

EVALUATION OF DIRECT AND STANDARD ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS ON SOME BACTERIA ISOLATED FROM AUTOMATED BLOOD CULTURES

OTOMATİZE KAN KÜLTÜRLERİNDE ÜREYEN BAKTERİLER İÇİN DİREKT VE STANDART DİSK DİFÜZYON TESTLERİNİN DEĞERLENDİRİLMESİ

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Anahtar Sözcükler: Otomatize kan kültürleri, bakteriler, direkt antimikrobiyal duyarlılık testi, standart antimikrobiyal duyarlılık testi, disk difüzyon yöntemi

SUMMARY

The aim of this study is to evaluate direct and standardized antimicrobial susceptibility testing methods for the detection of bacteria isolated from automated blood cultures. In accordance with the protocol of the study, 126 blood cultures were collected from the patients during a 12-month period in 2001-2002. The samples from positive bottles showing growth of Gram-negative bacilli or clustered Gram-positive cocci, determined by Gram staining, were used for direct inoculation onto Mueller Hinton agar. The negative smears which had positive cultures and the positive smears which showed mixed cultures were excluded from the study. The results of direct disk diffusion tests were compared with those of standard Kirby-Bauer disk diffusion method, and discrepancies were classified as very major, major, or minor errors. The overall agreement of the two methods for *Staphylococcus aureus* and coagulase-negative staphylococci, in terms of the interpretive categories (susceptible, intermediate, and resistant) were 92.2 % and 90.7%, respectively. There were 32 (2.5%) very major errors, 16 (1.3%) major errors and eight (0.6%) minor errors which were caused by the direct method for *Staphylococcus* spp. The overall agreement of the two methods for Enterobacteriaceae and *Pseudomonas* spp. in terms of the interpretive categories were 92.1% and 96.8%, respectively. There were 22 (1.5%) very major errors, 11 (0.7%) major errors and 19 (1.3%) minor errors caused by direct method for Gram-negative bacilli. The rapid method for the antimicrobial susceptibility testing of Gram-negative bacilli and oxacillin resistant *S. aureus* is simple and requires no centrifugation, preincubation, or standardization of the inocula. This method can be used for patients with bacteremia. The direct disk method is proposed as a test that can be used as a supplement to the standardized procedures for the earlier determination of the susceptibility patterns of aerobic Gram-negative bacilli and *Staphylococcus* spp. from blood cultures.

ÖZET

Bu çalışmada otomatize kan kültürlerinden izole edilen bakteriler için direkt ve standart antimikrobiyal duyarlılık testlerinin karşılaştırılması amaçlanmıştır. 2001-2002 yılında 12 aylık dönemde bakteremi kuşkulu 126 hastadan alınan kan kültürleri değerlendirmeye alınmıştır. Üreme sinyali veren şişelerden alınan örnekler Gram boyama sonucuna göre Gram-olumlu kümeli kok ya da Gram-negatif basil (GNB) olarak belirlenmiş ve Müller Hinton agara direkt inokülasyon

yöntemi ile antibiyogram yapılmıştır. Kültür sonucu pozitif olduğu halde Gram boyama sonucu negatif olan, kültürde birden fazla bakteri üreyen ve Gram boyama sonucu pozitif olan örnekler çalışma kapsamına alınmamıştır. Direkt difüzyon testi sonuçları standart Kirby-Bauer disk difüzyon testi sonuçları ile karşılaştırılmıştır. Her iki yöntem arasındaki uyumsuzluk çok büyük hata, büyük hata ve küçük hata olarak sınıflandırılmıştır. *Staphylococcus aureus* ve koagülaz-negatif stafilocoklar için iki yöntem arasında toplam uyum, sırasıyla, %92.2 ve %90.7 olarak bulunmuştur. Stafilocok türler için toplam çok büyük hata %2.5, büyük hata %1.3 ve küçük hata %0.6 olarak saptanmıştır. Gram-negatif basil ve *Pseudomonas* için toplam uyum sırasıyla, %92.1 ve %96.8 olarak belirlenmiştir. Gram-negatif basil için toplam çok büyük hata %1.5, büyük hata %0.7 ve küçük hata %1.3 olarak bulunmuştur. Sonuç olarak; direkt disk difüzyon testi uygulama kolaylığı, santrifüj, preinkübasyon ve inokulasyon standardizasyonu gerektirmemesi nedeniyle diğer hızlı yöntemlere üstünlüğü olan ve özellikle GNB ve oksasiline dirençli *S. aureus*'un neden olduğu bakteremilerde hızlı tan ve erken tedavi için yararlı olabilecek bir yöntemdir. Direkt yöntem, kan kültürlerinden izole edilen GNB ve stafilocok türleri için duyarlılık paterninin erkenden belirlenmesinde standart prosedürlere ek olarak kullanılabilir.

