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Direct Method to Determine the Antibiotic Susceptibility of Rapidly Growing Blood Pathogens

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Standardized direct disk diffusion antibiotic susceptibility testing on monomicrobial blood cultures is compared with the Food and Drug Administration method. The direct method yields acceptable data and may conserve 24 h in reporting results.

Disk diffusion antibiograms of bacterial blood pathogens are most appropriately determined by the Food and Drug Administration (FDA) recommended procedure (1-4), which requires isolated colonies to prepare inocula for susceptibility testing. The necessity to await pure cultures causes 48 to 72 h to elapse before antibiotic susceptibility data become available. Our studies suggest that this period may be shortened by 24 h when bacteremia is caused by a single, rapidly growing pathogen.

Fifty milliliters of B-D supplemented peptone vacutainer blood bottles was inoculated with 5 ml of suspected bacteremic blood, incubated at 35 C, and observed daily for 7 days. Suspicious cultures were sampled with a 3-ml syringe. One drop of culture fluid (ca. 0.01 ml) was inoculated into 2 ml of Columbia broth. Additional culture aliquots were Gram stained and inoculated onto 10% sheep blood agar plates.

Broth suspensions from blood cultures containing either staphlococci or gram-negative bacilli were incubated for 4 to 6 h at 37 C and were subsequently adjusted with 0.9% NaCl to

TABLE 1. *Comparison of direct and indirect disk diffusion methods for obtaining antibiograms of blood pathogens*

Antibiotic	Organism (no. of isolates)							
	Staphylococcus aureus (16)		*Escherichia coli* (33)		*Klebsiella pneumoniae* (11)		*Proteus mirabilis* (14)	
	Mean[a] difference (mm)	Range (mm)	Mean difference (mm)	Range (mm)	Mean difference (mm)	Range (mm)	Mean difference (mm)	Range (mm)
Vancomycin (30 μg)	0.6	0-2	—[b]	—	—	—	—	—
Novobiocin (30 μg)	0.7	0-3	—	—	—	—	—	—
Clindamycin (2 μg)	1.9	0-4	—	—	—	—	—	—
Oxacillin (1 μg)	0.9	0-3	—	—	—	—	—	—
Erythromycin (15 μg)	1.6	0-6	—	—	—	—	—	—
Penicillin (10 units)	0.4	0-2	—	—	—	—	—	—
Ampicillin (10 μg)	0.3	0-2	0.6	0-2	R[c]	R	1.1	0-2
Chloramphenicol (30 μg)	1.0	0-3	1.1	0-4	1.0	0-1	0.9	0-3
Tetracycline (30 μg)	0.7	0-2	0.4	0-2	0.5	0-1	R	R
Cephalothin (30 μg)	0.9	0-4	0.8	0-2	1.0	0-2	1.1	0-2
Kanamycin (30 μg)	—	—	0.7	0-2	0.9	0-2	1.1	0-3
Streptomycin (10 μg)	—	—	0.6	0-2	0.8	0-2	0.7	0-2
Colistin (10 μg)	—	—	0.6	0-2	0.1	0-1	R	R
Gentamycin (10 μg)	—	—	0.8	0-3	1.5	0-3	0.9	0-2
Carbenicillin (100 μg)	—	—	0.8	0-3	R	R	1.5	0-4
Polymixin B (300 units)	—	—	0.4	0-1	0.9	0-2	R	R

[a] The antibiotic zones of inhibition obtained with each blood isolate, by both the direct and indirect methods, were measured. Averages and ranges of the individual differences were then computed.

[b] —, Staphyloccocal isolates were not tested against kanamycin, streptomycin, colistin, gentamycin, carbenicillin, or polymixin B. Gram-negative isolates were not tested against vancomycin, novobiocin, clindamycin, oxacillin, erythromycin, or penicillin.

[c] The zones of inhibition were minimal in size with the majority of isolates tested.

match a 0.5 McFarland $BaSO_4$ standard. Mueller-Hinton agar plates were inoculated evenly with the standard suspensions, and appropriate antibiotic disks (BBL) were applied. After 18 to 24 h of incubation at 35 C, inhibition zone diameters were measured to the nearest whole millimeter using a linear scale. Polymicrobial blood cultures or those containing slow-growing bacteria were not considered.

Isolated colonies from the blood agar plates were used to prepare inocula for the FDA standardized, Bauer-Kirby procedure. Inhibition zone sizes for each isolate and drug tested by the direct and standard procedures were recorded and compared to interpretive standards established for the FDA method (2).

Seventy-four blood cultures containing a single, rapidly growing bacterial pathogen were evaluated. Antibiograms of 16 *Staphylococcus aureus*, 33 *Escherichia coli*, 14 *Proteus mirabilis*, and 11 *Klebsiella pneumoniae* were obtained by both methods.

Data (Table 1) compared for each isolate and antibiotic indicated that the average difference in zone sizes for the two methods was less than 2 mm in all instances and approximately 1 mm in most cases. Results obtained by the two methods necessitated a change in interpretive susceptibility category in only five instances (0.7%).

Attempts have been made in this laboratory to shorten the time required to obtain in vitro susceptibility data on blood pathogens by using the blood culture fluid as inoculum for disk diffusion testing. Results have correlated erratically with data obtained by the accepted technique because of the use of unstandardized inocula and the possible presence of factors influencing zone sizes. This study has shown that inoculation of a relatively tiny sample of blood culture fluid into a relatively large volume of nutrient broth followed by growth to a specific density results in a standardized inoculum and elimination by dilution of factors affecting zone size. This technique is not proposed as a replacement for the FDA method but is presented as a faster alternative method to determine antibiotic susceptibility of blood pathogens in clinically urgent situations.

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