care and earlier patient discharge (1, 2). Although rapid identification and antibiogram of pathogens can be made with automated blood culture, identification, and antibiogram systems, faster systems are needed. Therefore, over the years, microorganisms have been identified by various molecular methods and resistance genes have been identified (1, 2). Finally, with the introduction of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), rapid microbial identification and detection of some resistance phenotypes have become possible (3, 4).

This study used two multiplex tandem real-time PCR kits to rapidly diagnose Gram-positive cocci and Gram-negative bacilli that grew in blood cultures as sepsis agents and to detect resistance genes. The first one, the Gram-positive 12 (GP-12) kit (AusDiagnostics, Australia), detects *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., and antibiotic resistance genes (*mecA*, *vanA*, *vanB*) in them. The second one, Gram-negative 12 (GN-12) kit (AusDiagnostics, Australia), detects *Enterobacteriaceae*, *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and their antibiotic resistance genes (*Oxa-23* [carbapenem hydrolyzing beta-lactamase OXA-23], *pan-IMP* [IMP-1, metallo beta-lactamase IMP containing 4], *pan-vim* [metallo-beta-lactamase VIM-1, 2 or 3], *aadA1* [aminoglycoside nucleotidyltransferase *aadA1*], *aadB* [aminoglycoside nucleotidyltransferase *aadB*], *aac(6')IIC* [aminoglycoside-6' acetyltransferase *aac(6')IIC*]). We aimed to evaluate the performances of these two kits.

MATERIALS AND METHODS

Collection of samples

Blood samples taken from patients at the sepsis clinic were inoculated into BACTEC™ (Becton, Dickinson and Company, USA) and BACT/ALERT® (bioMérieux, France) blood culture bottles. The study included 132 blood culture bottles with positive signals. First, Gram staining from the blood culture bottles gave a positive signal. Seventy-eight

samples having Gram-negative bacilli in Gram staining were applied to the GP-12 panel. In Gram staining, 54 samples of Gram-positive cocci were applied to the GP-12 panel. At the same time, the passages of the samples were made and incubated. After that, identification and antibiograms were made in Phoenix™ automated system (Becton, Dickinson and Company, USA) and VITEK 2 Compact automated system (bioMérieux, France), and the results were evaluated.

DNA isolation

For DNA isolation, according to the recommendations of the kit manufacturer, the EZ1® Advanced XL (Qiagen, Germany) nucleic acid extraction device and EZ1® DNA Tissue Kit (Qiagen, Germany) were used. EZ1® DNA Tissue Kit (Catalog No: 953034; Qiagen, Germany) and EZ1® Advanced XL DNA Bacteria Card (Catalog No: 9018694; Qiagen, Germany) were used in the extraction device. For DNA extraction, 200 microliters of blood culture fluid from each sample were used as the starting material. DNA isolation took 17 minutes for 14 samples. The isolation was completed by following the instructions on the screen for the extraction device. At the end of the procedure, 50 microliters of DNA were obtained from each sample.

Pre-PCR and PCR

The study used a multiplexed Tandem PCR technique consisting of a two-step sequential PCR reaction. For each sample, five microliters of DNA were used as starting material. In the first step, a short 15-cycle multiplex pre-amplification reaction (pre-PCR) and PCR set-up steps were performed using homologous primers for all targets in the panel. Easy Plex pipetting

HIGHLIGHTS

- Rapid identification of bacteria in positive blood cultures and resistance genes is crucial for timely antibiotic treatment.
- It was also possible to detect polymicrobial growth with GP-12 and GN-12 panels.
- Multiplex tandem real-time PCR tests can be used to rapidly diagnose common Gram-positive cocci and Gram-negative bacilli, detect their commonly seen antibiotic resistance genes.

Table 1. Results of Gram-positive-12 kit and Phoenix/Vitek-2 Compact Systems.