INTRODUCTION

The detection of bacteremia is one of the most important functions of clinical microbiology laboratories. Due to the high morbidity and mortality associated with this disease process, rapid detection and identification of clinically relevant microorganisms in blood cultures remains the most important aspect (1). According to these demands, the development of continuously monitoring blood culture systems have been considered as one of the crucial issues. The BacT/Alert system (Becton Dickinson, Maryland, USA) can be accepted as one of the fully automated blood culture systems (2). The BacT/Alert software examines the readings from each bottle and determines whether there is an evidence for any kind of bacterial growth or not.

Rapid detection and identification of clinically relevant microorganisms in blood cultures still remain the most important matters (1). However, standard antimicrobial susceptibility test results of the isolated bacteria from blood cultures take long time. Rapid antimicrobial susceptibility testing of the isolates are very important for the management of the patients. This would then change the management of the patients with suspected sepsis.

The present study has been designed to evaluate direct and standard disk diffusion (Kirby-Bauer) methods of bacteria isolated from automated blood cultures. The aim of the study is to determine whether there is any difference in direct and standard antimicrobial susceptibility testing of various bacteria isolated from blood cultures and antimicrobials that are used for susceptibilities.

MATERIAL AND METHODS

This study was conducted from February 2001 to February 2002 at Abant İzzet Baysal University Hospital in Düzce. Blood specimens were collected from bacteremia suspected patients.

The BACTEC aerobic bottles (Becton Dickinson Microbiology Systems, Maryland, USA) were normally inoculated with 5 to 10 ml of blood from the patients, inserted into

BACTEC® 9050 instruments (Becton Dickinson Microbiology Systems, Maryland, USA), and incubated at 37° C. Samples from positive bottles showing the growth of Gram-negative bacilli (GNB) or clustered Gram-positive cocci, determined by Gram staining, were used for direct inoculation into Mueller-Hinton agar. The sample (1 ml) of positive blood culture was taken to the sterile tube and dipped a sterile cotton-wool into the suspension and removed the excess liquid by turning the swab against the side of the tube. The inoculum was spread evenly over the entire surface of the plate by swabbing in three directions. The plate was left to dry before applying disks. The disk should be applied to the surface of the agar within 15 minutes of inoculation (3). The negative smears of positive cultures and positive smears of mixed cultures were excluded from the study. Eleven antimicrobial agents were used for direct antimicrobial susceptibility testing of the clustered Gram-positive cocci and GNB as defined by the National Committee for Clinical Laboratory Standards (4). All blood isolates that were obtained on subculture plates were identified by conventional microbiological procedures. Gram-positive clustered cocci were identified according to the effect on mannitol and trehalose, colony morphology on blood agar, catalase and coagulase tests. Gram-positive bacteria identified as streptococci were excluded from the study. Gram-negative bacilli were identified with API 20E (bio Mérieux). Antimicrobial susceptibility testing of the isolates were determined by the standardized disk diffusion method (Kirby-Bauer) with Mueller-Hinton agar (bio Mérieux).

The results from direct disk diffusion tests were compared with those from standard Kirby-Bauer disk diffusion method, and discrepancies were classified as very major, major, or minor errors. A very major error was a susceptible result by the direct method and a resistant result by the standard method. A major error was a resistant result by the direct method and a susceptible result by the standard method. A minor error can be defined as any change involving an intermediate result (5).

