Measurement of Levels of Aminoglycosides and Vancomycin in Serum in the Presence of New β -Lactam Antibiotics

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We evaluated the effect of new β -lactam antibiotics (azlocillin, mezlocillin, piperacillin, cefotaxime, moxalactam, and cefoperazone) on assays for aminoglycosides and vancomycin. These antibiotics produced no interference in an immunoassay for gentamicin and tobramycin. The new penicillins produced no interference in a bioassay of amikacin and vancomycin with penicillinase incorporated into the assay agar. Bioassay in the presence of the cephalosporins required predigestion with cephalosporinase. We describe a method for accurate bioassay in the presence of the available cephalosporins, with the exception of moxalactam.

Aminoglycoside antibiotics and vancomycin have a narrow toxic-therapeutic ratio and require careful dosage adjustment in the presence of renal failure. Measurement of levels of these antibiotics in serum is a useful adjunct in monitoring therapy. Several methods are available for determining levels of antimicrobial agents in serum, including bioassay, radioenzymatic assay, high-pressure liquid chromatography, and a variety of immunoassays (5). Bioassay techniques are simple and inexpensive but time consuming, and other antibiotics present in the sample may interfere with the results. The immunoassays are much more rapid and generally free of interference by other antibiotics but are more expensive (8).

Several new β -lactam antibiotics have recently been released for clinical use. These include the new penicillins (azlocillin, mezlocillin, and piperacillin) and the new cephalosporins (cefotaxime, moxalactam, and cefoperazone). We have evaluated the effect of these new antibiotics on the measurement of levels of aminoglycosides and vancomycin in serum in a clinical laboratory. We assessed the effects of the new β -lactam antibiotics on the measurement of gentamicin and tobramycin levels by enzyme immunoassay and on the measurement of amikacin and vancomycin levels by bioassay.

Antimicrobial reference powders of known potency were supplied by the manufacturers. Penicillinase (derived from *Bacillus cereus*, 10^6 Kersey units per ml) was purchased from BBL Microbiology Systems, Cockeysville, Md., and was stored at 4°C until used. Cephalosporinase (derived from *Enterobacter cloacae*) was purchased from Miles Research Product Division, Miles Laboratories, Inc., Elkhart, Ind. The lot utilized had a potency of 2,559 U/ml. It was diluted 1:3, 1:10, and 1:30 in 0.1 M phosphate buffer (pH 8.0) and stored in portions at -20°C.

Gentamicin and tobramycin were assayed by a commercially available enzyme immunoassay (EMIT; Syva Corp., Palo Alto, Calif.), following the directions of the manufacturer (7). The instrumentation used included an Auto Carousel (Syva Corp.), a Gilford Stasar III spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), and a CP-5000 clinical processor (Syva Corp.). Gentamicin and tobramycin controls (1.25 and 7.2 μ g/ml, respectively) were prepared in pooled, antibiotic-free human serum and stored

in portions at -20°C. Fresh solutions of the new β -lactam antibiotics were prepared in distilled water at 5,000 μ g/ml. To determine the effect of the new β -lactam antibiotics on the enzyme immunoassay of gentamicin and tobramycin, 0.9 ml of each aminoglycoside control solution was added to 0.1 ml of each β -lactam solution (or 0.1 ml of distilled water). The mixtures were assayed in triplicate by EMIT, and the results for the mixtures containing the β -lactams were compared with those for the mixtures containing distilled water as control.

Amikacin and vancomycin were measured by an agar diffusion bioassay method previously described (10). The assay medium was modified Trypticase soy agar (BBL Microbiology Systems), and the indicator organism was *Bacillus globigii*. Penicillinase (1.5 ml) was added to each assay plate. Amikacin and vancomycin standards were prepared in serum (as described above) at doubling concentrations from 1.56 to 100 μ g/ml. A solution of oxacillin (100 μ g/ml) was included on each plate to verify the effectiveness of the penicillinase. Amikacin and vancomycin controls were prepared in serum at concentrations of 6.25 and 25 μ g/ml, respectively. To determine the effect of new penicillins on the bioassay, 0.9 ml of each amikacin or vancomycin control solution was added to 0.1 ml of the solutions of azlocillin, mezlocillin, or piperacillin (or 0.1 ml of distilled water). The resulting mixtures were assayed in triplicate by bioassay, and the results for the mixtures containing the penicillins were compared to those for the mixtures containing distilled water as control.

