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Comparison of Methodologies for Synergism Testing of Drug Combinations against Resistant Strains of *Pseudomonas aeruginosa*

DIANE M. CAPPELLETTY[1] AND MICHAEL J. RYBAK[1,2]*

Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital/University Health Center and College of Pharmacy and Allied Health Professions,[1] and Department of Internal Medicine, Division of Infectious Diseases, School of Medicine,[2] Wayne State University, Detroit, Michigan 48201

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The purpose of this study was to determine if synergism was maintained for various combinations of β-lactams with an aminoglycoside against four clinical strains and one laboratory strain of *Pseudomonas aeruginosa* which were resistant, according to the MICs, to the β-lactams and/or aminoglycoside. The results from both the checkerboard and killing curve methodologies were compared. The laboratory strain (ATCC 27853) was manipulated in vitro by serial passage onto agar containing increasing concentrations of each antibiotic to select for resistance. One clinical isolate (R61) was also serially passed to raise the MIC of piperacillin from 128 to 1,024 μg/ml. The fractional inhibitory concentration indices for all isolates indicated indifference for all combination therapies, with values ranging from 0.6 to 3. In contrast, killing curve results for all isolates demonstrated synergism with drug concentrations at either one-fourth or one-half the MIC for each organism. The MIC of piperacillin for the laboratory-manipulated clinical isolate R61 was 1,024 μg/ml, and synergism was still observed with concentrations of one-half the MIC of piperacillin and amikacin. For clinical isolate R166, which was β-lactam and tobramycin resistant, synergism continued to be demonstrated with concentrations of tobramycin (1/16 MIC) in combination with piperacillin and cefepime at 1/2 the MIC. The results of this study indicate that against *P. aeruginosa*, synergism is observed in spite of resistance to β-lactams and/or aminoglycosides. Synergism appears to be maintained even at very high MICs (piperacillin, 1,024 μg/ml; tobramycin, 128 μg/ml) with drug concentrations within achievable therapeutic ranges. With current definitions of synergism there was a complete lack of correlation between the results obtained by the checkerboard and killing curve methodologies, with the fractional inhibitory concentration indices showing indifference and killing curves resulting in synergism. The methodologies and definitions of synergism or antagonism are variable and not standardized and should be reevaluated.

Combinations of antimicrobial agents are considered to be synergistic if the effect of the combination is greater than the effect of either agent alone or greater than the sum of the effects of the individual agents. Antagonism results if the combination provides an effect worse than the effect of either agent alone or worse than the sum of the effects of the individual agents. There are many analytical methods established for examining the effect of drug combinations, and all are dependent upon the definition of additivity. Loewe defined additivity as the sum of each individual drug effect ($E_T = E_A + E_B$). This type of additivity occurs when two drugs bind to a similar site to produce an effect (12–14). Webb defined additivity as when two drugs bind to dissimilar sites and stated that if drug A inhibits 40% of a population, then drug B can assert its effect only on the remaining 60% of the population [$E_T = E_A + E_B (1 - E_A)$] (21). By both definitions, an additive effect is equal to one, synergism is less than one, and antagonism is greater than one. By Loewe's isobologram method, an isobologram would be linear only if the individual dose-response curves are linear or by using the similar-site assumption. A sigmoid dose-response curve or the dissimilar-site assumption causes the isobologram to curve at the ends; therefore, determination of synergism or antagonism may not be valid from nonlinear isobolograms. Three-dimensional (3-D) models using 3-D graphing techniques and mathematical equations are becoming more widespread for analyzing the effect curves for drug combinations. Greco et al. have established the URSA (universal response surface approach) approach, Prichard and Shipman have developed the MacSynergy II program, and several other investigators have a methodology as well for determining synergism using 3-D models (6, 19). The advantages of 3-D analysis include visualizing the entire dose-response surface, calculating additivity by either the similar-site or dissimilar-site method, and being able to quantitate the effect to allow for comparison of various drug combinations. These methodologies are frequently used in cancer and antiviral research.

Currently, in the infectious diseases research literature, there are two laboratory methods commonly used to determine synergism. The first utilizes fractional inhibitory concentrations (FICs) and FIC indices which are determined by either broth or agar checkerboard techniques. For an antibiotic combination to be synergistic by this method, there must be an at least fourfold reduction in the MIC of each antibiotic when the two agents are combined compared with the MIC of each antibiotic tested by itself. The other method uses time-kill curves to compare differences in colony counts of an organism over a predetermined time interval. Some investigators use 24 h postexposure to an antibiotic for comparing differences in colony counts; others have used the first 4 to 8 h and have

* Corresponding author. Mailing address: The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital, 4201 St. Antoine Blvd., Detroit, MI 48201. Phone: (313) 745-4554. Fax: (313) 993-2522.