The antimicrobial agents used in *Staphylococcus aureus* and coagulase-negative Staphylococci (CNS) were as follows: Penicillin (10 U), oxacillin (1 µg), vancomycin (30 µg), teicoplanin (30 µg), gentamycin (120 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), erythromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg) and tetracycline (30 µg). The antimicrobial agents used in Gram-negative bacilli were as follows: Ampicillin (10 µg), amoxicillin-clavulanic acid (20µg/10 µg), cefaclor (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), piperacillin (100µg), amikacin (30 µg), gentamycin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and ciprofloxacin (5 µg).

RESULTS

Fifty-eight automated blood cultures containing *S. aureus* and CNS were tested against eleven antimicrobial agents by using direct susceptibility and standard disk susceptibility techniques. The overall agreement of the two methods for *S. aureus* and CNS in terms of the interpretive categories (susceptible, intermediate, and resistant) were 92.2% and 90.7%, respectively. Direct and standard antimicrobial susceptibility testing results and agreement of *Staphylococcus* spp. are shown in Table 1.

There were 32 (2.5%) very major errors, 16 (1.3%) major errors and eight (0.6%) minor errors caused by direct method for *Staphylococcus* spp. (Table 2). Twenty-three very major errors were observed during testing with erythromycin (nine), oxacillin (five), trimethoprim-sulfamethoxazole (five), gentamycin (four).

Sixty-eight automated blood cultures containing GNB were tested against the eleven antimicrobial agents. The

Table 2. Error type distribution of direct and standard susceptibility methods according to the antibiotics used for *Staphylococcus* spp.

Antibiotics	No. of tests	No. of interpretive category errors		
		Very major	Major	Minor
Penicillin	116	1 (0.9)	1 (0.9)	2 (1.7)
Oxacillin	116	5 (4.3)	0	0
Vancomycin	116	0	0	0
Teicoplanin	116	0	0	0
Erythromycin	116	9 (7.7)	3 (2.6)	0
Gentamycin	116	4 (3.4)	2 (1.7)	1 (0.9)
Clindamycin	116	2 (1.7)	0	0
Ciprofloxacin	116	2 (1.7)	2 (1.7)	3 (2.6)
Trimethoprim/smz	116	5 (4.3)	5 (4.3)	0
Chloramphenicol	116	2 (1.7)	2 (1.7)	1 (0.9)
Tetracycline	116	2 (1.7)	1 (0.9)	1 (0.9)
Total	1276	32 (2.5)	16 (1.3)	8 (0.6)

overall agreement of the two methods for Enterobacteriaceae and *Pseudomonas* spp. in terms of the interpretive categories were 92.1% and 96.8%, respectively. Direct and standard antimicrobial susceptibility testing results of Gram-negative bacilli and agreement between the two methods are shown in Table 3.

There were 22 (1.5%) very major errors, 11 (0.7%) major errors and 19 (1.3%) minor errors caused by the direct method. Thirteen very major errors were observed when testing with ampicillin (five), amoxicillin-clavulanic acid (five), cefaclor (three) (Table 4).

Table 1. Direct and standard antimicrobial susceptibility testing results of *Staphylococcus* spp.

Antibiotics	Susceptibility and agreement					
	<i>S. aureus</i> n=21			CNS n=37		
	Direct %	Standard %	Agreement %	Direct %	Standard %	Agreement %
Penicillin	23.8	19.1	90.5	10.8	8.1	94.6
Oxacillin	71.4	71.4	100	51.4	64.9	86.5
Vancomycin	100	100	100	100	100	100
Teicoplanin	100	100	100	100	100	100
Erythromycin	71.4	57.1	85.7	45.9	54.1	73.5
Gentamycin	85.7	76.1	90.5	78.4	78.4	86.5
Chloramphenicol	83.3	75.0	91.7	71.4	75.0	96.4
Ciprofloxacin	85.0	90.0	95.0	78.4	89.2	83.8
Trimethoprim/smz.	70.0	75.0	76.2	55.6	58.3	86.1
Clindamycin	89.5	94.7	89.5	80.6	83.3	91.7
Tetracycline	31.3	31.3	87.5	44.4	51.9	92.6

CNS Coagulase-negative staphylococci

Table 3. Direct and standard antimicrobial susceptibility testing results of Gram-negative bacilli and agreement between the two methods

