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Comparison of Rapid Antibiotic Susceptibility Test Method Directly from Blood Culture Bottle with Standard Disc Diffusion Method

Kan Kültürü Şişesinden Doğrudan Yapılan Hızlı Antibiyotik Duyarlılık Testi Yönteminin Standart Disk Difüzyon Yöntemi ile Karşılaştırılması

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ABSTRACT

Aim: Early determination of antimicrobial susceptibility of sepsis pathogens is important. In this study, we aimed to compare the standard disc diffusion method with the rapid antimicrobial susceptibility testing (RAST) method performed directly from blood culture bottles.

Material and Methods: Bacteria isolated from samples that gave a positive signal on the blood culture device between April 2019 and September 2019 were included in the study, and antimicrobial susceptibilities were determined by the standard disc diffusion method and the RAST method. Categorical agreement, small error, large error, very large error, and area of technical uncertainty ratios were recorded.

Results: A total of 103 bacteria including 19 *S. aureus*, 10 *Enterococcus spp*. and 24 *E. coli*, 24 *K. pneumoniae*, 13 *P. aeruginosa*, and 13 *A. baumannii* were included in the study. When the RAST method was compared with the standard disc diffusion method, 100% agreement was found between the methods against imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole in *E. coli* isolates at all hours evaluated, and against meropenem in *K. pneumoniae* isolates at the 6th and 8th hour. For *S. aureus* and *P. aeruginosa* isolates, very major errors were found in the RAST results. For *A. baumannii* isolates, 100% agreement between methods was observed for many antibiotics.

Conclusion: It was concluded that the RAST method is a simple and inexpensive test for life-threatening infections such as sepsis. It was also felt that similar studies should be carried out with a large number of isolates, as compliance rates vary depending on the bacteria tested.

Keywords: Bacteremia; disc diffusion antimicrobial tests; blood culture.

ÖZ

Amaç: Sepsis etkenlerinin antimikrobiyal duyarlılıklarının erken belirlenmesi çok önemlidir. Bu çalışmada, standart disk difüzyon yöntemi ile kan kültür şişelerinden doğrudan yapılan hızlı antibiyotik duyarlılık testi (HADT) yönteminin karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Çalışmaya Nisan 2019 ile Eylül 2019 tarihleri arasında kan kültürü cihazında pozitif sinyal veren örneklerden izole edilen bakteriler dahil edilmiş ve antimikrobiyal duyarlıkları standart disk difüzyon yöntemi ve HADT yöntemi ile belirlenmiştir. Kategorik uyum, küçük hata, büyük hata, çok büyük hata ve teknik belirsizlik alanı oranları kaydedilmiştir.

Bulgular: Çalışmaya 19'u *S. aureus*, 10'u *Enterococcus spp*. ile 24'ü *E. coli*, 24'ü *K. pneumoniae*, 13'ü *P. Aeruginosa* ve 13'ü *A. baumannii* olmak üzere toplam 103 adet bakteri dahil edilmiştir. HADT yöntemi ile standart disk difüzyon yöntemi karşılaştırıldığında, *E. coli* izolatlarında imipenem, meropenem, gentamisin ve trimetoprim-sülfametoksazole karşı değerlendirilen tüm saatler için, *K. pneumoniae* izolatlarında ise meropeneme karşı 6. ve 8. saatler için yöntemler arasında %100 uyum bulunmuştur. *S. aureus* ve *P. aeruginosa* izolatlarında ise HADT sonuçlarında çok büyük hata saptanmıştır. *A. baumannii* izolatlarında birçok antibiyotik için yöntemler arasında %100 uyum olduğu görülmüştür.

Sonuç: HADT yönteminin sepsis gibi hayatı tehdit eden enfeksiyonlar için kullanımı kolay ve ucuz bir test olduğu sonucuna varılmıştır. Test edilen bakteriye göre değişen uyum oranları nedeniyle benzer çalışmaların çok sayıda izolatla yapılması gerektiği de düşünülmüştür.

Anahtar kelimeler: Bakteriyemi; disk diffüzyon antimikrobiyal testleri; kan kültürü.