TABLE 1. Susceptibility results

Strain	MIC/MBC (μg/ml) of:					
	Ceftazidime	Cefepime	Piperacillin	Piperacillin-tazobactam	Amikacin	Tobramycin
BRPA	50/100	50/50	128/256	256/256	1.56/1.56	ND[a]
ARPA	1.56/3.13	4/4	8/16	8/8	32/64	ND
R60	50/50	50/50	128/256	256/512	1.56/1.56	ND
R61	ND	ND	1,024/1,024	512/512	1.56/1.56	ND
R144	64/128	32/64	512/1,024	256/256	2/8	ND
K3	64/128	32/64	256/1,024	256/512	128/256	ND
R166	4/8[b]	128/256[b]	256/1,024	256/512	16/16	128/256

[a] ND, not done.
[b] Confirmed by E-test.

compared rates of killing between combination and monotherapy.

Neither of these two methods is without limitations; the checkerboard method has reproducibility problems (20), evaluates the effect of the combination at only a single time point (18 to 24 h after incubation), and provides information only about inhibitory concentrations and not about bactericidal effects of the combinations (15). Killing curves are time-consuming and lack an ability to detect an additive response by current guidelines, drug concentrations are fixed and do not decrease over the time course of the experiment as they would in vivo, evaluation of the interaction occurs at only a single time point, and if both antibiotics are potent against the pathogen, it may be difficult to demonstrate synergism. In addition, there are no standard concentrations at which antibiotics are tested. Some investigators use concentrations achievable in serum; others use subinhibitory concentrations. Different proportions or ratios of each antibiotic may be combined, and the results obtained with different ratios may be significantly different. Since neither method is standardized, variability in procedures from different laboratories using the same method of testing may cause interlaboratory comparison and interpretation of results to be inconsistent and unreliable. Also, there is often a lack of correlation between FICs and killing curves in obtaining a synergistic response (3, 18). One method may result in indifference, while the other method with the same isolate will show synergism. Since the FIC procedure which measures an inhibitory result is being compared with killing curves with a bactericidal result, a better correlation between methodologies may be demonstrated if both methods had a bactericidal endpoint. Therefore, comparing a fractional bactericidal concentration with killing curves may be more appropriate.

Infections caused by *Pseudomonas aeruginosa* are often difficult to treat and usually require a combination of two antimicrobial agents for effective treatment (commonly, a β-lactam and an aminoglycoside). The clinician selects the agents on the basis of the susceptibility results for the pathogen and often will choose only antibiotics to which the organism is interpreted as susceptible. This practice appears to assume that synergism is obtained only when the organism is susceptible to both antibiotics. In two separate studies, Baltch et al. (2) and Chin and Neu (4) both demonstrated synergism by the checkerboard method when *P. aeruginosa* was resistant to the β-lactam and/or aminoglycoside. In contrast, Lagast et al. determined killing curves using normal volunteer sera (1 h after dosing with piperacillin and amikacin) diluted in Mueller-Hinton broth against *P. aeruginosa* isolates resistant to both antibiotics and *Klebsiella pneumoniae* isolates resistant only to piperacillin. The results indicated no significant killing with combination therapy over that with monotherapy against *P. aeruginosa*; however, synergism was observed against *K. pneumoniae* (10). Contrasting results were obtained in a study by Arpi (1) using various β-lactams and netilimicin and the time-kill curve method. The results indicated synergism more frequently than any other response, and antagonism was not observed for any of the combinations against a variety of isolates, including *P. aeruginosa.*

The objectives of this study were (i) to determine if synergism was maintained for various combinations of β-lactams and aminoglycosides against *P. aeruginosa* isolates which were resistant according to the MIC of one or both drug classes; (ii) to evaluate the maintenance of synergism for the combinations when MICs were one, two, and four times the resistance breakpoint; and (iii) to assess the correlation of results obtained from both the checkerboard and killing curve methodologies.

MATERIALS AND METHODS

Bacterial strains. The following strains of *P. aeruginosa* were tested: ATCC 27853 and clinical isolates R60, R144, R166, and K3. By serial passage of the ATCC 27853 isolate through increasing concentrations of each antibiotic incorporated into tryptic soy agar (TSA), resistance to each β-lactam (isolate BRPA) and amikacin (isolate ARPA) was selected for in vitro (9). The R60 clinical isolate was passed onto agar containing piperacillin to further raise the MIC eightfold (isolate R61).

Antibiotics. Ceftazidime for injection (L-arginine formulation [lot Z90043EY]) and pentahydrate (lot 130632) supplied by Glaxo; cefepime (lot 903), amikacin for injection (lot C4K92A), and base R.S. (lot 20040) supplied by Bristol-Myers Squibb; piperacillin (lot 72-517) and tazobactam (lot 3C98) supplied by Lederle; tobramycin for injection (lot P3S003) supplied by Geneva; and tobramycin sulfate (lot 44H04451) supplied by Sigma were used. Analytical-grade antibiotics were used for susceptibility and FIC testing. If available, antibiotics for injection were used for killing curves.

