The serum bactericidal rate (SBR) assay was used to assess the antipseudomonal activity of gentamicin with or without piperacillin. Heat-inactivated donor serum was spiked with gentamicin, piperacillin, or a combination at concentrations representative of levels in serum and tested against five *P. aeruginosa* strains isolated from blood. The SBR assay was performed as follows: colony counts in test samples (control, gentamicin, piperacillin, and combination) were determined at 0, 2, 4, 6, and 8 h after inoculation of a test strain. Log$_{10}$ CFU per milliliter was plotted versus time, and linear regression analysis was performed; the slope of the regression line was defined as the SBR. The SBRs of agents alone and in combination were compared statistically. The SBR of gentamicin was dependent on the serum concentration. The combination of gentamicin and piperacillin always resulted in a higher SBR than did either drug alone. However, this difference was not statistically significant for highly gentamicin-susceptible strains (MIC, ≤2 μg/ml) until the gentamicin concentration was reduced below the MIC. For a gentamicin-resistant strain (MIC, 8 μg/ml), the combination of gentamicin and piperacillin produced mean SBRs similar to that found with gentamicin-susceptible strains. These results provide evidence that the SBR assay may be a useful method of evaluating antibiotic interactions, since it can be done by using serum and since it compares the antibacterial activity of drugs statistically rather than requiring arbitrary criteria to define interactions.

Various in vitro methods have been developed to analyze the effect of antibiotics in combination on a bacterial population. The timed killing curve method (11) and the checkerboard technique (1) are the most commonly used of these methods. Although these assays have provided useful information (6), they have several drawbacks: arbitrarily chosen antibiotic concentrations are often tested and may not necessarily reflect those achieved in vivo; tests are performed in protein-free broth; and the definitions used to evaluate results have been chosen arbitrarily, especially for the timed killing curve method.

Another approach to the in vitro evaluation of antibiotic interactions is the measurement of the rate of serum killing, or serum bactericidal rate (SBR) described by Drake et al. (4) in a study of the treatment of experimental *Staphylococcus aureus* endocarditis. Recently, there has been increasing interest in using the rate of serum killing as a measure of antibacterial activity of antibiotics (17, 24, 25). In these studies, the methodology was similar to the timed killing curve method except that serum, rather than broth, containing an antibiotic was used. The use of serum taken from a subject administered an antibiotic rather than the use of arbitrarily chosen antibiotic concentrations in broth takes into consideration antibiotic pharmacokinetics and protein binding. However, the criteria used to compare rates of killing of various antibiotics have been only vaguely defined.

In the present study, we describe a method to quantitate the serum killing rate or SBR of antibiotics. Quantitation of the SBR allows one to statistically compare rates of killing by various antibiotics rather than use arbitrary definitions to evaluate antibiotic interactions. To demonstrate this method, we have chosen to study the SBRs of gentamicin and piperacillin alone and in combination against several strains of *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS**

**Test bacteria.** Five strains of *P. aeruginosa* were selected for study. All were isolated from blood and were obtained from the Microbiology Laboratory of the Veterans Administration Medical Center, Buffalo, N.Y. These strains were maintained on Mueller-Hinton agar and subcultured weekly.

**Donor serum.** Serum was obtained from four healthy male volunteers who were being given no medication. The serum was inactivated by being heated to 56°C for 30 min to eliminate the possible confounding factor of intrinsic bactericidal activity against the test strains. It was kept frozen at −20°C until used.

**Antibiotics.** Antibiotics were kindly provided by their respective manufacturers: Schering Corp., Kenilworth, N.J. (gentamicin) and Lederle Laboratories, Pearl River, N.Y. (piperacillin). The antibiotic concentrations selected for testing were chosen such that they approximated those achieved in serum after intravenous administration of a single dose (5, 19). For gentamicin (dose of 2 mg/kg), the simulated concentrations in serum were 8, 5, 3, and 1 μg/ml. For piperacillin (dose of 4 g) simulated concentrations in serum were 300, 75, and 10 μg/ml. Gentamicin and piperacillin stock solutions were prepared by using donor serum and were kept frozen at −20°C until used. Mixtures of gentamicin and piperacillin were prepared just prior to use. Because of the lack of availability of a large volume of donor serum, stock solutions were prepared every 2 to 3 weeks. Gentamicin stock solutions were assayed prior to use to determine the exact concentration present. Piperacillin stock solutions were not assayed prior to use.