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INTRODUCTION

Accurate detection and rapid reporting of bloodstream infections are the two most important functions of the clinical microbiology laboratory (1). Bacteremia can lead to serious complications, including sepsis (2). Sepsis increases morbidity and mortality rates, particularly in patients who spend long periods in intensive care. To prevent this, urgent initiation of broad-spectrum antimicrobial treatment is mandatory (3,4). Identifying bacteria from positive bottles and performing antibiotic susceptibility testing takes 24-48 hours using standard methods. This leads to delays in treatment (5).

The most commonly used antimicrobial susceptibility testing method in clinical microbiology laboratories is disc diffusion, described by Bauer et al. (6) in 1966. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommended direct and rapid antimicrobial susceptibility testing (RAST), which requires a short incubation period from positive blood culture bottles for the major antimicrobials used in the treatment of sepsis. The method is based on the standard EUCAST disc diffusion method but with modified inoculum and incubation time. Undiluted blood culture water from the positive blood culture bottle was used as inoculum and the incubation time was shortened to 4, 6, and 8 hours. The antimicrobials tested were selected to cover the most important agents for the treatment of sepsis (5).

The aim of this study was to perform RAST according to EUCAST recommendations on blood culture bottles with the preliminary diagnosis of bacteremia and giving positive signals and to compare the results with the standard disk diffusion method.

MATERIAL AND METHODS

Blood culture samples sent to the Düzce University Faculty of Medicine Hospital Medical Microbiology Laboratory from different hospitals and outpatient clinics between April and September 2019 were included in the study. Microorganisms isolated from the samples that gave a positive signal on the BACTEC automated blood culture device (Becton Dickinson, USA) were identified by conventional methods and/or the VITEC 2 Compact® system (Biomerieux, France), antimicrobial susceptibilities were tested by the standard disc diffusion method and the results were recorded (7). Blood culture bottles with monobacterial growth were included in the study. A 125 µL blood sample taken from blood culture bottles giving positive signals was plated on Müller-Hinton agar (Condalap, Spain) in 9 cm petri dishes, and antibiotic discs according to EUCAST recommendations for each bacterium were placed on top. The susceptibility of the microorganisms was measured and recorded after 4, 6, and 8 hours according to EUCAST recommendations.

The recorded antimicrobial susceptibility results were compared with the results recorded in the standard disc diffusion test and the rates of categorical agreement (CA-same clinical category), minor error (mE-reporting a moderately susceptible result as susceptible/resistant), major error (ME-reporting a result that should be susceptible as resistant), very major error (VME-reporting a result that should be resistant as susceptible) and area of technical uncertainty (ATU) were recorded (8,9).

In the study, data were given as numbers and percentages.

RESULTS

A total of 103 bacterial isolates including 19 *S. aureus*, 10 *Enterococcus spp*., 24 *Escherichia coli*, 24 *Klebsiella pneumoniae*, 13 *Pseudomonas aeruginosa*, and 13 *Acinetobacter baumannii* were included in the study. When the RAST method was compared with the standard disc diffusion method, no major errors were detected in *E. coli* isolates and 100% agreement between the methods was found for all hours evaluated against imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole (Table 1). Similarly, a full agreement was found for *K. pneumoniae* isolates against meropenem at hours 6 and 8 (Table 2). The highest error rate was observed for tobramycin in *E. coli* isolates and imipenem in *K. pneumoniae* isolates.

For *S. aureus* isolates included in the study, minor errors in RAST results were not observed for any antibiotic, whereas VMEs were found for all antibiotics (Table 3).

For *Enterococcus spp*. isolates, a full inter-method agreement was found for gentamicin and linezolid, but the vancomycin result was identified as an ATU for all isolates tested (Table 4).

For *A. baumannii* isolates, no minor error was detected for any antibiotic, whereas 100% inter-method agreement was found for imipenem, meropenem, ciprofloxacin, levofloxacin and gentamicin (Table 5).

According to the results of the RAST method, VMEs, and ATUs were detected in *P. aeruginosa* isolates against many antibiotics. Minor errors were found only against amikacin (Table 6).

DISCUSSION

There are several studies based on direct inoculation from positive blood culture bottles to reduce the reporting time of bloodstream infections. Setting appropriate cut-off values is a prerequisite for the correct interpretation of early results. EUCAST has published guidelines on this topic. Many studies show that the RAST test is promising in this regard, although it detects erroneous findings (10,11).