Antibiotics	Susceptibility and agreement					
	Gram-negative enteric bacilli n=54			<i>Pseudomonas</i> spp. n=14		
	Direct %	Standard %	Agreement %	Direct %	Standard %	Agreement%
Ampicillin	40.7	33.3	88.9	0	0	100
Amox/Clav.	70.4	59.3	85.2	14.3	14.3	100
Cefaclor	67.3	61.5	92.3	14.2	7.1	92.9
Ceftriaxone	75.5	78.8	88.7	7.1	0	92.9
Ceftazidime	81.3	77.1	93.9	21.4	21.4	100
Imipenem	98.1	100	96.3	64.3	57.1	85.7
Gentamycin	88.9	83.3	90.7	0	0	100
Amikacin	90.7	94.4	96.3	21.4	21.4	100
Ciprofloxacin	87.0	88.9	92.6	78.6	85.7	92.9
Trimethoprim/smz	69.4	67.3	93.9	14.3	14.3	100
Piperacillin	70.4	74.1	88.9	29.3	36.4	92.9

Table 4. Error type distribution of direct and standart antimicrobial susceptibility methods according to the antibiotics used for Gram-negative bacilli

Antibiotics	No. of tests	No. of interperative category errors		
		Very major	Major	Minor
Ampicillin	136	5 (3.7)	1 (0.7)	0
Amoksisillin/Clav.	136	5 (3.7)	0	1 (0.7)
Cefaclor	136	3 (2.2)	0	1 (0.7)
Ceftriaxone	136	1 (0.7)	0	6 (4.4)
Ceftazidime	136	2 (1.4)	0	1 (0.7)
Imipenem	136	0	1 (0.7)	3 (2.2)
Gentamycin	136	1 (0.7)	1 (0.7)	3 (2.2)
Amikacin	136	0	1 (0.7)	1 (0.7)
Ciprofloxacin	136	1 (0.7)	3 (2.2)	1 (0.7)
Trimetoprim/smz	136	2 (1.4)	1 (0.7)	0
Piperacillin	136	2 (1.4)	3 (2.2)	2 (1.4)
Total	1496	22 (1.5)	11 (0.7)	19 (1.3)

DISCUSSION

The isolation of any significant microorganism from a blood culture requires careful evaluation by the clinician, and prompt action is usually necessary. If the results of clinical microbiological analyses are to contribute in a meaningful way to the diagnosis and management of the patients with bacteremia, they must be made available to the clinician in a relevant time frame (6, 7). Most clinical laboratories use liquid media for the detection of microorganisms in blood, and the antimicrobial susceptibility tests are performed with colonies obtained on subculture plates. After a positive blood culture is detected, the

standard procedures may take approximately two days to provide the susceptibility results. Due to this fact, efforts have been made to devise analytical procedures which can provide earlier results.

Rapid techniques for testing the susceptibilities of organisms in blood cultures include the direct disk diffusion test (8, 9) and automated or semiautomated instrument systems. Direct disk diffusion susceptibility testing of the organisms in positive blood cultures have been considered as reliable for most microorganisms and antimicrobial agents (8, 10). This technique can save 18 to 24 hours when compared with the duration required for the standardized protocols.

Under most conditions, the susceptibility results were available within three to six hours after inoculation by the direct method whereas by routine procedures results are available in an average of 40 to 48 hours. This provided the susceptibility patterns to be available on the same day. In many studies, it has been reported that the agreement rate of the two methods should be over 90% (11). In this study, the overall agreement of the two methods for *Staphylococcus* spp. and GNB were 90.7% and 92.5%, respectively. These results suggest that, direct disk diffusion method can be used for susceptibility testing of *Staphylococcus* spp. and GNB isolated from automated blood cultures.