Medium. Mueller-Hinton broth (Difco, Detroit, Mich.) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) was used for all susceptibility and killing curve experiments. TSA (Difco) was used for performing colony counts.

Susceptibility and FIC testing. MICs and MBCs were determined for each drug by broth microdilution according to standards of the National Committee for Clinical Laboratory Standards (17). Synergism by the checkerboard method was defined as an FIC index of ≤ 0.5, indifference was defined as an FIC index of >0.5 to 4, and antagonism was defined as an FIC index of >4 (15). Concentrations within the FIC panel were such that the MIC of each antibiotic was in the middle of the range of concentrations tested (15). A suspension (0.5 McFarland standard) of organism was prepared in 0.9% saline from bacterial growth on a 24-h-old TSA plate. This was diluted such that an inoculum of approximately 5×10^5 CFU/ml was obtained.

Kill curves. Kill curves were determined in 15-ml sterile conical tubes containing a total volume of 8 ml. Antibiotics were tested alone and in combination. Antibiotics were tested at one-fourth the MIC for each isolate, and concentrations were increased incrementally until synergism was obtained or a maximal concentration of four times the MIC was reached. A suspension (0.5 McFarland standard) of organism was prepared in 0.9% saline from bacterial growth on a 24-h-old TSA plate. This was diluted such that an initial inoculum of 10^6 CFU/ml was obtained for all killing curves. Samples (0.1 ml) were taken at 0, 2, 4, 8, and 24 h; suitable dilutions were made in 0.9% sodium chloride; and 20 μl was plated in triplicate on TSA. The plates were incubated at 37°C for 18 to 24 h, and colony

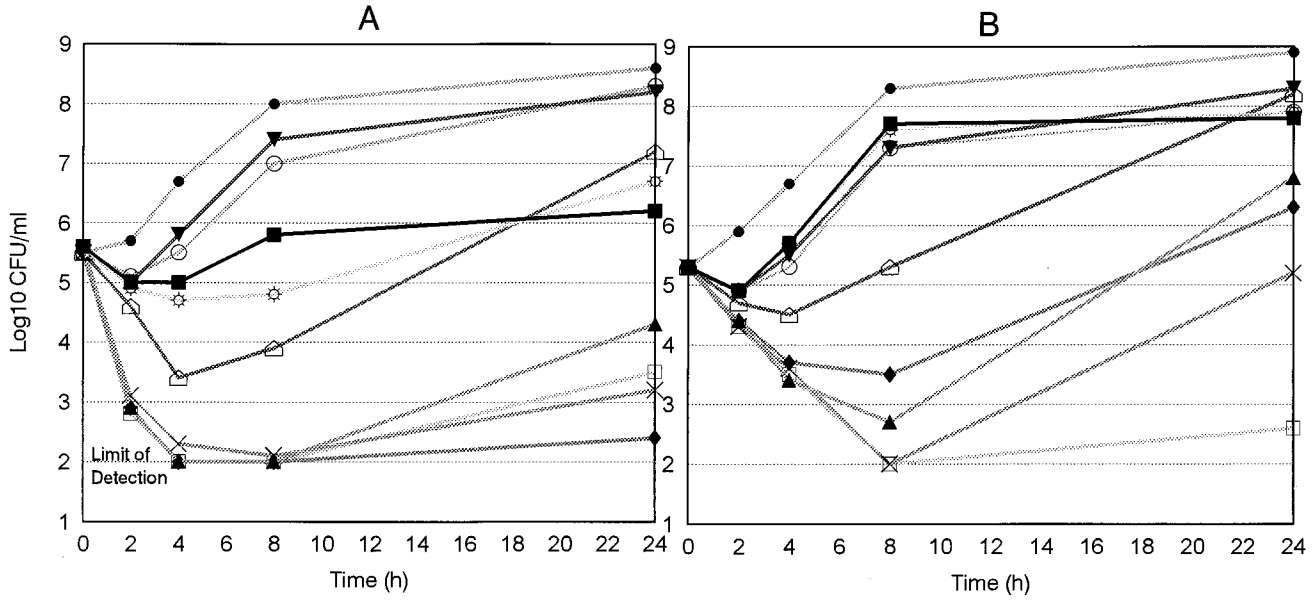

FIG. 1. Killing curve at one-fourth the MIC for BRPA (A) and ARPA (B). ■, ceftazidime; ☼, cefepime; ▼, piperacillin; ○, piperacillin-tazobactam; ⌓, amikacin; ▲, ceftazidime plus amikacin; ◆, cefepime plus amikacin; ×, piperacillin plus amikacin; □, piperacillin-tazobactam plus amikacin; ●, growth control.

counts were performed. All experiments were run in duplicate, and the tubes were incubated at 37°C in a Glas-Col rotator for 24 h. Killing curves were constructed by plotting $\log_{10}$ CFU per milliliter against time over 24 h. Synergism was defined as a decrease in colony count of ≥100 CFU/ml at 24 h with the combination in comparison with the count obtained with the most active single agent. Antagonism was an increase in colony count of ≥100 CFU/ml at 24 h (15).