**MIC and MBC determinations.** MICs and MBCs of gentamicin and piperacillin for *P. aeruginosa* were determined by using the standard macrodilution procedure (15). The bacterial inoculum ranged from $10^7$ to $10^8$ CFU/ml (mean, $6.5 \times 10^7$ CFU/ml). The MIC was defined as the lowest concentration of antibiotic that prevented turbidity after overnight incubation at 37°C. The MBC was defined as the lowest
antibiotic concentration that decreased the inoculum by >99.9% within 18 h, as determined by subculture of 0.01 ml from tubes with no turbidity onto Mueller-Hinton agar (18).

Experimental design. Each gentamicin concentration was tested with each of three concentrations of piperacillin for a given test strain of *P. aeruginosa*. Each experiment was performed in the following manner. Four test samples of 0.9 ml of serum were prepared: (i) heat-inactivated serum only (control); (ii) serum containing gentamicin alone; (iii) serum containing piperacillin alone; and (iv) serum containing gentamicin plus piperacillin at the same concentrations as in (ii) and (iii). Serum-supplemented broth (0.1 ml) (21) containing a test strain was added to each test sample.

SBR assay. A test strain of *P. aeruginosa* was grown overnight in Mueller-Hinton broth at 37°C. The optical density of the broth was adjusted to a predetermined level, and the culture was diluted 1:100 in cation-serum-supplemented Mueller-Hinton broth diluent (21). A 0.1-ml portion of the 1:100 dilution was added to 0.9 ml of test serum. This provided a final inoculum of approximately 10^5 to 10^6 CFU/ml. Test samples were vortexed and incubated at 37°C without shaking. At 0, 2, 4, 6, and 8 h after inoculation, colony counts of bacteria in each test sample were determined as follows. After the samples were vortexed, a 0.05-ml portion was removed from each sample and serially diluted in sterile phosphate-buffered saline, and 0.05 ml of each dilution was subcultured onto Mueller-Hinton agar. Colony counts were determined after 18 h of incubation at 37°C. The minimal CFU/ml detectable was 200. For each serum sample tested, the log_{10} CFU of viable bacteria remaining per ml was plotted versus time of incubation.

To attempt to minimize antibiotic carryover on subculture plates, two methods were used. First, from each dilution, a 0.05-ml portion was subcultured by being placed in a single streak on an agar plate and allowed to dry for 10 min before being spread over the agar (20). Second, from time zero to 4 h of incubation, all samples were diluted 100- to 10,000-fold (12). At 6 and 8 h of incubation, colony counts decreased to a level at which such high dilutions (≥1,000-fold) could not be used in some experiments. In this situation, a 0.05-ml portion of a test sample was subcultured directly onto an agar plate without dilution; 10- and 100-fold dilutions of the test sample were also made and 0.05 ml of each dilution was subcultured. The colony count from an undiluted test sample was compared with that resulting from dilutions of the same test sample. We usually found that the concentration of bacteria calculated by using colony counts in undiluted subculture samples was somewhat less than that calculated by using results from the dilutions. This difference was always less than 1 log_{10} unit and probably represented effects of antibiotic carryover in the subculture of the undiluted test sample. In such instances, the bacterial concentration was calculated by using the results of colony counts in the 10- and 100-fold dilutions.