In our study, the number of samples with growth at 4, 6, and 8 hours and the susceptibility patterns were investigated using the RAST method for the antibiotics and bacteria recommended by EUCAST. For all strains included in the study, it was observed that the number of samples with growth and evaluated samples, especially at 4 and 6 hours, was less than the number of samples processed, and the number of samples that could be evaluated increased with increasing incubation time. This situation was accepted as a natural consequence of bacteriological culture but was considered to be a limiting situation in studies to be performed with the RAST method.

In a study comparing the RAST method and the standard disc diffusion method in *E. coli* isolates the categorical agreement rate between the two tests was found as <90% for piperacillin-tazobactam, levofloxacin, and tobramycin, whereas it was found as ≥90% for all other antibiotics (9). In our study, the inter-method agreement was found to be 100% for imipenem, meropenem, gentamicin, and trimethoprim-sulfamethoxazole in *E. coli* isolates. When the same comparison was made for *K. pneumoniae* isolates, the agreement rate was ≥90% in all time periods for cefotaxime, ceftazidime, meropenem, gentamicin, and trimethoprim-sulfamethoxazole. For *K. pneumoniae* isolates, the concordance rates for imipenem were 62.5%,

Table 1. Comparison of RAST and disc diffusion methods in *E. coli* isolates (n=24)

Antibiotics / Hours	4 hours	6 hours	8 hours
Piperacillin-tazobactam			
Number of growth	17	22	24
CA, n (%)	11 (64.7)	18 (81.8)	21 (87.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	2 (11.8)	1 (4.5)	1 (4.2)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	4 (23.5)	3 (13.6)	2 (8.3)
Cefotaxime			
Number of growth	15	19	21
CA, n (%)	14 (93.3)	18 (94.7)	20 (95.2)
mE, n (%)	1 (6.7)	1 (5.3)	1 (4.8)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime			
Number of growth	15	22	24
CA, n (%)	14 (93.3)	21 (95.5)	23 (95.8)
mE, n (%)	1 (6.7)	1 (4.5)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem			
Number of growth	16	17	17
CA, n (%)	16 (100)	17 (100)	17 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem			
Number of growth	17	22	24
CA, n (%)	17 (100)	22 (100)	24 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	16	21	24
CA, n (%)	15 (93.8)	19 (90.5)	22 (91.7)
mE, n (%)	0 (0.0)	1 (4.8)	1 (4.2)
ME, n (%)	1 (6.3)	1 (4.8)	1 (4.2)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Levofloxacin			
Number of growth	16	18	18
CA, n (%)	15 (93.8)	16 (88.9)	16 (88.9)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	1 (6.3)	1 (5.6)	1 (5.6)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	1 (5.6)	1 (5.6)
Amikacin			
Number of growth	16	22	24
CA, n (%)	16 (100)	22 (100)	22 (91.7)
mE, n (%)	0 (0.0)	0 (0.0)	2 (8.3)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 2. Comparison of RAST and disc diffusion methods in *K. pneumoniae* isolates (n=24)

Antibiotics / Hours	4 hours	6 hours	8 hours
Piperacillin-tazobactam			
Number of growth	17	22	24
CA, n (%)	15 (88.2)	19 (86.4)	21 (87.5)
mE, n (%)	0 (0.0)	1 (4.5)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	2 (11.8)	2 (9.1)	2 (8.3)
Cefotaxime			
Number of growth	14	20	22
CA, n (%)	13 (92.9)	18 (90.0)	20 (90.9)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	1 (7.1)	2 (10.0)	2 (9.1)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime			
Number of growth	14	22	22
CA, n (%)	14 (100)	20 (90.9)	20 (90.9)
mE, n (%)	0 (0.0)	1 (4.5)	1 (4.5)
ME, n (%)	0 (0.0)	1 (4.5)	1 (4.5)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem			
Number of growth	16	19	20
CA, n (%)	10 (62.5)	13 (68.4)	14 (70.0)
mE, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
ME, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
VME, n (%)	4 (25.0)	4 (21.1)	3 (15.0)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (5.0)
Meropenem			
Number of growth	17	23	24
CA, n (%)	16 (94.1)	23 (100)	24 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (5.9)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	17	23	24
CA, n (%)	15 (88.2)	21 (91.3)	23 (95.8)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	2 (11.8)	2 (8.7)	1 (4.2)
Levofloxacin			
Number of growth	16	19	19
CA, n (%)	15 (93.8)	17 (89.5)	18 (94.7)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (6.3)	2 (10.5)	1 (5.3)
Amikacin			
Number of growth	16	22	24
CA, n (%)	14 (87.5)	20 (90.9)	22 (91.7)
mE, n (%)	1 (6.3)	1 (4.6)	1 (4.2)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (6.3)	1 (4.6)	1 (4.2)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 1. Comparison of RAST and disc diffusion methods in *E. coli* isolates (n=24) *continued*