RESULTS

Susceptibility results. The MICs and MBCs for each isolate are shown in Table 1. The ATCC 27853-derived isolate BRPA had a stable resistance selected, with the MICs and MBCs increasing 32- to 50-fold from baseline levels. The ATCC 27853-derived isolate ARPA had a 16-fold increase in the amikacin MIC which was stable through 10 passes; however, after 20 passes the MIC reverted back to its baseline value. The clinical isolate R61 that was manipulated in the laboratory also had a stable resistance, with an MIC of piperacillin of 1,024 μg/ml. All clinical isolates were resistant to each β-lactam, while two isolates, K3 and R166, were resistant to amikacin and tobramycin, respectively, as well as each β-lactam.

FIC results. The FIC indices for all isolates are shown in Table 2. All results by the microtiter checkerboard method indicated indifference for all combination therapies.

Killing curves. (i) ATCC 27853-derived strains. All combination regimens at one-fourth the MIC for the BRPA isolate resulted in synergism by our definition and also provided a higher rate of killing over the first 2 to 4 h (Fig. 1A). For ARPA, the amikacin-resistant isolate, at one-fourth the MIC, synergism at 24 h was observed only for amikacin-piperacillin and amikacin-piperacillin-tazobactam combinations. All four combinations had higher rates of killing during the first 4 h than did the monotherapy regimens (Fig. 1B). Because the MIC for this isolate reverted to the baseline MIC, we were unable to determine killing curves at one-half the MICs to determine if synergism would be obtained at the higher concentrations. The variability in colony counts for all regimens against BRPA and ARPA was ≤0.5 $\log_{10}$ and ≤0.4 $\log_{10}$ CFU/ml, respectively.

(ii) β-Lactam-resistant clinical strains. At one-fourth the MIC of each agent for the R60 isolate synergism was not obtained. The concentrations were increased to one-half the MICs, which resulted in synergism for all combination regimens (Fig. 2A). The laboratory manipulation of R61 isolate which resulted in an eightfold increase in the piperacillin MIC (from 128 to 1,024 μg/ml) also resulted in synergism for the combination of piperacillin or piperacillin-tazobactam with amikacin at one-half the MICs (Fig. 2B). The rates of killing for the combination regimens over the first 4 h were higher than those for any monotherapy regimen against both of these isolates. All combinations at one-fourth the MIC of each agent resulted in synergism for the R144 isolate (data not shown). The variability in colony counts was ≤0.5 $\log_{10}$ CFU/ml for all regimens and isolates.

(iii) β-Lactam- and aminoglycoside-resistant clinical strains. Figure 3A shows the results of the killing curves for the K3 isolate tested against antibiotics at one-fourth the MICs. Synergism was obtained at 24 h; however, the rates of killing of the combination regimens over the first 4 h were not distinguishable from those of amikacin by itself. The R166 isolate, which was resistant to tobramycin (MIC, 128 μg/ml), required the antibiotic concentrations to be raised to one-half the MICs for synergism to be observed (Fig. 3B). The rates of killing for the combination regimens against this isolate in contrast to those against K3 were higher than those for any of the monotherapy regimens over the first 4 h. Since the concentration of tobramycin utilized in this experiment was far above what is achievable clinically, we repeated the experiment using tobramycin concentrations of 8 and 16 μg/ml (equivalent to 1/16 and 1/8 the MIC) with piperacillin and cefepime at 1/2 the MIC (Fig. 4A and B, respectively). At these low increments of the MIC of tobramycin, synergism and the high rate of killing were still observed for both combination regimens. The variability in colony counts was ≤0.8 $\log_{10}$ CFU/ml for all regimens and both isolates.