**Table 1. MICs and MBCs for *P. aeruginosa* strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gentamicin (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>Piperacillin (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3918</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2446</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2435</td>
<td>1.56</td>
<td>3.15</td>
<td>1.56</td>
<td>3.15</td>
</tr>
<tr>
<td>2586</td>
<td>1.56</td>
<td>3.15</td>
<td>1.56</td>
<td>3.15</td>
</tr>
<tr>
<td>3975</td>
<td>1.56</td>
<td>3.15</td>
<td>1.56</td>
<td>3.15</td>
</tr>
</tbody>
</table>

Statistical analysis. By using the Statistical Package for the Social Sciences, multivariate repeated measures analysis of variance (ANOVA) with polynomial transformation (2, 8) was performed on the data generated for gentamicin and piperacillin alone and in combination. This analysis evaluated the trend of each curve to determine which equation (linear, quadratic, cubic, or quartic) best described the relationship between log_{10} CFU per milliliter and time of exposure to one or both agents. On the basis of analysis of curve trends (given in Results), linear regression analysis was used to determine the SBR. The SBR was defined as the slope of the regression line, and its units were the change in the log_{10} CFU per milliliter per hour of exposure to an agent(s). For purposes of comparison, the more negative the slope, the faster the rate of bacterial killing. There was always a positive slope (indicating growth) in serum without antibiotic (control) for all strains tested. The slope of the control samples ranged from +0.056 to +0.278 (mean, +0.182).

ANOVA was used to compare mean SBRs by using data pooled from experiments with several test strains. For analysis of experiments with a single strain (strains 2446 or 3918), the SBRs for each agent alone and in combination were compared by using a one-tailed t test (3). For all statistical analyses, *P* ≤ 0.05 was considered significant.

Aminoglycoside assay. The gentamicin concentration in stock solutions was determined by using a fluorescence polarization immunoassay technique on the Abbott TDx system (Abbott Diagnostics, Irving, Tex.) in the Clinical Chemistry Laboratory at the Veterans Administration Medical Center, Buffalo, N.Y. During several experiments, serum containing both gentamicin and piperacillin (at 300 µg/ml only) was assayed at time zero and at the end of an 8-h incubation period. There was no difference in the gentamicin concentration at these times. This indicated that there was no inactivation of gentamicin by piperacillin during the incubation period.

**RESULTS**

Microbiological activity. The MICS and MBCs of piperacillin and gentamicin for the five strains of *P. aeruginosa* are listed in Table 1. Strain 3918, for which the gentamicin MIC is 8 µg/ml, was considered resistant to this agent, while the other four strains were susceptible. All strains were susceptible to piperacillin.

Relationship between inoculum size and SBR. The bacterial inoculum for 224 separate SBR determinations ranged from 1.0 × 10^5 to 1.8 × 10^6 CFU/ml (mean, 6.7 × 10^5 CFU/ml). To determine whether there was any correlation between initial inoculum size and the resulting SBR, linear regression analysis was performed on scatter plots of inoculum size versus SBR of gentamicin or piperacillin alone for each strain of *P. aeruginosa*. For four of the five test strains of *P. aeruginosa*, no significant correlation was noted. However, for strain 2446, there was a significant positive correlation (*r* = +0.648; *P* < 0.05) for piperacillin. This indicated that as the inoculum of this strain increased, the rate of killing decreased (7).

Analysis of curve trends. Multivariate repeated measures ANOVA with polynomial transformation was performed on data pooled from experiments with three *P. aeruginosa* strains for which the gentamicin MIC was ≤2 µg/ml. All the curves for gentamicin or piperacillin alone at all concentrations tested (except piperacillin at 75 µg/ml) showed a significant linear trend, with *P* ranging from 0.000001 to 0.05.
FIG. 1. Representative examples of curves illustrating a significant linear trend only (piperacillin at 300 μg/ml; linear, P = 0.000001; quadratic, P = 0.094; and cubic and quartic, P > 0.5); a significant linear, quadratic, and cubic trend (gentamicin at 5 μg/ml; P = 0.00001; P = 0.00009, and P = 0.00015, respectively); and no best fit (piperacillin at 75 μg/ml; linear, P = 0.107; quadratic, P = 0.25, cubic and quartic, P > 0.3). The points for each curve have been connected to allow distinction between them and are not meant to indicate the curve trend. Abbreviations: Pip, piperacillin; Gent, gentamicin.