Bioassay of amikacin and vancomycin in the presence of the new cephalosporins required predigestion of the samples with cephalosporinase. We first determined the amount of cephalosporinase needed to inactivate a variety of cephalosporins. Diluted solutions of cefotaxime, moxalactam, and cefoperazone (500 μ g/ml), as well as 500- μ g/ml solutions of cephalothin, cefazolin, cefamandole, and cefoxitin, were mixed 1:1 with each dilution of cephalosporinase and incubated for 1 h at 37°C. The mixtures were analyzed by bioassay, and the amount of cephalosporinase needed to inactivate each cephalosporin was recorded. We then added 0.9 ml of each amikacin or vancomycin control solution to 0.1 ml of the solutions of cefotaxime, moxalactam, and cefoperazone (or 0.1 ml of distilled water). These mixtures were predigested for 1 h at 37°C by diluting them 1:1 with the appropriate concentration of cephalosporinase as determined above; the control mixture containing distilled water

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TABLE 1. Bioassay measurements of amikacin in the presence of azlocillin, mezlocillin, or piperacillin

β -Lactam antibiotic added (0.1 ml) ^a	Bioassay measurements (mean ^b $\pm$ SD (μ g/ml)) of amikacin control (0.9 ml)	
	No. 1	No. 2
None (0.1 ml of distilled water added)	4.9 $\pm$ 0.5	20 $\pm$ 1
Azlocillin	5.1 $\pm$ 0.2	23 $\pm$ 1
Mezlocillin	5.2 $\pm$ 0.2	21 $\pm$ 2
Piperacillin	5.1 $\pm$ 0.5	21 $\pm$ 2

^a Final concentration of antibiotic in mixture was 500 μ g/ml.^b Mean level in mixture of amikacin control plus second antibiotic or distilled water.

received the 1:30 dilution of cephalosporinase. The predigested samples were assayed in triplicate by bioassay, and the results for the mixtures containing the cephalosporins were compared to those for the mixtures containing distilled water as control. Bioassay results were multiplied by 2 to take into account the dilution by the cephalosporinase.

Using the EMIT system, we detected no interference in the measurement of gentamicin or tobramycin controls by the presence of new β -lactam antibiotics (azlocillin, mezlocillin, piperacillin, cefotaxime, moxalactam, and cefoperazone) at concentrations (500 μ g/ml) higher than those likely to be achieved clinically.

None of the new penicillins produced zones of inhibition around standard antimicrobial reference disks in the bioassay system as described above. In addition, the presence of these three new penicillins did not affect accurate measurement of levels of amikacin (Table 1) or vancomycin (data not shown) in serum by bioassay.

In contrast, antimicrobial disks of the three new cephalosporins (cefotaxime, moxalactam, and cefoperazone) produced large zones of inhibition in the bioassay system. Similarly, disks of these new cephalosporins produced substantial zones of inhibition with the cephalothin-resistant *Klebsiella pneumoniae* bioassay indicator strain described by Lund et al. (3). Therefore, it was necessary to include a predigestion step with cephalosporinase before quantitating amikacin and vancomycin by bioassay in the presence of the new cephalosporins.