PCR						Phoenix™ and VITEK 2 Compact				
ID		n	Mec A	Van A	Van B	ID	n	FOX-R	VA-R	
Pan-Staphylococcus	<i>S. aureus</i> (nuc+femA+)	21	12			<i>S. aureus</i>	21	12		
	CoNS	12	11			<i>S. epidermidis</i>	7	5		
						<i>S. haemolyticus</i>	4	4		
						<i>S. hominis</i>	1	1		
Pan-Streptococcus	<i>S. pneumoniae</i> (LytA-Strep+)	2				<i>S. pneumoniae</i>	2			
Pan-Staphylococcus + Pan-Streptococcus	CoNS	1	1			<i>S. epidermidis</i>	1	1		
Pan-Enterococcus	<i>E. faecium</i> (ddl+)	2				<i>E. faecium</i>	2			
	<i>E. faecalis</i> (ddl+)	3				<i>E. faecalis</i>	3			
Pan-Streptococcus + Pan-Enterococcus	CoNS	9	9	5		<i>S. epidermidis</i> + <i>E. faecium</i>	2	2	2	
						<i>S. epidermidis</i>	1	1		
	<i>E. faecalis</i> (ddl+) + CoNS	4	4				<i>E. faecium</i>	6		3
							<i>E. faecalis</i>	3		
							<i>E. faecalis</i> + <i>S. epidermidis</i>	1	1	
TOTAL		54	37	5	0	TOTAL	54	27	5	

FOX: Cefoxitin, VA: Vancomycin, mecA: Methicillin-resistance gene, vanA: Vancomycin resistance gene A, vanB: Vancomycin resistance gene B, nuc: *Staphylococcus aureus* thermostable nuclease gene, femA: *Staphylococcus aureus* femA gene, CoNS: Coagulase-negative staphylococci, lyt-A Strep: *Streptococcus pneumoniae* autolysin gene, ddl: D-Ala-D-Ala ligase gene

robot (AusDiagnostics, Australia) was used for pre-PCR and PCR set-up stages. There was no difference in procedure between Gram-positive and Gram-negative panels. For the pre-PCR and PCR set-up stages, in line with the manufacturer's instructions, the required filter pipette tips, 72 rotor disc, oil and water tubes used for pre-PCR, master mix 1, master mix 2 tubes and dilution plate were loaded into the Easy Plex pipetting robot. The products formed after the first step were diluted into the wells for the second real-time PCR reaction using the nested inside primers of the primers used in the first step.

After DNA isolation and pre-PCR stages, the materials were applied to the GP-12 and GN-12 panels (AusDiagnostics, Australia). The second step of the PCR reaction was performed in the Rotor-Gene Q

(Qiagen, Germany). DNA amplification was detected by measuring the increase in fluorescence using EvaGreen™ dye (Biotium, USA). A 72-position Easy-Plex™ ring (AusDiagnostics, Australia) was used for the second step of PCR.

After approximately one hour of pipetting and pre-PCR steps, the 72-rotor disk was taken, and the reaction wells were covered with sealing film. Then, the 72-rotor disk was loaded into Rotor-Gene Q (Qiagen, Germany) real-time PCR device and amplified according to the kit manufacturer's instructions.

Culture, identification, and antibiogram

After Gram staining from BACTEC™ (Becton, Dickinson and Company, USA) and BACT/ALERT® (bioMérieux, France) blood culture bottles that gave

a positive signal, the 5% sheep blood agar and eosin methylene blue (EMB) agar media were inoculated from the bottles. The next day, cultures were evaluated, and antibiograms of grown microorganisms were identified using Phoenix™ automated system (Becton, Dickinson and Company, USA) and VITEK 2 Compact automated system (bioMérieux, France).

RESULTS

After the multiplex tandem real-time PCR, results were compared to those obtained from automated systems. All the results are given in Table 1 and Table 2.