However, for gentamicin alone at concentrations of 8, 5, and 3 μg/ml, there was also a significant quadratic or cubic trend. For gentamicin at all test concentrations combined with any concentration of piperacillin, the curves also displayed a significant linear trend (0.009 < P < 0.05), but some combinations also had a significant quadratic or cubic trend. The only situations in which the analysis showed no best fit were for piperacillin at 75 μg/ml alone and piperacillin at 75 μg/ml combined with gentamicin at 1 μg/ml. Examples of curves which were best described by linear regression only or by quadratic or cubic equations as well as linear regression or for which there was no best fit are shown in Fig. 1. Since almost all the curves had a significant linear trend, linear regression analysis was used to evaluate all the data to simplify the mathematical approach. Using linear regression, we calculated a single slope or SBR for each drug alone and in combination at all test concentrations for each of five test strains of P. aeruginosa. We admit that some curves may be better described by quadratic or cubic equations. However, our goal was not to specifically mathematically characterize the curves but rather to verify that linear regression analysis would be valid.

**SBR of piperacillin or gentamicin alone.** The mean SBRs of piperacillin at the three concentrations tested (300, 75, and 10 μg/ml) for each P. aeruginosa test strain are shown in Table 2. By ANOVA, there was no statistical difference among the mean SBRs of piperacillin at 300 μg/ml when data for all five test strains were collectively analyzed. Similarly, there was no statistical difference among the mean SBRs of piperacillin at 75 or 10 μg/ml. For strains 3918, 2446, and 2435, there was no significant difference between the mean SBR of piperacillin at 300 and at 75 μg/ml (Student’s t test). However, for strains 2586 and 3975, there was a significant difference between the mean SBR of piperacillin at 300 and at 75 μg/ml. These results were in agreement with the broth dilution susceptibility tests listed in Table 1 in that for these two strains, a concentration of 300 μg/ml was above the MBC while 75 μg/ml was below the MBC.

The mean SBR of gentamicin at the four test concentrations for each P. aeruginosa test strain are shown in Table 3. Mean SBRs of each gentamicin concentration for each test strain were compared by ANOVA (data for gentamicin at 1 μg/ml were not included). When all five strains were analyzed, there was a significant difference among the mean SBRs of gentamicin at 8, 5, and 3 μg/ml, with P < 0.001, <0.005, and <0.0025, respectively. The analysis was repeated, excluding the data for gentamicin-resistant strain (3918); for gentamicin at 8 or 5 μg/ml, no significant difference among the mean SBRs was found for the four test strains but there was a significant difference among the mean SBRs of gentamicin at 3 μg/ml (P < 0.025). The analysis was repeated, excluding the data for strain 2446 (gentamicin MIC, 4 μg/ml); for the three strains included in this last analysis, the gentamicin MIC is ≤2 μg/ml. Using just results with these three susceptible strains, we found no significant difference among the mean SBRs of gentamicin at 8, 5, or 3 μg/ml.

On the basis of the ANOVA evaluation, results with the three gentamicin-susceptible strains (MIC ≤2 μg/ml) were pooled for further analysis. The mean SBR of gentamicin at each concentration was compared to determine whether there was a relationship between the magnitude of the SBR and the gentamicin concentration. The mean SBR of gentamicin at 8 μg/ml (−0.396) for the three strains was significantly higher than that of gentamicin at 5 μg/ml (−0.238; P < 0.01), which, in turn, was significantly higher than that of gentamicin at 3 μg/ml (−0.133; P < 0.025). Thus, for these highly gentamicin-susceptible strains of P. aeruginosa, the greater the gentamicin concentration, the higher the SBR at concentrations exceeding the MIC.