DISCUSSION

The susceptibility profile or MICs are the primary tools or information utilized by clinicians in selecting appropriate antimicrobial therapy for a particular infection. It is standard

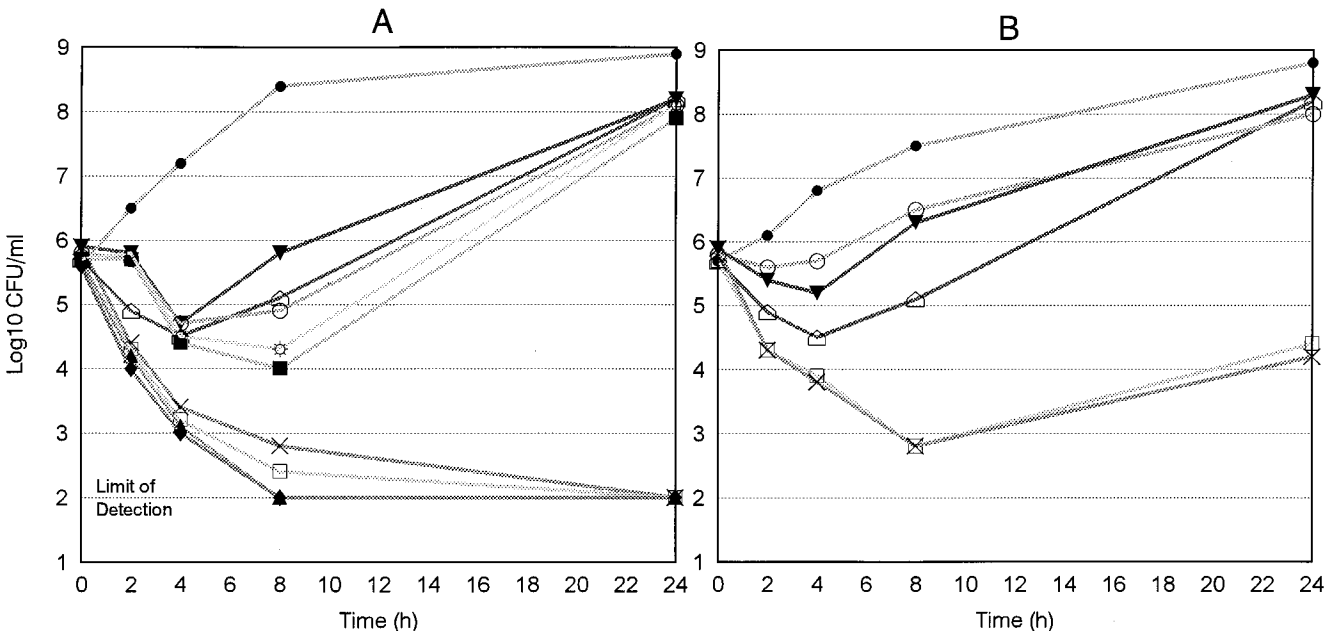

FIG. 2. Killing curves at one-half the MIC for R60 (A) and R61 (B). ■, ceftazidime; ✲, cefepime; ▼, piperacillin; ○, piperacillin-tazobactam; □, amikacin; ▲, ceftazidime plus amikacin; ◆, cefepime plus amikacin; ×, piperacillin plus amikacin; □, piperacillin-tazobactam plus amikacin; ●, growth control.

practice to use two agents to treat an infection caused by *P. aeruginosa*, and the clinician's selection of antimicrobial agents is guided by the susceptibility results for the pathogen(s). Unfortunately, with the increased incidence of multidrug-resistant organisms, the clinician is often faced with only one agent (or one class of agents) that the pathogen is susceptible to (on the basis of the MICs) and monotherapy may be chosen, which might result in suboptimal treatment of the infection and even treatment failure. Selecting only antibiotics to which the organism is interpreted as being susceptible and not those to which it is considered to be resistant by the MIC implies that the benefit of combination therapy (synergism) is only obtained when the organism is susceptible to both agents. In contrast, killing curve guidelines for synergism testing assume that one of the agents tested does not kill or minimally inhibits the organism when it is tested by itself (15). This assumption does not state whether this lack of activity is due to resistance and therefore to high MICs or whether subinhibitory concentrations of a drug are being tested. The exact mechanism(s) of synergism with different drug combinations is not known for sure; however, for aminoglycosides and β-lactams mutual enhancement of drug uptake appears to be involved. Miller and colleagues examined the early effects of β-lactams on aminoglycoside uptake and found that the β-lactams increased the uptake of subinhibitory concentrations of tobramycin in *P. aeruginosa* within the first couple of hours post-antibiotic exposure, which also resulted in a higher rate of killing for the combination therapies (16). Aminoglycoside permeabilization of the outer membrane increasing β-lactam uptake was characterized by Hancock and associates (8). They measured the rate of hydrolysis of nitrocefin and found it to increase from 1.7- to 3.5-fold, depending upon the concentration of gentamicin. They concluded that the aminoglycoside displacing Mg^{2+} in the outer membrane increased the uptake of β-lactams.