Firstly, all the pathogens were successfully identified by using these two multiplex tandem real-time PCR kits. Twenty-one *S. aureus* and twelve methicillin-resistant strains were identified using GP-12 panels and the Phoenix™ and VITEK 2 Compact automated systems. Twelve coagulase-negative staphylococci (CoNS) found with GP-12 panels were also detected with Phoenix™ and VITEK 2 Compact automated systems. However, while the *mecA* gene was found in eleven samples with the GP-12 panel, methicillin resistance was detected in ten samples in automated systems. Two strains of *S. pneumoniae* were detected as the same in both GP-12 panels and automated systems. Two *E. faecium* and three *E. faecalis* were found to be the same in both GP-12 panels and automated systems. However, a difference was found between the GP-12 panel and the automated systems in the samples with mixed infection. While pan-Staphylococcus, pan-Streptococcus, and coagulase-negative staphylococci (CoNS) carrying the *mecA* gene were detected in one sample, an automated system identified one methicillin-resistant *Staphylococcus epidermidis* in the same sample. In nine samples, methicillin-resistant coagulase-negative staphylococci (MRCoNS) and nine *E. faecium* associations were detected, five of which carried the *vanA* gene. In the same samples, MRCoNS and two vancomycin-resistant *E. faecium* coexistence in two samples, MRCoNS in one sample, and six *E. faecium* in six samples, six of which were resistant to vancomycin, were found in automated systems. While one *E. faecium* and six MRCoNS strains could not be detected with automated systems, all vancomycin-resistant *E. faecium* strains were also de-

tected with automated systems. While MRCoNS and *E. faecalis* were detected in four samples, only *E. faecalis* was found in three. Methicillin-resistant *S. epidermidis* and *E. faecalis* association was found in one sample with automated systems.

In Table 2, the GN-12 panel and automated systems are compared. According to the results obtained from the GN-12 panel, the results obtained under three headings, pan-Enterobacteriaceae, pan-Pseudomonas, and pan-Enterobacteriaceae + pan-Pseudomonas, were compared with the results obtained with automated systems.

In the first group, pan-Enterobacteriaceae and twelve *E. coli*, three of the OXA-23 gene and one *aadA1* gene were detected by the GN-12 panel. In comparison, automated systems isolated eleven *E. coli* and one *Acinetobacter baumannii* in the same samples. Ertapenem resistance was found in one of the *E. coli* isolates; gentamicin resistance was found in four, while the *A. baumannii* strain was resistant to carbapenems and gentamicin. The absence of *A. baumannii* in the panel leads to the chance of predicting one of the two gentamicin resistances while detecting OXA-23 in three samples, which was considered a positive aspect.

OXA-23 gene was found in one of fifteen strains identified as pan-Enterobacteriaceae and *K. pneumoniae* with the GN-12 panel, and the *aadA1* gene was found in four of them. Although fifteen *K. pneumoniae* strains were isolated in the same samples by automated systems, resistance to carbapenems was detected in two and resistance to gentamicin in eight. Although the panel identified all strains, it was weak in determining the resistance profiles.

The *aadA1* gene was found in one of four samples isolated from pan-Enterobacteriaceae and *E. coli* + *K. pneumoniae* with the GN-12 panel. *E. coli* in two samples, *K. pneumoniae* in one and *E. coli* + *K. pneumoniae* with the *aadA1* gene, were found in one of the same samples with automated systems. The panel was evaluated positively. *E. coli* + *K. pneumoniae* + *P. aeruginosa* containing pan-*vim* and *aadA1* genes were detected in one sample with pan-Enterobacteriaceae with GN-12 panel. *E. coli* + *K. pneumoniae* was detected in this sample using automated systems.

No carbapenem or aminoglycoside resistance was detected. The panel was found to be superior in polymicrobial infections.

With the GN-12 panel, only pan-*Enterobacteriaceae* could be detected in three samples, and no identification at the species level could be made. The *aadA1* gene was detected in one of these samples. With automated systems, two samples were identified as *Enterobacter cloacae* and one as *Enterobacter aerogenes*. Although identification could not be made at the species level, it was determined that the infectious agents were members of *Enterobacteriaceae*. In addition, the *aadA1* gene was found in one of them.

In the second group, pan-*Pseudomonas*, fifteen *P. aeruginosa* strains were identified, one of which had the pan-*vim* gene. Automated systems isolated fifteen *P. aeruginosa* strains; two were resistant to carbapenems and gentamicin, and three were resistant to only imipenem.

In the third group, pan-*Enterobacteriaceae* + pan-*Pseudomonas*, seven *E. coli* with one *aadA1* and *oxa23* genes were detected. At the same time, seven *E. coli* strains, two of which were resistant to gentamicin, were identified by automated systems. Automated systems identified eight *K. pneumoniae* strains, including one *Klebsiella oxytoca* and seven *K. pneumoniae* resistant to five gentamicin and one ertapenem. One *P. aeruginosa* strain was detected as *P. aeruginosa* resistant to imipenem and meropenem by automated systems.