**SBR of gentamicin plus piperacillin.** In Table 4, the mean SBRs of gentamicin and piperacillin alone are compared with the SBRs of gentamicin plus piperacillin by using pooled data for the three gentamicin-susceptible strains (MIC ≤2 μg/ml). For gentamicin concentrations of 8, 5, or 3 μg/ml, there was an increase in the SBR (more negative) with the addition of any test concentration of piperacillin compared with that of gentamicin or piperacillin alone. However, these differences were statistically significant (ANOVA) only for the combination compared with piperacillin alone but not

![Table 2: SBRs of piperacillin for five strains of P. aeruginosa](image)

<table>
<thead>
<tr>
<th>Piperacillin conc (μg/ml)</th>
<th>3918</th>
<th>2446</th>
<th>2435</th>
<th>2586</th>
<th>3975</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>-0.153 ± 0.034</td>
<td>-0.110 ± 0.081</td>
<td>-0.079 ± 0.053</td>
<td>-0.146 ± 0.028&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.174 ± 0.056&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>-0.120 ± 0.061</td>
<td>-0.063 ± 0.084</td>
<td>-0.067 ± 0.044</td>
<td>-0.058 ± 0.048&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.054 ± 0.047&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>+0.091 ± 0.011</td>
<td>+0.060 ± 0.042</td>
<td>-0.135 ± 0.041</td>
<td>+0.087 ± 0.062</td>
<td>+0.056 ± 0.080</td>
</tr>
</tbody>
</table>

* Change in log<sub>10</sub> CFU per milliliter per hour of antibiotic exposure. Four SBR determinations per piperacillin concentration. Results are expressed as mean ± standard deviation.

<sup>a</sup> P < 0.01 by Student’s t test.
compared with gentamicin alone. At a gentamicin concentration of 1 μg/ml, the addition of piperacillin at any of the test concentrations significantly increased the SBR compared with that for gentamicin alone or piperacillin at 10 μg/ml but not piperacillin at 300 or 75 μg/ml alone.

Although there was a measurable killing rate in serum containing gentamicin at 1 μg/ml plus piperacillin, only the SBR of gentamicin at 1 μg/ml plus piperacillin at 300 μg/ml was not significantly different from the mean SBRs of higher gentamicin concentrations plus piperacillin. A comparison of the mean SBRs achieved with the addition of piperacillin (at any test concentration) to a given concentration of gentamicin revealed no significant difference by ANOVA. Also, there was no correlation between the magnitude of the SBR of gentamicin plus piperacillin and the concentration of gentamicin (excluding gentamicin at 1 μg/ml).

There was a relationship between the susceptibility of a test strain to gentamicin and the frequency with which there was a significant increment of the SBR when piperacillin was added compared with that of gentamicin alone. This is shown in Table 5 with strains 2446 (gentamicin MIC, 4 μg/ml) and 3918 (gentamicin MIC, 8 μg/ml). For strain 2446, when the gentamicin concentration decreased below the MIC, there was more likely to be a significant increase in the SBR of gentamicin when any concentration of piperacillin was added compared with that of gentamicin alone. For strain 3918, at all gentamicin concentrations tested (which were at or below the MIC for this strain), the addition of any concentration of piperacillin significantly increased the SBR compared with that of gentamicin alone. It should be noted that the SBRs of gentamicin plus piperacillin for the gentamicin-resistant strain (3918) were the same order of magnitude as that of the gentamicin-susceptible strains (Table 4).

**DISCUSSION**

The checkerboard and the timed killing curve assays have been the most commonly used in vitro methods to evaluate antibiotic interactions (11). Along with the previously noted methodologic problems associated with these assays, another drawback is that they have not been used to directly evaluate samples from patients, such as serum or other body fluids containing antibiotics. In contrast, the SBR assay has been specifically designed to use serum containing antibiotics. Drake et al. (4) first described the SBR assay in a study evaluating the treatment of experimental *S. aureus* endocarditis. More recently, several studies have been reported in which the rate of serum killing has been used to evaluate the antimicrobial activity of serum from volunteers given an antibiotic (17, 24, 25). In the last three studies, the SBR assay was a 24-h timed killing curve with serum samples containing antibiotics. However, in none of these studies was the rate of killing actually quantitated.