The results of this study show that in spite of the MICs indicating resistance to the β-lactams and/or aminoglycoside, synergism was still observed by the time-kill curve methodology. Even with a high level of resistance as seen with the R61, K3, and R166 isolates of *P. aeruginosa*, synergism was obtained by killing curves at a fraction of the MICs. For all of the isolates except for K3, synergism was observed at 6 to 8 h, showing a higher rate of killing with combination therapy over monotherapy as well as a ≥ 2 $\log_{10}$ decrease in CFU per milliliter at 24 h. Synergism was not observed for K3 at 8 h because of the potent activity of amikacin monotherapy against this isolate. The R166 isolate of *P. aeruginosa* was resistant to tobramycin and susceptible to amikacin (MIC = 16 μg/ml). As a result of this pattern of susceptibility to the aminoglycosides it is probable that the mechanism of resistance to tobramycin is by intracellular inactivation and therefore the mechanism of synergism may be increased uptake of the β-lactam because of outer membrane changes induced by tobramycin. This isolate (R166) also expressed an unusual pattern of susceptibility to the β-lactams. All MICs were retested by microdilution method, and in addition, cefepime and ceftazidime were tested by E-strips. The results were confirmed, and currently we cannot explain the mechanism(s) responsible for the resistance profile observed. We do not know the mechanism of resistance

TABLE 2. FIC indices

Strain	FIC index of:			
	Ceftazidime-amikacin	Cefepime-amikacin	Piperacillin-amikacin	Piperacillin-tazobactam-amikacin
BRPA	2.5	1.2	3	1.5
ARPA	1	1	1	1
R60	1.2	2.1	0.6	2.5
R61	ND[a]	ND	2.5	2.5
R144	1	0.8	1	1.5
K3	1.5	1.5	2.1	1.2
R166[b]	1.5	2	1.5	2

[a] ND, not done.
[b] Tobramycin used instead of amikacin.

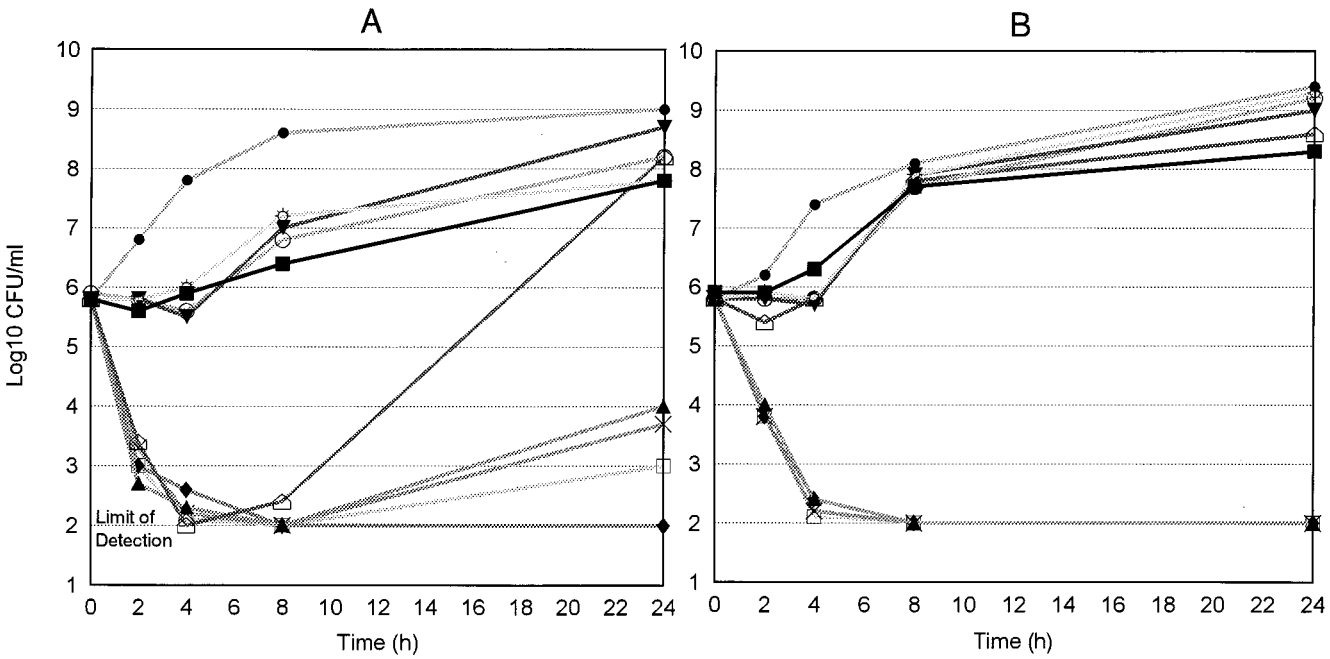

FIG. 3. Killing curves at one-fourth the MIC for K3 with amikacin (A) and one-half the MIC for R166 with tobramycin (B). ■, ceftazidime; ✲, cefepime; ▼, piperacillin; ○, piperacillin-tazobactam; ⌓, amikacin or tobramycin; ▲, ceftazidime plus amikacin or tobramycin; ◆, cefepime plus amikacin or tobramycin; ×, piperacillin plus amikacin or tobramycin; □, piperacillin-tazobactam plus amikacinv or tobramycin; ●, growth control.

for the K3 isolate with respect to the aminoglycosides. The isolate was resistant to amikacin, tobramycin, and gentamicin, and therefore, we cannot postulate as to the mechanism of synergism observed against this isolate.