Gentamicin			
Number of growth	13	18	20
CA, n (%)	13 (100)	18 (100)	20 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Tobramycin			
Number of growth	16	19	21
CA, n (%)	14 (87.5)	16 (84.2)	17 (80.9)
mE, n (%)	0 (0.0)	0 (0.0)	1 (4.8)
ME, n (%)	2 (12.5)	3 (15.8)	2 (9.5)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (4.8)
Trimethoprim-sulfamethoxazole			
Number of growth	16	20	20
CA, n (%)	16 (100)	20 (100)	20 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 2. Comparison of RAST and disc diffusion methods in *K. pneumoniae* isolates (n=24) *continued*

Gentamicin			
Number of growth	15	21	23
CA, n (%)	14 (93.3)	20 (95.2)	21 (91.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (6.7)	1 (4.8)	1 (4.3)
ATU, n (%)	0 (0.0)	0 (0.0)	1 (4.3)
Tobramycin			
Number of growth	16	21	23
CA, n (%)	15 (93.8)	16 (76.2)	18 (78.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (6.3)	5 (23.8)	5 (21.7)
Trimethoprim-sulfamethoxazole			
Number of growth	16	19	20
CA, n (%)	15 (93.8)	18 (94.7)	19 (95.0)
mE, n (%)	1 (6.3)	1 (5.3)	1 (5.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 3. Comparison of RAST and disc diffusion methods in *S. aureus* isolates (n=19)

Antibiotics / Hours	**4 hours**	**6 hours**	**8 hours**
Cefoxitin			
Number of growth	6	9	19
CA, n (%)	6 (100)	9 (100)	17 (89.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	2 (10.5)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Clindamycin			
Number of growth	5	9	19
CA, n (%)	5 (100)	8 (88.9)	17 (89.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	1 (11.1)	2 (10.5)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Gentamicin			
Number of growth	4	7	16
CA, n (%)	4 (100)	6 (85.7)	14 (87.5)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	1 (6.3)
VME, n (%)	0 (0.0)	1 (14.3)	1 (6.3)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Norfloxacin			
Number of growth	5	9	19
CA, n (%)	4 (80.0)	7 (77.8)	16 (84.2)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	1 (20.0)	2 (22.2)	3 (15.8)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Table 4. Comparison of RAST and disc diffusion methods in *Enterococcus spp*. strains (n=10)

Antibiotics / Hours	**4 hours**	**6 hours**	**8 hours**
Ampicilin			
Number of growth	5	7	10
CA, n (%)	5 (100)	6 (85.7)	10 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	1 (14.3)	0 (0.0)
Gentamicin*			
Number of growth	2	3	7
CA, n (%)	2 (100)	3 (100)	7 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Vancomycin			
Number of growth	-	-	-
CA, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	4 (100)	7 (100)	10 (100)
Linezolid			
Number of growth	2	6	10
CA, n (%)	2 (100)	6 (100)	10 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

68.4%, and 70% at 4, 6, and 8 hours, respectively, whereas these rates were 94.1%, 100%, and 100% for meropenem, respectively.

Erdoğan et al. (12) reported the lowest categorical agreement 91.9% for piperacillin-tazobactam and 92.4% for tobramycin among all antimicrobials tested in their study comparing the RAST method with the standard disc diffusion method. In the same study, it was found that the number of tests concluded at the 4th hour was less than the number of tests concluded at the 6th and 8th hours in *E. coli* and *K. pneumoniae* isolates, and they reported that the EUCAST RAST method is applicable in routine laboratories, can be used to give rapid results with low test cost, but the results should be confirmed by standard methods due to the presence of very large errors. Cao et al. (13) found that the rate of VME in *E. coli* and *K. pneumoniae* isolates was 0.8% in the 4th hour, while no VME was detected in the 6th hour. In the aforementioned study, the advantages of the RAST method such as ease of application and rapid results were emphasized, but it was also reported that further studies were needed.

