Laboratory Evaluation of Amikacin Susceptibility Testing by the AutoBac I System

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A total of 319 clinical isolates were compared for amikacin susceptibility by AutoBac I and Bauer-Kirby disk diffusion methods. There was an overall agreement of 98.5% between AutoBac I and Bauer-Kirby. An additional 23 strains resistant to amikacin, obtained from Bristol Laboratories, registered a 65% overall agreement. The sensitivity readings differed between the two methods for 13 organisms: 11 were minor discrepancies, intermediate by one of the two methods, and 2 were very major discrepancies, resistant by disk diffusion and susceptible by AutoBac I.

As new antibiotics reach the clinical stage of development, each must be individually examined by generally accepted *in vitro* methods to determine standard values for susceptibility testing. In our study, susceptibility to commercially prepared amikacin disks by the Bauer-Kirby method was compared to results obtained with the AutoBac I system.

MATERIALS AND METHODS

Organisms. Over 500 clinical isolates were collected from three hospitals on a daily basis. In addition, 50 seldom-isolated organisms were selected from clinical stock culture collections of these hospitals. Combined, these organisms represented 36 species, 20 genera, 6 families, and 4 groups classified as "miscellaneous gram-negative rods." The isolates were transferred daily to fresh tryptic soy agar with 5% sheep blood plates, incubated at 35°C, and tested within 48 h of transfer.

Because 96.3% of the clinical isolates, other than *Enterococcus*, from contributing hospitals were susceptible to amikacin by both methods tested (Table 1), we obtained 23 strains resistant to amikacin from Bristol Laboratories, Syracuse, N. Y. These organisms represented eight species, seven genera, and three families, including ten isolates of *Pseudomonas aeruginosa*.

Susceptibility test methods. Susceptibility of each organism to amikacin was tested, in duplicate, by the AutoBac I (Pfizer Diagnostic Co., New York, N. Y.), according to the manufacturer's instructions and the Bauer-Kirby procedure. Fresh 150-mm Mueller-Hinton agar plates, calcium and magnesium adjusted (Clinical Standards Laboratories, Carson, Calif.), were used for all disk diffusion testing.

P. aeruginosa (ATCC 27853) and *Escherichia coli* (ATCC 24922) were utilized as control organisms for the disk diffusion method, and *P. aeruginosa* (CDC-F12) and *E. coli* (ATCC 25922) were utilized as control

organisms for the AutoBac I method. The controls were run with each experimental series and always fell within the acceptable range for the Bauer-Kirby method, or were sensitive with LSIs of 0.85 to 1.00 by the AutoBac I method.

Zones of inhibition were measured with a Fisher-Lilly antibiotic zone reader (Fisher Scientific, St. Louis, Mo.) to the nearest 0.1 mm. The zone reader is used in our laboratory because it reduces intertechnician variability. The reader measures zone sizes slightly smaller than by the caliper method; this did not affect the outcome of this investigation.

Bauer-Kirby zone sizes for amikacin were recorded as resistant if less than 11 mm, intermediate if between 12 and 13 mm, and sensitive if 14 mm or more (4).

Amikacin disks assayed by the Food and Drug Administration at 11.70 µg were obtained from Pfizer,

TABLE 1. Susceptibility of clinical isolates to amikacin

No. of isolates	Organism	% Susceptible
77	<i>E. coli</i>	97.5
63	<i>P. aeruginosa</i>	95.3
29	<i>Enterobacter</i> sp.	100
26	<i>Klebsiella</i> sp.	100
22	<i>Staphylococcus aureus</i>	100
20	<i>Enterococcus</i>	0
18	<i>Proteus mirabilis</i>	100
12	<i>Proteus</i> sp.	100
12	<i>Serratia</i> sp.	92
9	<i>Citrobacter</i> sp.	100
9	<i>Pseudomonas</i> sp.	100
5	<i>Salmonella</i> sp.	100
2	<i>P. maltophilia</i>	0
2	<i>Shigella</i> sp.	100
2	<i>S. epidermidis</i>	50
11	Other gram-negative rods	82

TABLE 2. Overall discrepancies, by organism, between Autobac I and the disk diffusion test

No.	Organism	Source	Test result ^a		Class of discrepancy ^b	MIC ^c
			Bauer-Kirby	AutoBac I		
81	<i>P. aeruginosa</i>	Wound	I	S	Minor	1.56
100	<i>E. coli</i>	Urine	S	I	Minor	1.56
180	<i>Achromobacter xylosoxidans</i>	Wound	I	R	Minor	64-128
196	<i>P. aeruginosa</i>	Bristol	I	R	Minor	8
197	<i>P. aeruginosa</i>	Bristol	R	S ^d	Very Major	16-32
198	<i>P. aeruginosa</i>	Bristol	R	S ^d	Very Major	32
200	<i>S. marcescens</i>	Bristol	I	R	Minor	8
210	<i>P. mirabilis</i>	Bristol	R	I	Minor	32
211	<i>K. pneumoniae</i>	Bristol	I	S	Minor	32
217	<i>P. aeruginosa</i>	Bristol	R	I	Minor	16
218	<i>P. aeruginosa</i>	Bristol	I	R	Minor	16
232	<i>P. aeruginosa</i>	Urine	I	S	Minor	1-2
233	<i>C. diversus</i>	Urine	I	S	Minor	0.5-4.0

^a S, Susceptible; I, intermediate; R, resistant.

^b Very major indicates susceptible by AutoBac I, resistant by disk diffusion; minor indicates intermediate by one of the two methods.

^c MIC, Minimal inhibitory concentration, in micrograms per milliliter.

^d Resistant after agitation.

and amikacin standard powder was supplied by Bristol Laboratories. When sensitivity determination of the two methods disagreed, broth dilution determination for minimal inhibitory concentration to amikacin was performed.

RESULTS AND DISCUSSION

The results of 319 of the 500 clinical isolates tested are reported; duplicate isolates obtained on successive days from the same patient were eliminated. Agreement between the two methods was demonstrated for 98.5% of the clinical isolates. Of the organisms supplied by Bristol Laboratories, the methods were in agreement 65% of the time. The discrepancies between the two methods were rated as very major, major, or minor by the criteria of Thornsberry et al. (3).

Discrepancies between the two methods are listed in Table 2. Of these discrepancies, 85% were classified as minor, whereas 15% were considered very major. There were no organisms in the major discrepancies category. These results were similar to the data of Thornsberry et al. and Cleary and Maurer (1) in their comparisons of the AutoBac I and disk diffusion susceptibility testing of the aminoglycoside antibiotics.

The two organisms demonstrating very major discrepancies (no. 197 and 198) were apparent failures of the AutoBac I system. These organisms were noted to grow in the cuvette chamber, but precipitated out during incubation and were not detected by the photometer. These organisms registered as resistant if the cuvette

was agitated manually before reading. Although the AutoBac I manual does not allude to this problem with *Pseudomonas*, it appears to be of concern when testing amikacin. Therefore, when testing *Pseudomonas* with amikacin, we suggest the cuvette be inspected for precipitation and agitated manually before reading in the AutoBac I.

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