It is reported that a new susceptibility method should have very major error lower than 1.5% for each antibiotic (11). In this study for overall antibiotics, there were 32 (2.5%) very major errors, 16 (1.3%) major errors and eight (0.6%) minor errors caused by direct method for *Staphylococcus* spp. (Table 2). Twenty-three very major

errors were observed in testing with erythromycin (nine), oxacillin (five), trimethoprim-sulfamethoxazole (five) and gentamycin (four). One of the most common isolated microorganisms from blood cultures is *S. aureus*. When *S. aureus* is found in blood cultures, it is usually representative of the significant clinical disease (12). In this study, no major discrepancies were observed for *S. aureus* during testing oxacillin. All of the very major errors belonging to oxacillin were observed for CNS. Due to the high rate of the isolation of MRSA from bacteremic episodes, rapid disk diffusion method for this bacterium may be useful. It is reported that the rapid detection of oxacillin-resistant *S. aureus* in blood cultures by using an impedance method can be useful and may allow proper antimicrobial treatment almost 36 hours before the result of the conventional culture methods (13). Results of direct susceptibility testing for erythromycin, trimethoprim-sulfamethoxazole and gentamycin were observed unreliable.

Due to the high rate of the isolation of aerobic GNB from patients with bloodstream infection, a rapid method for the antimicrobial susceptibility testing of GNB is crucial. In the present study, there were 22 (1.5%) very major errors, 11 (0.7%) major errors, 19 (1.3%) minor errors caused by direct disk susceptibility method for GNB. Ten very major errors were observed during testing ampicillin (five) and amoxicillin-clavulanic acid (five). Lower (1.5%) very major error rates were observed in the direct method of GNB than *Staphylococcus* spp. (2.5%). The impedance method has been proposed for the patients with GNB bacteremia (14). The results in the present study indicate that the agreement of the direct and standardized method is similar to those of new methods. The direct disk method is proposed as a test that can be used as a supplement to the standardized procedures for the earlier determination of the susceptibility patterns of aerobic GNB and *Staphylococcus* spp. from blood cultures.

The impact of the rapid antimicrobial susceptibility testing on infectious disease outcome has been systematically assessed by Doern et al. (15). The benefits include the significant reduction in the numbers of the microbiology tests, subsequent positive blood cultures, serum antibiotic assays, some imaging procedures, and days of incubation and reductions in the length of time spent in an intensive care area. It was important to find that the mortality rate was much lower (8.8%) for the rapid test group than for the control group (15.3%) for which conventional overnight techniques. Trenholme et al. (16) also reported that rapid susceptibility testing of blood isolates could result

in an earlier initiation of an appropriate therapy or a change to the use of more effective and less expensive antibiotics. In addition, the rapid availability of the susceptibility information was more likely to be followed by the treatment of the patients by clinicians. The direct disk diffusion susceptibility testing of the organisms observed in blood cultures have been considered to be reliable for most of the microorganism-antimicrobial agent combinations (10).

Before direct inoculation, some researchers proposed that positive blood samples should be subcultured in a liquid broth followed by the adjustment of the inoculum density. However, several studies which were done with inocula taken directly from positive blood bottles also obtained better results, but an incubation period of 16 to 20 hours is normally required for the direct disk diffusion test [8]. Several instrument-assisted susceptibility test systems have been developed, and these systems are aimed to provide results in a matter of hours rather than days. These instruments include MicroScan, the Vitek Automicrobic system, and the Cobasbact system (17-20). However, several steps including sample centrifugation, blood cell lysis, and standardization of the inoculum are recommended before direct inoculation is applied into these systems (21-23). The detection principles for these systems are usually based on the measurement of changes in optical properties (turbidity or fluorescence) and are more sensitive to interferences from the blood specimens. The rapid disk diffusion method for the antimicrobial susceptibility testing of GNB and oxacillin-resistant *S. aureus* which is simple and requires no centrifugation, preincubation, or standardization of the inocula might be preferred for the patients with bacteremia. It can be suggested that many laboratories can use direct disk diffusion method easily without using extra equipment.

The result of this present study would be desirable if the direct method is used to guide a clinician in starting antimicrobial therapy for the patients. Because of the rates of the very major errors which were lower than 1.5% for oxacillin testing of *S. aureus* and the antibiotics which were used frequently for GNB, rapid method can be useful and may provide proper antimicrobial treatment almost 36 hours earlier than the result of the conventional culture methods for septicemic patients. The direct disk method is proposed as a test that can be used as a supplement to the standardized procedures for the earlier determination of the susceptibility patterns of aerobic GNB and *Staphylococcus* spp. from blood cultures.

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