Martins et al. (14) reported that the majority of zone diameters for *E. coli* and *K. pneumoniae* isolates could be read appropriately after 6 hours of incubation, as highlighted in several studies (15,16). Kansak et al. (17) found that there were more antibiotic and isolate reading errors for *E. coli* and *K. pneumoniae* isolates in the 4th-hour evaluation compared to the 6th- and 8th-hour evaluations, and that the categorical agreement rate increased by 25% for *E. coli* isolates and 50% for *K. pneumoniae* isolates in the 6th-hour evaluation. Only piperacillin-tazobactam had a categorical agreement rate of 84.4% and 88.2% and a minor error rate of 15.6% and 11.8% for *E. coli* and *K. pneumoniae* isolates, respectively. As a result, due to the high minor error rate in the 4th and 6th hours, it was recommended that preliminary reports should be given after the 8th-hour evaluations.

Soo et al. (11) reported that error rates decreased with time in *P. aeruginosa* isolates using the RAST method and that VME was not detected for all antibiotics at the 8th hour, and the authors reported that it would be appropriate to evaluate studies with a large number of isolates. In their study, Kansak et al. (17) found the categorical agreement rate for piperacillin-tazobactam, ceftazidime, and meropenem to be 75% at hour 6 and the categorical agreement rate for all other antibiotics to be ≥90% in *P. aeruginosa* isolates. In the same study, the categorical agreement rate for the antibiotics tested was ≥90% for *A. baumannii* isolates, most of which were multidrug-resistant isolates, and no difference was observed between the 4th and 8th hours. In our study, 92% categorical agreement was found for tobramycin and ciprofloxacin against *P. aeruginosa* isolates at the 8th hour, while the categorical agreement rate was <90% for the other antibiotics at both incubation times. In our study, for *A. baumannii* isolates, the categorical agreement between methods was 37.5% and 70% for amikacin disc at the 4th and 6th hour, and growths detected against sulfamethoxazole at the 4th hour were determined as ATU. Categorical agreement was ≥90% for all other antibiotics and incubation times. The low categorical agreement for *P. aeruginosa* isolates in our study is a remarkable finding and studies with a large number of isolates related to these bacteria are needed. The

Table 5. Comparison of RAST and disc diffusion methods in *A. baumannii* isolates (n=13)

Antibiotics / Hours	4 hours	6 hours	8 hours
Imipenem			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Levofloxacin			
Number of growth	8	10	13
CA, n (%)	8 (100)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Amikacin			
Number of growth	8	10	13
CA, n (%)	3 (37.5)	7 (70.0)	11 (84.6)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	1 (10.0)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	5 (62.5)	2 (20.0)	1 (7.7)
Gentamicin			
Number of growth	8	9	12
CA, n (%)	8 (100)	9 (100)	12 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Tobramycin			
Number of growth	8	10	13
CA, n (%)	8 (100)	9 (90)	12 (92.3)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	1 (10.0)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole			
Number of growth	8	10	13
CA, n (%)	0 (0.0)	10 (100)	13 (100)
mE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
ATU, n (%)	8 (100)	0 (0.0)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

values found for *A. baumannii* suggest that the RAST test can be used in routine laboratory applications.

Kansak et al. (17) reported in their study of 20 *S. aureus* isolates that zone diameters were easily assessed at the 4th hour except for two isolates, VME and minor error were not detected, but an 11.1% minor error rate was observed for cefoxitin and gentamicin at the 4th hour. In the aforementioned study, the authors could not detect categorical compliance for vancomycin in *Enterococcus spp*. isolates and VME could not be detected as there were no resistant strains. However, they did detect major errors and VME and therefore characterized the results of vancomycin in *Enterococcus spp*. isolates as categorical non-agreement. Jasuja et al. (9) investigated RAST and Vitek MIC concordance in *S. aureus* isolates and found no VME and minor error for cefoxitin and a BH rate of less than 1%. In the same study, the VME rate for ampicillin in *Enterococcus spp*. isolates was less than 1%, no VME and minor error were detected, only one VME rate (4.2%) was detected for vancomycin, and VME and minor error rates were not reported.

Researchers have reported that the RAST method is rapid and reliable for highly resistant bacteria such as MRSA and VRE (9). In our study, similar to other studies, the categorical agreement rate of cefoxitin susceptibility was ≥90% in all *S. aureus* isolates except for two isolates grown in the 8th hour. Our results suggest that the RAST method can be used in routine laboratories, especially for early detection of MRSA strains and for treatment guidance, but the fact that VME was detected in two isolates of *S. aureus* on cefoxitin disc suggests that studies with larger numbers of isolates are needed and the test should be controlled by the standard disc diffusion method.