The present study was developed to investigate a method of quantitating the rate of serum killing and to use these objective data to statistically evaluate antibiotic interactions. There are two major differences between the method of measuring the SBR in the present study and the methods used in other studies (4, 17, 24, 25). First, unlike the method used in these other studies, our method did not involve dilution of serum samples prior to measuring the SBR. Dilution of serum containing an antibiotic will lower the concentration of antibiotic depending on the degree of dilution. As we have shown in the present study and as others have shown previously (9), the SBR of aminoglycosides is concentration dependent, i.e., the higher the aminoglycoside concentration in serum, the higher the SBR at concentrations above the MIC. Thus, lowering the concentration of aminoglycoside by diluting a sample will result in a reduction of the SBR regardless of which methodology is used. Since aminoglycoside concentrations in serum are usually not much higher than two- to threefold the concentration needed to kill susceptible bacteria, dilution of serum could markedly reduce the SBR. This dilution effect is not as pronounced for β-lactams, since the concentration of these agents in serum usually greatly exceeds the concentration necessary to kill susceptible organisms. We have attempted

### Table 3: SBR of gentamicin for five strains of *P. aeruginosa*

<table>
<thead>
<tr>
<th>Gentamicin concn (μg/ml)</th>
<th>3918</th>
<th>2446</th>
<th>2435</th>
<th>2586</th>
<th>3975</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>-0.099 ± 0.070</td>
<td>-0.296 ± 0.050</td>
<td>-0.377 ± 0.050</td>
<td>-0.43 ± 0.037</td>
<td>-0.379 ± 0.073</td>
</tr>
<tr>
<td>5</td>
<td>-0.053 ± 0.046</td>
<td>-0.146 ± 0.003</td>
<td>-0.291 ± 0.020</td>
<td>-0.218 ± 0.077</td>
<td>-0.206 ± 0.077</td>
</tr>
<tr>
<td>3</td>
<td>+0.068 ± 0.032</td>
<td>-0.003 ± 0.58</td>
<td>-0.117 ± 0.026</td>
<td>-0.156 ± 0.053</td>
<td>-0.125 ± 0.037</td>
</tr>
<tr>
<td>1</td>
<td>+0.037</td>
<td>+0.089</td>
<td>+0.039</td>
<td>+0.068</td>
<td>+0.110</td>
</tr>
</tbody>
</table>

* Change in log_{10} CFU per milliliter per hour of antibiotic exposure. There were three SBR determinations per gentamicin concentration per strain except for gentamicin at 1 μg/ml, for which only one determination was performed per strain. Results are expressed as mean ± standard deviation for all gentamicin concentrations except 1 μg/ml.

### Table 4: SBR of gentamicin and piperacillin alone or in combination for three gentamicin-susceptible strains

<table>
<thead>
<tr>
<th>Piperacillin concn (μg/ml)</th>
<th>SBR* for gentamicin at following concn (μg/ml):</th>
<th>0</th>
<th>8</th>
<th>5</th>
<th>3</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>-0.133 ± 0.049</td>
<td>-0.395 ± 0.03</td>
<td>-0.238 ± 0.179</td>
<td>-0.133 ± 0.021</td>
<td>+0.077 ± 0.044</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>-0.056 ± 0.002</td>
<td>-0.636 ± 0.265</td>
<td>-0.428 ± 0.135</td>
<td>-0.435 ± 0.086</td>
<td>-0.131 ± 0.082</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>+0.199 ± 0.014</td>
<td>-0.811 ± 0.194</td>
<td>-0.499 ± 0.079</td>
<td>-0.466 ± 0.221</td>
<td>-0.021 ± 0.013</td>
<td></td>
</tr>
</tbody>
</table>

* Strains 2435, 2586, and 3975, for which the gentamicin MIC is ≥2 μg/ml.
* Change in log_{10} CFU per milliliter per hour of antibiotic exposure. SBRs of combinations containing gentamicin at 8, 5, or 3 μg/ml were not significantly higher than for gentamicin alone but were significantly higher than for all test concentrations of piperacillin alone.
* P < 0.01 compared with SBR of gentamicin at 1 μg/ml.
* P < 0.025 compared with SBR of gentamicin at 1 μg/ml.
to minimize the dilution factor by making no dilution of serum samples and adding the test strains in as small a volume as possible to test samples.