We first determined the concentration of cephalosporinase needed to inactivate solutions of cephalosporins at concentrations of 500 μ g/ml. A 1:30 dilution of cephalosporinase (final concentration, 85.3 U/ml) was sufficient to inactivate solutions containing cefoperazone, cephalothin, cefazolin, or cefamandole. In contrast, the 1:3 dilution of cephalosporinase (final concentration, 853 U/ml) was necessary to inactivate solutions of cefotaxime or cefoxitin. No cephalosporinase concentration tested (including undiluted cephalosporinase incubated for 4 h) was sufficient to inactivate moxalactam. Utilizing cephalosporinase predigestion, we tested the feasibility of measuring amikacin and vancomycin levels in the presence of the new cephalosporins. Amikacin could be measured reliably in the presence of cefotaxime or cefoperazone by predigesting the cephalosporin with the appropriate concentration of cephalosporinase (as determined above) (Table 2). Similar data were obtained for the measurement of vancomycin in the presence of these two cephalosporins. We were unable to determine amikacin or vancomycin levels by bioassay in the presence of moxalac-

tam because of the inability to predigest moxalactam with the cephalosporinase used.

The EMIT assays for gentamicin and tobramycin have been previously shown to be quite specific. No cross-reaction has been detected with a variety of β -lactam antibiotics, unrelated aminoglycosides, and a number of other antimicrobial agents (1, 6, 7). We have extended these observations to include the new β -lactam agents (azlocillin, mezlocillin, piperacillin, cefotaxime, moxalactam, and cefoperazone).

Despite the rapidity and specificity of immunoassay techniques, microbiological assays for antimicrobial agents still remain useful. One of the major limitations of bioassays is interference with the measurement of one antibiotic in a sample by the presence of another antibiotic. In the past, this problem has been overcome by using an indicator organism resistant to the second antibiotic (3) or by physically or enzymatically inactivating the second drug (9). Degradation of β -lactam antibiotics by the addition of β -lactamase is the most commonly used inactivation in the bioassay of antimicrobial combinations. Our studies demonstrate that the addition of penicillinase to the assay agar is adequate for inactivating the three new penicillins, allowing accurate bioassay of aminoglycosides or vancomycin in the presence of these drugs.

The cephalosporins, however, are resistant to hydrolysis by the penicillinase used in our bioassay. Certain new cephalosporins, particularly cefoxitin, cefotaxime and moxalactam, are especially resistant to β -lactamase hydrolysis (2). We report a procedure in which cephalosporinase is used for inactivating many of the commonly used cephalosporins. The cephalosporinase predigestion step allows the accurate bioassay of aminoglycosides or vancomycin in the presence of cephalosporin antibiotics, including the new agents cefoxitin, cefotaxime, and cefoperazone (but not moxalactam).

A bioassay procedure for measuring tobramycin in the presence of moxalactam has been previously described (4). This procedure utilized predigestion of the sample for 30 to 60 min with the enzyme β -lactamase II from *B. cereus*. After predigestion, the bioassay was carried out by using *Bacillus subtilis* as the indicator organism. Unfortunately, this procedure was not effective for inactivating cefoxitin, and the preparation of the β -lactamase used was more complex than the system described here. The bioassay of antimicrobial mixtures containing moxalactam is still somewhat problematic for the clinical laboratory.

TABLE 2. Bioassay measurements of amikacin in the presence of cefotaxime or cefoperazone

β -Lactam antibiotic added (0.1 ml) ^a	Bioassay measurements (mean ^b $\pm$ SD (μ g/ml)) of amikacin control (0.9 ml)	
	No. 1	No. 2
None (0.1 ml of distilled water added)	6.1 $\pm$ 0.5	26 $\pm$ 1
Cefotaxime	6.2 $\pm$ 0.0	23 $\pm$ 0
Cefoperazone	7.1 $\pm$ 0.1	24 $\pm$ 2

^a Final concentration of antibiotic in mixture was 500 μ g/ml, before the addition of cephalosporinase.^b Mean level in mixture of amikacin control plus second antibiotic or distilled water (corrected for 1:1 dilution with cephalosporinase).

We thank George A. Jacoby and John S. Wolfson for helpful criticism of the manuscript and Amelia Costigan for secretarial assistance.

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