

## Rapid, Specific Microbiological Assay for Amikacin (BB-K8)

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The emergence of a strain of *Providencia stuartii* resistant to multiple antibiotics with the exception of amikacin provided a test organism for a microbiological assay for this new semisynthetic aminoglycosidic aminocyclitol. Results can be read at 4 h and are specific for amikacin. The resistance of *P. stuartii* to all currently used antibiotics allows the utilization of this technique in the presence of other concurrently administered antibiotics and therefore eliminates the need for their inactivation in the assay procedure. The rapidity, specificity, and simplicity of this microbiological assay may provide a technique for routine clinical monitoring of patients on therapeutic regimens and could be utilized by institutions unable to perform the radioimmunoassay or radioassay techniques.

The need to directly monitor potentially toxic antibiotics in individual patients has been stressed by Sabath et al. (9, 10). The search for rapid and specific procedures has led to the development of radioenzyme and radioimmunoassay techniques to determine gentamicin concentrations in sera (6, 11, 12). In addition, similar methods are currently being developed for amikacin. However, the need remains for rapid, simple, and specific assays that could be performed by those laboratories not equipped to perform these specialized techniques.

Many microbiological assays for the determination of concentrations of the aminoglycosidic aminocyclitol class of antibiotics in sera have been described (9, 10). Most are complicated by a requirement for inactivation of concomitantly administered antimicrobial agents and/or by the slow growth of the test microorganisms, thereby requiring 18 to 24 h before results are available. The emergence (7) of resistance in a strain of *Providencia stuartii* to all commonly used antimicrobial agents except amikacin prompted the use of a representative isolate of this strain as the test microorganism for an assay procedure. Utilization of this strain incorporated the features of rapidity, specificity for amikacin, and simplicity into a microbiological assay. Values for the serum concentrations of amikacin obtained with the microbiological assay to be described were compared with those obtained in the same sera by using both the radioenzyme assay and radioimmunoassay techniques.

### MATERIALS AND METHODS

**Test strain and characterization.** A strain of *P. stuartii* was a representative clinical isolate of the *Providencia* infecting patients in the burn ward at Los Angeles County/University of Southern California Medical Center, and its biochemical characterization has been reported in detail previously (5, 7). Susceptibility studies to all commonly used antimicrobial agents were performed by standardized methods (1, 2). This strain was susceptible only to amikacin (minimal inhibitory concentration, 0.8 µg/ml) and resistant to streptomycin, kanamycin, neomycin, gentamicin, tobramycin, penicillin, ampicillin, carbenicillin, methicillin, cephalothin, cefazolin, vancomycin, erythromycin, lincomycin, clindamycin, and chloramphenicol. Inhibitory concentrations ranged from a low of 50 µg of gentamicin per ml to more than 1,400 µg of kanamycin per ml.

**Preparation of assay plates.** The method for preparation of assay plates was a modification of that described by Bennett et al. (3). Antibiotic medium no. 11 (Difco control no. 580502) was autoclaved and cooled in a water bath at 50 C. To bottles containing 40 ml of melted agar, 0.2 ml of a well-dispersed 18-h culture of *P. stuartii* (strain no. 73-197) grown in Mueller-Hinton broth was added. The optical density of bacterial suspensions was read on a Coleman Junior spectrophotometer and adjusted to 0.15 at 540 nm by dilution with Mueller-Hinton broth. Viable colony counts of the inoculum were approximately  $1.3 \times 10^9$  colony-forming units per ml.

Forty milliliters of the "seeded" agar was poured into plastic plates (150 by 15 mm; Falcon) to provide a uniform layer 4 mm in depth. After solidification of the seeded agar, 30 wells (5.5 mm in diameter and 4 mm deep) were made with a metal cylinder. Agar plates were stored at 4 C until used.

**Preparation of antibiotic standards.** A stock solution of amikacin containing 1,000  $\mu\text{g/ml}$  was prepared in distilled water and sterilized by passage through a membrane filter (Millipore Corp.). The stock solution was diluted in sterile pooled human serum to prepare standard concentrations of 12.5, 6.25, 3.13, and 1.6  $\mu\text{g/ml}$ , respectively. Standard solutions were stored in portions at  $-20^\circ\text{C}$  and thawed immediately before use.

**Bioassay procedure.** Each standard with known amikacin concentrations and serum specimens obtained from patients receiving amikacin therapy was delivered by an individual capillary pipette (Corning) to an agar well. Specimens from patients were diluted in sterile pooled human serum and were assayed undiluted in addition to dilutions of 1:2 and 1:3. All samples were run in duplicate. Assay plates were incubated in an upright position at  $37^\circ\text{C}$ . After incubation for 4 h, clear zones of inhibition around agar wells were read to the nearest 0.1 mm with a calibrated hand lens (Bausch & Lomb). The well-defined zones of inhibition in the bacterial lawn are demonstrated in Fig. 1. Standard curves were obtained for each assay plate on semilogarithmic paper by plotting the standard concentrations of amikacin (12.5, 6.25, 3.13, and 1.6  $\mu\text{g/ml}$ ) versus diameters of the zones of inhibition. Thereafter, the concentrations of serum samples were determined by converting the mean diameter of inhibition of each sample to a concentration in micrograms per milliliter by extrapolation from the derived standard graph.