The second major difference is the manner in which the data generated are analyzed. In previous studies, viable bacterial counts have been plotted versus time to exposure to an agent(s) and colony counts have been compared at the end of the exposure period (4, 17, 25) or mean colony counts have been compared at various times after inoculation by using the Wilcoxon matched-pairs rank test (24). None of these studies has actually measured the rate of killing. In contrast; we have statistically evaluated the trend of 8-h killing curves generated after exposure of *P. aeruginosa* to gentamicin, piperacillin, or a combination. Although complex equations such as quadratic and cubic equations were found to best describe some of the curves generated (gentamicin alone or in combination with piperacillin), almost all the curves had a significant linear trend as well. Thus, we took the liberty of using linear regression analysis for all curves. This was done because it allowed us to work with one slope, which, in turn, could be compared with other slopes statistically. Implicit in this approach is that the rate of killing is constant during the period of exposure. This is, of course, not strictly the case, especially if the exposure is prolonged (i.e., 18 to 24 h). However, during the 8 h of exposure used in the present study, the rate of killing of *P. aeruginosa* by gentamicin or piperacillin alone or in combination had a significant linear trend. Thus, we believe that the use of linear regression analysis to determine the rate of killing over an 8-h period is valid.

The significant quadratic or cubic trend of curves generated with gentamicin either alone or combined with piperacillin may be related, at least in part, to the relatively rapid onset (within 2 h) of killing of susceptible strains by aminoglycoside either alone or combined with a β-lactam (15) followed by a plateau in the SBR as exposure is prolonged (4 to 8 hours). In contrast, exposure of test strains to piperacillin alone at all test concentrations resulted usually in a significant linear trend only, but SBRs tended to be lower than for gentamicin alone or for the combination. The lower rate of killing by piperacillin of susceptible strains of *P. aeruginosa* has been described for some β-lactams and may be related to the mechanism by which these agents kill bacteria (13). In a few circumstances, the multivariate analysis revealed no definite trend; the explanation for this is not clear.

For *P. aeruginosa* strains for which the gentamicin MIC is ≤2 μg/ml, addition of piperacillin to serum containing 8, 5, or 3 μg of gentamicin per ml produced a higher SBR than did addition of either agent alone. However, this difference in the SBRs was not significant. It was not until the gentamicin concentration was reduced to 1 μg/ml that the addition of piperacillin produced a significantly higher SBR than gentamicin alone. This indicates that for highly gentamicin-resistant strains of *P. aeruginosa*, there was no significant advantage, in terms of the rate of killing, in the addition of piperacillin to gentamicin until the gentamicin concentration was below the MIC. For strains for which the gentamicin MIC is ≤2 μg/ml, this would usually occur toward the end of a dosing interval. On the basis of these findings with highly gentamicin-resistant strains of *P. aeruginosa*, several approaches to the administration of gentamicin and piperacillin in the treatment of infections due to these organisms can be postulated. Piperacillin might be administered only when the gentamicin concentration in serum decreased to a level below the MIC for the infecting strain. This should usually occur late in the dosing interval of gentamicin for very susceptible strains. This method of administering piperacillin would most probably decrease the amount of drug administered per day compared with conventional dosing regimens and would, in turn, decrease the cost of therapy. Another approach might be to try to take advantage of the higher SBRs (although not significantly higher) observed with gentamicin plus piperacillin compared with gentamicin alone. This could be done by administering gentamicin intermittently, as is presently done, while giving a continuous infusion of piperacillin. On the basis of the results of the present study, this approach would theoretically produce a relatively constant SBR throughout the gentamicin-dosing interval.