In the pan-*Enterobacteriaceae* + pan-*Pseudomonas* group, two samples, in which one *E. coli* and *K. pneumoniae* with *aadA1* gene were detected together, were identified as one *E. coli* and one as *K. pneumoniae* by automated systems. *E. coli* and *P. aeruginosa* were detected in one sample, and automated systems could only detect *E. coli*. The association of *P. aeruginosa* and *K. pneumoniae* with two *aadA1* and *oxa23* genes was detected in five samples. Automated systems isolated two *K. pneumoniae* and two *P. aeruginosa* from one sample; two were resistant to gentamicin, and one was resistant to ertapenem. Also, *K. pneumoniae* and *Serratia marcescens* were isolated from the same sample; they were resistant to gentamicin, imipenem, meropenem, and ertapenem.

In the pan-*Enterobacteriaceae* + pan-*Pseudomonas* group, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were detected in three samples. At the same time, two of them had *aadB*, three *aadA1*, and one pan-*vim* gene. Two *E. coli*, one resistant to gentamicin, and *E. coli* and *Morganella morganii* in one sample, both resistant to gentamicin, were identified by automated systems. One specimen in this group could not be identified at the species level, but the *aadA1* gene was detected. In automated systems, this sample was defined as *E. coli*.

DISCUSSION

Many studies have been conducted on the rapid identification of microorganisms grown in blood cultures using molecular methods and the detection of resistance genes, and the clinical benefits of these tests have been reported in terms of results. These tests, which provide early detection of essential resistance phenotypes, are practical in rapidly optimizing antimicrobial therapy by reducing off-label antibiotics (5-7). For this purpose, Gosiewski et al. (7) have tried to detect Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*S. aureus*), yeast (*Candida albicans*), and filamentous fungi (*Aspergillus fumigatus*) in blood by nested-multiplex real-time PCR. According to the results, they stated that the method they designed allowed the detection of bacteria in whole blood samples, was much more sensitive than the culture method, and allowed the detection of the main groups of microorganisms within a few hours.

Wolk et al. (8) used the GenMark Dx ePlex blood culture identification Gram-negative panel in their study, identifying Gram-negative pathogens and determining the resistance genes (CTX-M, KPC, IMP, VIM, NDM, and OXA [OXA-23 and OXA 48]). They stated that the panel they recommend was also valuable for finding pathogens that might be missed in Gram staining containing the pan-Gram-positive and pan-*Candida* regions.

In their study, MacVane and Nolte (9) diagnosed and followed up patients with growth in blood culture using conventional methods and a rapid multiplex PCR panel and compared the results. Their results showed no difference between the two groups

Table 2. Results of Gram-negative-12 kit and Phoenix™ and VITEK 2 Compact automated systems.