**Effect of other antimicrobial agents.** The effect on zones of inhibition by antimicrobial agents other than amikacin was analyzed by the addition of vancomycin (20  $\mu\text{g/ml}$ ; Lilly), methicillin (50  $\mu\text{g/ml}$ ; Bristol), ampicillin (30  $\mu\text{g/ml}$ ; Bristol), clindamycin (20  $\mu\text{g/ml}$ ; Upjohn), carbenicillin (70  $\mu\text{g/ml}$ ; Roerig), cephalothin (30  $\mu\text{g/ml}$ ; Lilly), chloramphenicol (30

$\mu\text{g/ml}$ ; Parke-Davis), or tetracycline (20  $\mu\text{g/ml}$ ; Lederle) to standard concentrations of amikacin (12.5, 6.25, 3.13, and 1.6  $\mu\text{g/ml}$ ). Zones obtained by addition of the above antibiotics were compared with those obtained with amikacin in controls.

## RESULTS

Concentrations of amikacin were determined by this 4-h microbiological assay with *P. stuartii* in 19 sera collected from patients on therapeutic regimens with this antibiotic (Table 1, first column). Amikacin concentrations in these same sera were compared with those determined by the radioenzyme assay and the radioimmunoassay techniques (Table 1, second and third columns). Product-moment correlation coefficients obtained by pairing results with those obtained with the radioenzyme assay and radioimmunoassay techniques were 0.96 and 0.93, respectively. Zones of inhibition were not affected by the addition of other currently used antibiotics.

## DISCUSSION

Amikacin is a new semisynthetic aminoglycosidic aminocyclitol antibiotic derived from kanamycin (8). Existing indirect methods available for calculation of kanamycin dosages include either the utilization of computerized information based on changing creatinine clearance or an estimation based on serum creatinine concentration or creatinine clearance. The pharmacokinetics of amikacin in humans has been reported to parallel that of kanamycin (4), and

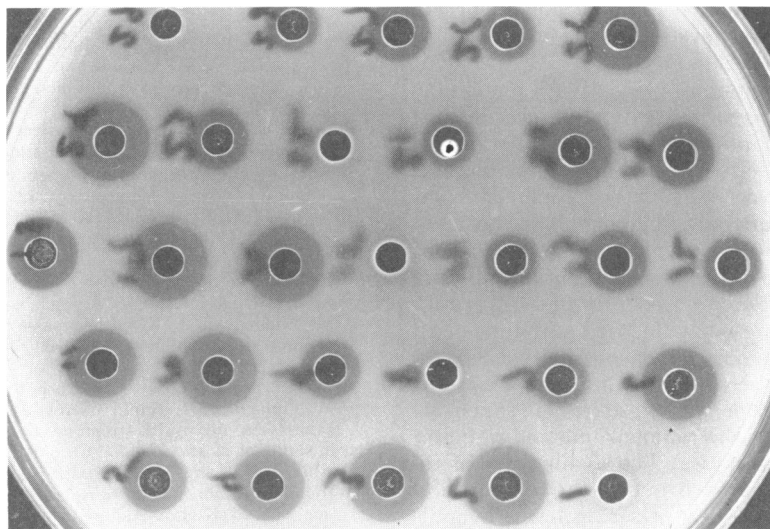


FIG. 1. Demonstration of the well-defined zones of inhibition at 4 h on seeded bacterial lawns of *Providencia stuartii*.

TABLE 1. Comparison of amikacin concentrations in sera by various techniques

Sample no.	Amikacin concn ( $\mu\text{g/ml}$ ) by:		
	Microbiological assay	Radioenzyme assay	Radioimmunoassay
1	1.3	2.3	0.5
2	1.7	1.5	1.7
3	10.8	12.0	17.0
4	11.7	15.0	15.0
5	12.6	14.5	11.0
6	16.8	17.0	15.0
7	16.8	18.0	18.0
8	19.5	16.0	15.2
9	21.0	17.5	21.0
10	22.5	21.5	15.0
11	24.3	24.0	32.0
12	25.2	29.5	32.0
13	26.4	28.0	32.0
14	29.1	35.0	30.0
15	29.4	28.5	28.0
16	31.8	36.0	34.5
17	36.0	33.5	40.0
18	39.0	33.5	40.0
19	39.0	44.0	33.0

therefore either method to calculate dosages may be applicable for amikacin. However, indirect methods for determining dosages of the aminoglycosidic aminocyclitol class of antibiotics have been unreliable (9, 10). In addition, the narrow margin between toxic and therapeutic levels of these agents requires close monitoring of serum concentrations despite the careful calculation of dosages. These factors point to the necessity for direct measurements to prevent renal or ototoxicity and to document the presence of serum levels that are inhibitory to infecting microorganisms.

It has been possible to inactivate penicillins (9, 10), cephalosporins (excluding cephalixin) (9, 10), and chloramphenicol with either broad-spectrum or specific enzymes in microbiological assays. However, antibiotics such as vancomycin, clindamycin, lincomycin, and tetracycline cannot be inactivated and may present problems when other test microorganisms are used. The resistance of the described strain of *P. stuartii* to all presently licensed and unlicensed antimicrobial agents lends specificity solely for amikacin. Further, the microbiological assay is equivalent in specificity to more expensive methods such as the radioenzyme and radioimmunoassay techniques. The availability of reproducible data in 4 h makes the assay useful for routine monitoring of serum levels in individual patients at particular risk of developing drug toxicity. In addition, the high degree of correlation of results by these three techniques

will allow meaningful interpretation of pharmacokinetic data with amikacin regardless of the method utilized.

The microbiological assay described here provides a rapid, highly specific, and simplified technique for the measurement of amikacin concentrations in body fluids. It can be applied in laboratories not equipped to do the radioenzyme or radioimmunoassays and may be applicable to routine monitoring of serum levels in patients on therapeutic regimes.

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