In contrast to studies in the literature, in our study, ATU was detected in all incubation times for vancomycin and in only one isolate at the 6th hour for ampicillin in *Enterococcus spp*. isolates and the categorical agreement was 100% for all other antimicrobials. The high level of categorical agreement for *Enterococcus spp*. isolates for antimicrobials other than vancomycin suggest that RAST can be used efficiently in routine laboratory applications.

CONCLUSION

It was concluded that the RAST method is easy to use and does not cause additional work and economic burden in life-threatening infections such as sepsis. The results obtained at the end of the 8th hour suggested that the antibiotics tested by the RAST method could guide the clinician in the use of antibiotics in treatment. However, the results for tobramycin and pipersiline tazobactam for *E. coli* and *K. pneumoniae* isolates, imipenem for *K. pneumoniae* isolates, and norfloxacin for *S. aureus* should be interpreted with caution. For *P. aeruginosa* isolates, susceptibility increased with increasing incubation time for all antibiotics, and for *A. baumannii* isolates, the RAST method gave acceptable and reliable results for all antimicrobials at the end of the 8th hour. Despite the high number of positive results in our data, the fact that compliance rates were low for some antimicrobials supports the idea that such studies should be performed with a larger number of isolates and a larger number of antibiotics.

Table 6. Comparison of RAST and disc diffusion methods in *P. aeruginosa* isolates (n=13)

Antibiotics / Hours	6 hours	8 hours
Piperacillin-tazobactam		
Number of growth	7	12
CA, n (%)	4 (57.1)	9 (75.0)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	3 (25.0)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	0 (0.0)
Ceftazidime		
Number of growth	7	13
CA, n (%)	4 (57.1)	10 (76.9)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	1 (7.7)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	2 (15.4)
Imipenem		
Number of growth	7	12
CA, n (%)	4 (57.1)	10 (83.3)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	1 (14.3)	0 (0.0)
VME, n (%)	1 (14.3)	1 (8.3)
ATU, n (%)	1 (14.3)	1 (8.3)
Meropenem		
Number of growth	7	13
CA, n (%)	5 (71.4)	11 (84.6)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	1 (14.3)	0 (0.0)
VME, n (%)	0 (0.0)	1 (7.7)
ATU, n (%)	1 (14.3)	1 (7.7)
Ciprofloxacin		
Number of growth	7	13
CA, n (%)	5 (71.4)	12 (92.3)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	0 (0.0)	1 (7.7)
ATU, n (%)	2 (28.6)	0 (0.0)
Tobramycin		
Number of growth	7	12
CA, n (%)	6 (85.7)	11 (91.7)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	1 (14.3)	1 (8.3)
ATU, n (%)	0 (0.0)	0 (0.0)
Sefepim		
Number of growth	7	7
CA, n (%)	4 (57.1)	4 (57.1)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	2 (28.6)
VME, n (%)	0 (0.0)	0 (0.0)
ATU, n (%)	1 (14.3)	1 (14.3)
Levofloxacin		
Number of growth	7	7
CA, n (%)	4 (57.1)	4 (57.1)
mE, n (%)	0 (0.0)	0 (0.0)
ME, n (%)	2 (28.6)	2 (28.6)
VME, n (%)	0 (0.0)	1 (14.3)
ATU, n (%)	1 (14.3)	0 (0.0)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

Ethics Committee Approval: The study was approved by the Non-invasive Clinical Research Ethics Committee of Düzce University (15.04.2019, 2019/94).

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Table 6. Comparison of RAST and disc diffusion methods in *P. aeruginosa* isolates (n=13) *continued*

Antibiotics / Hours	6 hours	8 hours
Amikacin		
Number of growth	7	7
CA, n (%)	3 (42.9)	4 (57.1)
mE, n (%)	1 (14.3)	1 (14.3)
ME, n (%)	0 (0.0)	0 (0.0)
VME, n (%)	1 (14.3)	1 (14.3)
ATU, n (%)	2 (28.6)	1 (14.3)

RAST: rapid antibiotic susceptibility test, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, ATU: area of technical uncertainty, *: high level

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