		PCR							Phoenix™ and VITEK 2 Compact						
ID	n	OXA 23	Pan- <i>vim</i>	Pan- <i>imp</i>	<i>aadA1</i>	<i>aadB</i>	<i>aac(6')IIC</i>	ID	n	ETP	IMP	MEM	AK	CN	
Pan-Enterobacteriaceae	<i>E. coli</i>	12	3		1			<i>E. coli</i>	11	1				4	
								<i>A. baumannii</i>	1	IR	1	1		1	
	<i>K. pneumoniae</i>	15	1		4			<i>K. pneumoniae</i>	15	2	2	2		8	
	<i>E. coli</i> + <i>K. pneumoniae</i>	4				1			<i>E. coli</i>	2					
									<i>K. pneumoniae</i>	1					
	<i>E. coli</i> + <i>K. pneumoniae</i> + <i>P. aeruginosa</i>	1		1		1			<i>E. coli</i> + <i>K. pneumoniae</i>	1					1
	Nonidentified	3				1			<i>E. cloacae</i>	2					
<i>E. aerogenes</i>									1						
Pan-Pseudomonas	<i>P. aeruginosa</i>	15		1				<i>P. aeruginosa</i>	15	IR	5	2		2	
Pan-Enterobacteriaceae + Pan-Pseudomonas	<i>E. coli</i>	7	1		1			<i>E. coli</i>	7					2	
	<i>K. pneumoniae</i>	8						<i>K. pneumoniae</i>	7	1				5	
								<i>K. oxytoca</i>	1						
	<i>P. aeruginosa</i>	1						<i>P. aeruginosa</i>	1	-	1	1			
	<i>E. coli</i> + <i>K. pneumoniae</i>	2				1			<i>E. coli</i>	1					
									<i>K. pneumoniae</i>	1					
	<i>E. coli</i> + <i>P. aeruginosa</i>	1						<i>E. coli</i>	1						
	<i>K. pneumoniae</i> + <i>P. aeruginosa</i>	5	1			2			<i>K. pneumoniae</i>	2	1				2
									<i>P. aeruginosa</i>	2	-				
									<i>K. pneumoniae</i> + <i>S. marcescens</i>	1	1	1	1		1
<i>E. coli</i> + <i>K. pneumoniae</i> + <i>P. aeruginosa</i>	3		1		3	2		<i>E. coli</i> + <i>M. morgani</i>	1					1	
								<i>E. coli</i>	2					1	
Nonidentified	1				1			<i>E. coli</i>	1						
TOTAL	78	6	3	0	16	2	0	TOTAL	78	6	10	7	0	29	

AK: Amikacin, CN: Gentamicin, IMP: Imipenem, MEM: Meropenem, ETP: Ertapenem, OXA 23: Carbapenem-hydrolyzing beta-lactamase OXA-23, pan-*vim*: Metallo-beta-lactamase *vim*1, 2 or 3., Pan-IMP: Metallo beta-lactamase 1 and 4., *aadA1*: Aminoglycoside nucleotidyltransferase *aadA1*, *aadB*: Aminoglycoside nucleotidyltransferase *aadB*, *aac(6')IIC*: Aminoglycoside-6'-acetyltransferase *aac(6')IIC*.

regarding mortality, 30-day readmission, length of stay in the intensive care unit, hospitalization period after culture, or costs. However, it was stated that the time to effective treatment was shortened in the group using molecular diagnosis compared to the group using conventional methods alone, and the use of antimicrobials was further developed.

Granato et al. (10) also conducted a qualitative study to detect *mecA*, *vanA*, and *vanB* resistance genes of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *E. faecalis*, and *E. faecium* responsible for bacterial bloodstream infections. They compared the multiplex test, the iC-GPC Assay (iCubate, USA), with the Verigene Gram-positive blood culture (BC-GP) assay (Luminex Corp., USA). They stated that the iC-GPC Test showed a 95.5% agreement with the BC-GP test. They emphasized that the iC-GPC Test is accurate and reliable for detecting the five common Gram-positive bacteria and their resistance genes.

Wang et al. (11) compared multiplex real-time PCR and PCR reverse blot hybridization (PCR-REBA) tests with each other and with conventional culture methods. While PCR-REBA was superior in detecting the *mecA* gene, Gram-positive bacteria, Gram-negative bacteria, and *Candida* spp. were equivalent for both tests. They emphasized that using these two molecular methods is fast and reliable for the characterization of causative pathogens in bloodstream infections since the results from the culture can take up to 48-72 hours, while all two methods take about three hours.

Pilarczyk et al. (12) used LightCycler® SeptiFast test M Grade (Roche Molecular Systems, Germany), a real-time multiplex PCR test capable of detecting twenty-five bacterial and fungal pathogens, to diagnose bloodstream infections after cardiothoracic surgery early. They found the test valid for Gram-negative pathogens. Still, they recommended its use with conventional blood culture methods, as it was weak in detecting Gram-positive pathogens and could not detect antibiotic susceptibility.

Lee et al. used the BD MAX StaphSR Assay (SR assay; BD, USA) to distinguish *S. aureus* from CNoS and detect methicillin resistance in positive blood cul-

tures (13). As a result, they stated that the range of 0-27 cycle threshold (Ct) for positivity with the *nuc/mec* test was helpful for rapid detection of methicillin-resistant *S. aureus* (MRSA) and MRCNoS. They also emphasized genotypic *v mecA*-positive and oxacillin-susceptible isolates.