The lack of standardization for the killing curve synergism procedure has given rise to many debates over what drug concentrations should be used, what inoculum size is most appropriate, and what time frame is best for determining synergism by this method. The problem with analyzing rates of killing between monotherapy and combination therapy is that there are no definitions for changes in rates to determine synergism or antagonism. Lerner et al. determined killing curves over 8 and 24 h. They found β-lactams and aminoglycosides to be synergistic at 4 h by killing rate and in the colony count at 24 h even against aminoglycoside-resistant *P. aeruginosa* (11). However, there was some regrowth with the killing curves at 24 h, resulting in a greater mix of synergistic and indifferent responses. Glew and Pavuk found synergism 74% of the time within 7 h of drug exposure with multiple-drug-resistant members of the family *Enterobacteriaceae* using the time-kill curve methodology (5). Even though both of these studies refer to higher rates of killing, both assessed colony counts and required a decrease in colony count by ≥100 CFU/ml. Currently one of the most referenced definitions of synergism is by Lorian, "≥100-fold increase in killing activity at 24 h (as measured by colony counts) with the combination, in comparison to the most active single drug" (15). Should the definition have a fixed time point at which the results are interpreted, or should the results of the killing curve be modeled by one of the 3-D methods and the entire response surface be analyzed? The time parameter of 24 h can limit or alter results of the experiment if regrowth occurs with one or both antibiotics alone or in combination. This regrowth can be caused by several factors. Use of subinhibitory concentrations of antibiotics allows regrowth of the organism for monotherapy experiments, and regrowth is variable for combination regimens. Emergence of resistant subpopulations may account for the regrowth, or regrowth may be due to bacteria that had adhered to the surface of the tubes and were subsequently released in the media (7). Another factor that may affect regrowth of the organism is inactivation of the antibiotics in vitro. If the organism is producing β-lactamase, then the β-lactam antibiotics may not be present in the test tube at 24 h, or drug-drug interaction may result in the inactivation of one of the agents. We did not assess the emergence of resistant subpopulations for all experiments; however, we did recheck the MICs for the R166 isolate at the end of the killing curve experiment from the tubes containing ceftazidime in combination with tobramycin. The MICs of ceftazidime, cefepime, piperacillin, and tobramycin did not change, indicating that a more resistant subpopulation was not predominating.

The lack of correlation between the checkerboard and killing curve methods is also substantiated by the results of this study. The FIC indices for all combinations against all isolates resulted in indifference, while synergism was observed by the killing curves (as early as 6 to 8 h and also at 24 h). These discrepancies have been documented for many years by different investigators with both gram-negative and gram-positive organisms. Norden used several different criteria for defining a synergistic endpoint for checkerboard and killing curve methods against *K. pneumoniae* and found a lack of correlation in the frequency of synergism by the two methods for the same strains and that the results varied with the definition criteria utilized (18). Chandrasekar and colleagues tested *P. aeruginosa* by both methodologies and did not find any consistency in the results between the checkerboard method and 6- as well as 24-h killing curves (3). Problems with the killing curve methodology already discussed can account for some of these discrepancies in comparing the two methods. The checkerboard method has a reproducibility problem which has been studied by Rand and colleagues. They found that 25% of the replicates gave different classifications of results and that at least five