Further testing of these approaches to administering piperacillin and gentamicin to human volunteers is required to verify our results with simulated serum concentrations of these agents. Nevertheless, the SBR assay as described in this study may allow for evaluation of antibiotic interactions during dosing intervals in a manner not previously performed. For example, for a patient receiving an aminoglycoside plus a second agent such as piperacillin, one can obtain a serum sample at the time of the peak (or trough) concentration of the aminoglycoside. One can chemically manipulate (22) this sample such that three aliquots result: one containing aminoglycoside only, one containing β-lactamonly, and the third containing both agents. The bacterial strain infecting the patient is added to each aliquot, and an abbreviated (8-h) timed killing curve assay, as described in the present study, is performed. The SBR of each aliquot is calculated, and the rates are statistically compared. Using this approach, one can determine, at the time the serum sample was obtained, whether there is an advantage in terms of the SBR of the combination versus that of either drug alone. We have successfully used this approach with a small number of samples from patients (unpublished data). Use of the SBR assay in this manner may lead to the development of treatment regimens that are more patient-specific.
of antibiotic-dosing schedules which are based not only on the pharmacokinetics of each individual agent but also on the microbiologic effects of agents in combination.

An important finding in this study was the magnitude of the SBR achieved with the combination of gentamicin and piperacillin for a gentamicin-resistant (MIC, 8 μg/ml) P. aeruginosa strain (Table 5). At the four gentamicin concentrations tested, the addition of piperacillin at any test concentration resulted in a significantly higher SBR than that achieved with the combination against gentamicin-susceptible strains (Table 4). A similar favorable interaction between gentamicin and carbenicillin against gentamicin-resistant P. aeruginosa by using the checkerboard technique has been reported (10). Although a favorable killing rate was achieved, it is premature to suggest that one could clinically use the combination of a β-lactam and gentamicin to treat P. aeruginosa infections due to strains resistant to gentamicin. However, the precedent for such an approach has been previously established for treatment of enterococcal endocarditis (23). The enterococcus is frequently resistant to aminoglycosides, yet the combination of penicillin and aminoglycoside produces rapid killing of the organism in vitro (14). In vivo, the use of this combination has markedly improved the outcome of treatment of enterococcal endocarditis compared with that of penicillin treatment alone (23). The SBR assay would potentially provide a method to evaluate treatment of infections due to organisms resistant to available antibiotics.

It should be noted that there are several possible limitations of this study. First, owing to the lack of a large initial pool of donor serum, antibiotic stock solutions were prepared periodically. This might tend to produce some variability in the SBR assay results. In an attempt to minimize this, gentamicin stock solution concentrations were assayed prior to use. Analysis of the results with piperacillin and gentamicin alone revealed some variability, but, overall, the results were in agreement with susceptibility data. Second, the SBR method required a relatively large volume of serum (1.0 ml), but one should be able to modify the method for use with microtiter techniques. Finally, we did not compare the SBR assay with other commonly used assays of antibiotic interactions. Norden et al. (16) have previously described little agreement between the timed killing curve and checkerboard methods. Since the SBR assay is actually an abbreviated timed killing curve, it is unlikely that there would be close agreement with the checkerboard method.

The SBR assay has several advantages over the checkerboard and the standard timed killing curve methods for evaluating antibiotic interactions. The most important of these are the potential for using patient serum containing antibiotics and a statistical approach to comparison of bactericidal effects. The SBR assay may be more useful in developing or assessing dosing regimens for antimicrobial combinations. Patients being treated for endocarditis or for bacteriologically proven infection (in granulocytopenic patients) appear to be likely candidates for the SBR assay. It should be stressed that at present there is no information concerning the relationship between the magnitude of the SBR and the efficacy of an antibiotic regimen in humans. On the basis of the results of this study, further evaluation of the SBR assay appears warranted.

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LITERATURE CITED