Similar to our study, Hazelton et al. evaluated the performance of the GP-12 multiplex tandem PCR (MT-PCR) test (AusDiagnostics, Australia) (14). A total of 673 aerobic and anaerobic positive blood culture bottles showing Gram-positive cocci morphology on microscopy were included in the study and compared with traditional phenotypic methods. They reported that the internal control amplification was inhibited in 11.7% of the samples. However, 96.6% (537/556) of the remaining monomicrobial samples obtained results consistent with the genus level and 100% (172/172) at the species level. They reported that for 94.7% (36/38) of the polymicrobial samples, the results matched the phenotypic methods, and the methicillin and vancomycin susceptibility result fully agreed with the phenotypic methods. They rated the results positively because of the accuracy of over 90%. They reported that this test, in which Gram-positive pathogens and their primary antibiotic resistance markers could be obtained within three hours, was reliable.

Sze et al. used the Accelerate Pheno® system (Accelerate Diagnostics, Inc., Tucson, AZ), which uses fluorescence in situ hybridization probes and a new rapid test that determines rapid identification and antibiotic susceptibility from positive blood cultures (15). They were compared with the FilmArray Blood Culture Identification (BCID) (Biofire, USA) and FilmArray BCID 2 (Biofire, USA) panels that perform nested PCR. They found rapid methods successful and stated that tests suitable for laboratory conditions could be used.

In another study conducted with positive blood culture samples using fluorescence in situ hybridization probes, Reitz et al. emphasized that the FISH technique, which they found helpful for laboratories with limited resources without advanced rapid diagnostic methods such as mass spectrometry, was a good option (16).

Bryant et al. used the ePlex system (GenMark Diagnostics, USA), a random-access multiplex PCR platform developed for syndromic diagnosis in the diagnosis of bacteremia and fungemia (17). They stated that sensitive and reliable results could be obtained with the ePlex BCID panels, where they could identify fifty-six pathogens and ten resistance genes. Simultaneously, the transfer of results was necessary to measure the effect on mortality and length of stay.

In our study, after Gram staining, 54 vials with Gram-positive cocci were applied to the GP-12 panel, 78 vials with Gram-negative bacilli were applied to the GN-12 panel, and the results obtained by PCR and conventional methods were compared. All pathogens identified with the Phoenix™ automated system (Becton, Dickinson and Company, USA) and VITEK 2 Compact automated system (bioMérieux, France) were successfully identified with these two multiplex tandem real-time PCR kits. However, both panels were found to be more effective in the diagnosis of polymicrobial infections. Thirteen *mecA* genes (methicillin resistance) that conventional methods could not detect, one pan-*streptococcus*, one *E. faecium*, and nine CNoS GP-12 panels could be detected. Similarly, nine *K. pneumoniae*, three *E. coli*, and eight *P. aeruginosa* were detected by GN-12 panels as the polymicrobial infection agent that conventional methods could not detect. Although two *E. cloacae*, one *E. aerogenes*, one *S. marcescens*, one *M. morgani*, and one *E. coli* could not be detected as a species, it was found to be pan-*Enterobacteriaceae* in the panel. One *A. baumannii* strain could not be detected because it was not found in the panel. Although the number of strains resistant to carbapenems was twelve, six OXA-23 and three Pan-*vim* could be detected with GN-12 panels. At the same time, one of the aminoglycoside resistance genes was found in sixteen of twenty-nine strains resistant to any of the aminoglycosides. Different susceptibility phenotypes might have been captured with larger content panels. Generally, the panel was found beneficial.

Rapid identification of pathogens from blood cultures is gaining more and more importance with new resistance phenotypes that are developing and increasing in frequency. The number of carbapen-

em-resistant, extended-spectrum beta-lactamases (ESBL), methicillin, and vancomycin-resistant isolates is constantly increasing, and their early detection is vital for reducing mortality and morbidity. Therefore, multiplex PCR, real-time multiplex PCR, DNA-microarray-based hybridization technology, in situ hybridization-based methods, nucleic acid amplification-based methods, and combined platforms are used (17-20).