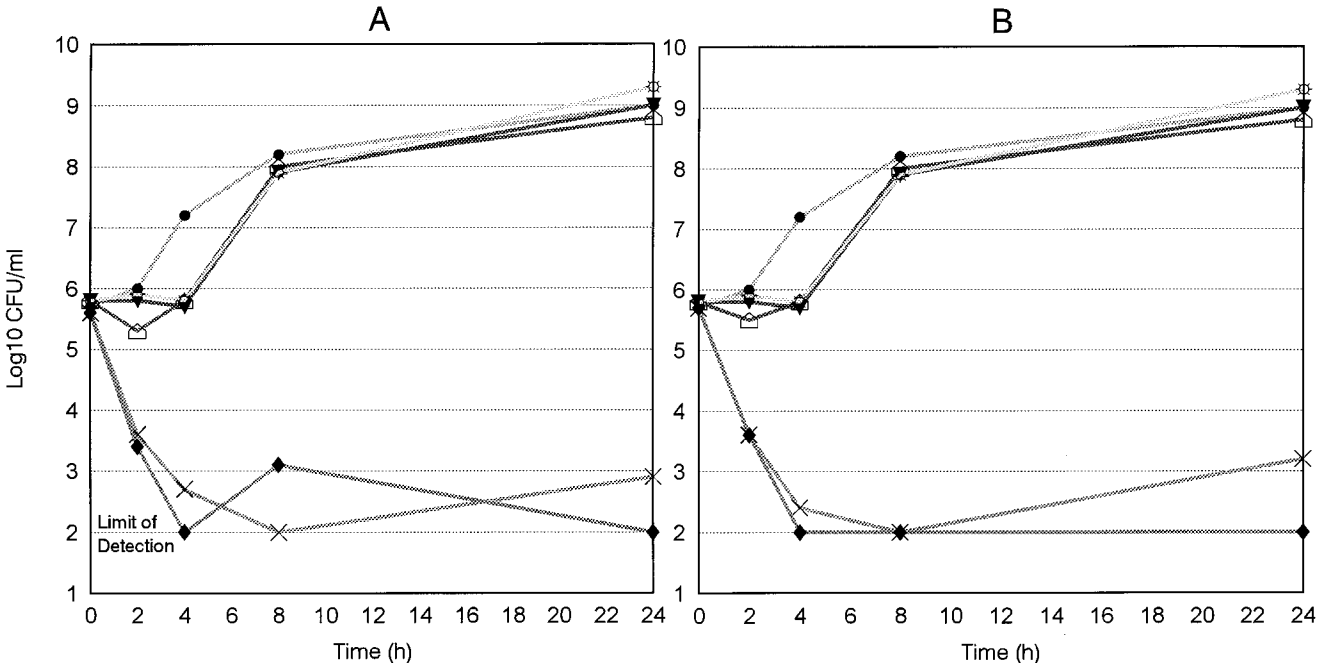

FIG. 4. Killing curve for R166 with tobramycin at 8 μg/ml (A) or 16 μg/ml (B). ✲, cefepime; ▼, piperacillin; ⌓, tobramycin; ◆, cefepime plus tobramycin; ×, piperacillin plus tobramycin; ●, growth control.

replicates must be set up to achieve a ≥80% agreement in the results (20). Also of concern in interpreting the results from the literature utilizing the checkerboard method are the definitions that have been used over the years to define synergism, indifference, and antagonism. Synergism has been defined as an FIC index of ≤0.5 and ≤0.6, and some have stated that an index of ≤0.25 is "marked" synergism (3, 11, 15, 18, 20). Antagonism has been defined as an FIC index of >1, ≥1, >2, and >4 (3, 11, 15, 18). Therefore, by definitions alone, a result may be reported as indifferent or antagonistic. As for the procedure itself, there is an inherent 1-dilution (or twofold) variability in the process of performing serial dilutions in the microtiter system. Since there needs to be a fourfold reduction in the MIC of each antibiotic to obtain an index of 0.5, this twofold variability affecting the results is commonplace.

With no standardization for either methodology, comparing results within each method or between methods becomes a difficult task. These unstandardized tests have been performed for at least 15 years and have helped determine the clinical practice of combining antibiotics to treat infections because of reported synergism in vitro. How accurate are some of these recommendations when the results may not be verified by another laboratory using the same methodology or even by the two methods within the same laboratory? With combination therapy becoming more prevalent because of severity of illness or infection and the emergence of resistant organisms, standardization of synergism testing is necessary. Currently the only synergy screening performed routinely in the clinical laboratory is for gentamicin or streptomycin with ampicillin against enterococci. The synergy screen determines if the organism is highly resistant to streptomycin or gentamicin (MIC > 2,000 mg/liter) but does not actually combine two agents in a single well for testing. Inhibition of the organism by this concentration of gentamicin (or streptomycin) strongly correlates with synergism of the combination of ampicillin and the aminoglycoside. Our data and the data of others suggest that a similar correlation test (if one can be established) or routine synergism testing would be beneficial for a select group of problematic gram-negative organisms. The results of this study indicate that synergism is still observed even for combinations of antibiotics that *P. aeruginosa* is resistant to and even at very high MICs. However, only a small number of clinical isolates were tested, and further work should be performed on larger numbers of isolates (pseudomonads and *Enterobacteriaceae*) to confirm these results or determine the frequency of synergism. With the increased prevalence of multidrug-resistant organisms, synergism testing becomes a potentially powerful tool to aid in selection of appropriate antibiotic therapy. Unfortunately, although for over 20 years synergism definitions have been employed and synergism testing procedures have been performed, they are still not standardized, and these issues need to be addressed.

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