This study has several limitations. One significant limitation is the requirement for trained molecular microbiology technicians or specialists, who may not always be available outside regular working hours or on public holidays. Additionally, the study is constrained by its sample size and the inability to detect fungal agents. For these reasons, syndromic sepsis panels are recommended for routine laboratory use because they do not require specially trained technicians and can be used by other laboratory personnel.

In conclusion, the two kits proved rapid (approximately three hours) and reliable in determining common Gram-negative and Gram-positive sepsis pathogens and resistance genes. As in many studies, using multiplex tandem real-time PCR kits helped detect sepsis agents and resistance genes. Considering all aspects, such as early initiation of treatment, a more successful and shorter treatment, reduced complications, and reduced mortality and morbidity, it is possible to say that rapid tests are not an extra burden in terms of cost. Today, with the widespread use of sophisticated technologies such as MALDI-TOF MS and FilmArray, different molecular tests can be used for laboratories that do not have these facilities. We believe that different multiplex tandem real-time PCR tests, which provide rapid identification and detect antibiotic resistance genes, will be beneficial depending on the possibilities of the laboratories.

Ethical Approval: The Ethics Committee of Erzincan Binali Yıldırım University approved the study on May 28, 2013, with decision number 03-03.

Informed Consent: N.A.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – B.G., A.Ç., M.A.T., F.K.; Design – B.G., A.Ç., M.A.T., F.K.; Supervision – B.G., F.K.; Fundings – B.G.; Materials – B.G., A.Ç., M.H., T.Ö.; Data Collection and/or Processing – B.G., S.A.; Analysis and/or Interpretation – B.G., A.Ç., S.A.; Literature

Review – B.G., A.Ç., M.A.T., F.K., M.H., T.Ö.; Writer – B.G., S.A., F.K.; Critical Reviews – B.G., A.Ç., M.A.T., M.H., T.Ö., S.A., F.K.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The Erzincan Binali Yıldırım University Scientific Research Projects Coordination Unit supported the study.

Scientific Presentation: This study was presented at the FEMS2015, the 6th Congress of European Microbiologists, which took place in Maastricht, the Netherlands, from June 7 to 11, 2015.

REFERENCES

- Schumann J, Johanns U, Ahmad-Nejad P, Ghebremedhin B, Woebker G. The impact of the FilmArray-based detection of microbial pathogens from positive blood culture vials on the time to optimal antimicrobial regimen in intensive care units of the Helios University Clinic Wuppertal, Germany. *J Clin Med*. 2021;10(24):5880. [\[CrossRef\]](#)
- Verroken A, Despas N, Rodriguez-Villalobos H, Laterre PF. The impact of a rapid molecular identification test on positive blood cultures from critically ill with bacteremia: A pre-post intervention study. *PLoS One*. 2019;14(9):e0223122. [\[CrossRef\]](#)
- Faron ML, Buchan BW, Ledebner NA. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for use with positive blood cultures: Methodology, performance, and optimization. *J Clin Microbiol*. 2017;55(12):3328-38. [\[CrossRef\]](#)
- Dai Y, Xu X, Yan X, Li D, Cao W, Tang L, et al. Evaluation of a rapid and simplified protocol for direct identification of microorganisms from positive blood cultures by using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Front Cell Infect Microbiol*. 2021;11:632679. [\[CrossRef\]](#)
- Sparks R, Balgahom R, Janto C, Polkinghorne A, Branley J. Evaluation of the BioFire Blood Culture Identification 2 panel and impact on patient management and antimicrobial stewardship. *Pathology*. 2021;53(7):889-95. [\[CrossRef\]](#)
- Peri AM, Stewart A, Hume A, Irwin A, Harris PNA. New microbiological techniques for the diagnosis of bacterial infections and sepsis in ICU including point of care. *Curr Infect Dis Rep*. 2021;23(8):12. [\[CrossRef\]](#)
- Gosiewski T, Jurkiewicz-Badacz D, Sroka A, Brzywczy-Włoch M, Bulanda M. A novel, nested, multiplex, real-time PCR for detection of bacteria and fungi in blood. *BMC Microbiol*. 2014;14:144. [\[CrossRef\]](#)
- Wolk DM, Young S, Whitfield NN, Reid JL, Thornberg A, Carroll KC, et al. A multicenter clinical study to demonstrate the diagnostic accuracy of the GenMark Dx ePlex Blood Culture Identification Gram-Negative Panel. *J Clin Microbiol*. 2021;59(9):e0248420. [\[CrossRef\]](#)
- MacVane SH, Nolte FS. Benefits of adding a rapid PCR-based blood culture identification panel to an established antimicrobial stewardship program. *J Clin Microbiol*. 2016;54(10):2455-63. [\[CrossRef\]](#)
- Granato PA, Unz MM, Widen RH, Silbert S, Young S, Heflin KL, et al. Clinical evaluation of the iCubate iC-GPC assay for detection of Gram-positive bacteria and resistance markers from positive blood cultures. *J Clin Microbiol*. 2018;56(9):e00485-18. [\[CrossRef\]](#)
- Wang HY, Kim S, Kim J, Park SD, Kim HY, Uh Y, et al. Comparison of multiplex real-time PCR and PCR-reverse blot hybridization assay for the direct and rapid detection of bacteria and antibiotic resistance determinants in positive culture bottles. *J Med Microbiol*. 2016;65(9):962-74. [\[CrossRef\]](#)
- Pilarczyk K, Rath PM, Steinmann J, Thielmann M, Padosch SA, Dürbeck M, et al. Multiplex polymerase chain reaction to diagnose bloodstream infections in patients after cardiothoracic surgery. *BMC Anesthesiol*. 2019;19(1):59. [\[CrossRef\]](#)
- Lee J, Park YJ, Park DJ, Park KG, Lee HK. Evaluation of BD MAX Staph SR assay for differentiating between *Staphylococcus aureus* and coagulase-negative staphylococci and determining methicillin resistance directly from positive blood cultures. *Ann Lab Med*. 2017;37(1):39-44. [\[CrossRef\]](#)
- Hazelton BJ, Thomas LC, Unver T, Iredell JR. Rapid identification of Gram-positive pathogens and their resistance genes from positive blood culture broth using a multiplex tandem RT-PCR assay. *J Med Microbiol*. 2013;62(Pt 2):223-31. [\[CrossRef\]](#)
- Sze DTT, Lau CCY, Chan TM, Ma ESK, Tang BSF. Comparison of novel rapid diagnostic of blood culture identification and antimicrobial susceptibility testing by Accelerate Pheno System and BioFire FilmArray Blood Culture Identification and BioFire FilmArray Blood Culture Identification 2 panels. *BMC Microbiol*. 2021;21(1):350. [\[CrossRef\]](#)
- Reitz A, Poppert S, Rieker M, Frickmann H. Evaluation of FISH for blood cultures under diagnostic real-life conditions. *Eur J Microbiol Immunol (Bp)*. 2018;8(4):135-41. [\[CrossRef\]](#)
- Bryant S, Almahmoud I, Pierre I, Bardet J, Touati S, Maubon D, et al. Evaluation of microbiological performance and the potential clinical impact of the ePlex® Blood Culture Identification Panels for the rapid diagnosis of bacteremia and fungemia. *Front Cell Infect Microbiol*. 2020;10:594951. [\[CrossRef\]](#)
- De Angelis G, Grossi A, Menchinelli G, Boccia S, Sanguinetti M, Posteraro B. Rapid molecular tests for detection of antimicrobial

- icrobial resistance determinants in Gram-negative organisms from positive blood cultures: a systematic review and meta-analysis. *Clin Microbiol Infect.* 2020;26(3):271-80. [\[CrossRef\]](#)
- 19** Peker N, Couto N, Sinha B, Rossen JW. Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. *Clin Microbiol Infect.* 2018;24(9):944-55. [\[CrossRef\]](#)
- 20** Demir M, Telli-Dizman G, Hazırolan G, Uzun Ö, Metan G. The impact of reporting the same-day identification and antibiotic susceptibility test results on the treatment of bloodstream infections. *Infect Dis Clin Microbiol.* 2024;6(2):123-32. [\[CrossRef\]](#)