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Determination of the Range of Antibacterial Activity by Use of Viable Counts

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The activity of three aminoglycosides and six beta-lactam antibiotics on strains of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*, and enterococci was studied. The minimal inhibitory concentrations (MICs), the minimal bactericidal concentrations (MBCs), and the minimal antibiotic concentrations (MACs) were determined after 5 h of incubation in broth cultures by colony-forming-unit counts. The MICs were also determined by agar dilution after 24 h of incubation. The MICs on agar after 24 h of incubation were higher than those in broth after 5 h of incubation. The differences ranged from 1.1- to 14.2-fold, but in most cases were only three- to fivefold ($P < 0.05$ to <0.001). The MBCs at 5 and 24 h were comparable in 71% of tests. For current practice, the MBC of enterococci can be determined after 5 h of incubation with antibiotics. The aminoglycosides showed MBCs which were closer to the MICs than were those of the beta-lactam antibiotics, which required a higher multiple of the MIC to show a bactericidal effect. The MBCs of oxacillin and cefamandole for *S. aureus* after 5 h of incubation were >128 times the respective MICs. The MACs ranged from 1/1.5 to 1/7 of the 5-h MICs. The three endpoints, MIC, MBC, and MAC, indicate the antibacterial range of an antibiotic in terms of inhibition of growth and bacterial survival. The data suggest that the antibacterial range of an antibiotic is similar for most strains of a given species and is, to some extent, a characteristic of similar antibiotics.

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) have been considered valuable endpoints of antibacterial activity to serve as guides for effective therapy of bacterial infections.

Prolonged broth incubation can alter the nutrient value of medium, the pH, and the activity of antibiotics as well as the output of bacterial inactivating enzymes (5, 7). The regrowth of bacteria in broth with beta-lactam antibiotics when incubated for a period in excess of 8 h is another factor which influences the MIC determinations (8).

With the exception of continuous infusion of an antibiotic, the usual administration of the drug will result in a peak body fluid level within 1 to 2.5 h, which is thereby followed by various rates of decline characteristic for the drug and its relationship to the metabolic and excretory functions of the patient.

To closer replicate the in vivo exposure of bacteria to peak antibiotic concentrations and to minimize the opportunities for the in vitro alterations described, a short incubation period is desirable.

The MIC can be determined by automated procedures 3 to 5 h after inoculation instead of the recommended overnight incubation; an adjustment in the inoculum size made these early results comparable to the overnight incubation (13). Short incubation periods for the determination of MBCs have also been used. The MBCs of groups A and B streptococci were determined after 3 to 6 h of incubation rather than the usual 24 h (2).

Most methods for the rapid determination of the MIC use turbidity (11, 21) or particle counts (13) rather than the viable bacterial counts as indicators of bacterial growth. This paper reports the activity of various antibiotics in terms of the MIC, MBC, and minimal antibiotic concentration (MAC) on six species of bacteria. These endpoints were determined by the number of colony-forming units (CFU) after 5 and 24 h of incubation with the drugs.

MATERIALS AND METHODS

Antibiotics. The antibiotics used included amikacin, ampicillin, oxacillin (Bristol Laboratories); tobramycin, cefamandole (Eli Lilly & Co.); gentamicin (Schering Corp.); carbenicillin (Pfizer Inc.); and ticarcillin (Beecham Laboratories).

Bacterial strains. Ten strains each of *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseu-*

domonas aeruginosa, Staphylococcus aureus, and enterococci were isolated from patients at the Bronx Lebanon Hospital Center and identified by routine methods (14). Only strains which were susceptible by the Bauer-Kirby technique (4) to the antibiotics listed in Table 1 were investigated.

Determination of MICs, MACs, and MBCs. The MICs for each strain and each antibiotic were first determined by a twofold agar dilution technique, using Trypticase soy agar (TSA; BBL Microbiology Systems); 0.04 ml of a 1:100 dilution of an 18-h culture in Trypticase soy broth (TSB; BBL Microbiology Systems) was spotted on a series of TSA plates containing no drug as well as twofold dilutions of the antibiotic to be tested. The plates were incubated for a period of 24 h at 36°C. The MIC was the lowest concentration of the drug which showed no growth on the TSA plates.

The 5-h MICs, MACs, and MBCs as well as the 24-h MBCs were determined by a broth dilution technique and CFU counts. Each organism was incubated in TSB at 36°C for 18 h. For each culture, a 0.5-ml sample of a 1:10 dilution in TSB was added to 200 ml of TSB and incubated in a water bath at 36°C for 1 h (for *Pseudomonas aeruginosa, S. aureus*, and enterococci, the incubation time was 1 h 45 min). Samples of 4.5 ml of each culture were then added to 0.5 ml of TSB containing antibiotic concentrations calculated to yield final concentrations ranging from 1/128 to 16 times the MIC for each strain. Controls grown in TSB with no drugs were also prepared. All tubes were incubated in a water bath at 36°C for 24 h. At 0 and 5 h for the control and at 5 and 24 h for the tubes containing antibiotics, 10-fold dilutions from each tube were made in 0.85% NaCl (up to 10^{-7} for turbid and 10^{-4} for clear tubes). A 0.2-ml amount of the last four dilutions were planted on TSA plates (or MacConkey agar for *Proteus mirabilis*). The inoculum was spread over the entire surface of the agar and incubated for 20 h.

The 5-h MIC was that concentration which resulted in a CFU count after 5 h of incubation which was comparable to the 0-h control CFU count ± 0.3 log, which is the normal variation range observed in our laboratory.

The MAC was defined as the lowest concentration of an antibiotic which produced a 1-log (90%) decrease in the number of CFU per milliliter after 5 h of incubation, as compared with the control grown in drug-free broth (15).

The 5- and 24-h MBC was that concentration which showed a 3-log decrease from the 0-h control after 5 and 24 h of incubation, respectively (14).

RESULTS

The number of CFU exposed to antibiotics was approximately 10^5 organisms per spot on agar or per milliliter of broth. The MICs at 5 and 24 h for the drugs and strains tested are shown in Table 1. The MICs which were determined in broth after 5 h of incubation were lower than the MICs determined by agar dilution after 24 h of incubation. A few MICs at 5 h were slightly lower (1.1 to 1.2 times lower for enterococci with cefamandole or ampicillin [$P < 0.1$]), and some MICs showed large differences (11.4 and

TABLE 1. Mean MICs of 10 strains of each species determined at 5 and 24 h of incubation

Antibiotic	Mean (± SD) MIC (μg/ml) of 10 strains of following species at indicated time (h)[a]:											
	E. coli		*Klebsiella*		*Proteus mirabilis*		*Pseudomonas aeruginosa*		*S. aureus*		Enterococci	
	5	24	5	24	5	24	5	24	5	24	5	24
Amikacin	5.1 (2.2)	13 (5)	2.2 (1.2)	5.7 (3.5)	3.4 (2.1)	39 (11)	2.6 (1.1)	12 (12)	1.1 (0.6)	6.3 (3.7)	58 (62)	119 (68)
Gentamicin	2.9 (3.7)	4.5 (1.5)[b]	0.8 (0.5)	2.4 (2.3)	1.2 (0.6)	10 (5.5)	1.8 (0.5)	4.2 (1.2)	0.3 (0.2)	2.0 (1.2)	11 (9.9)	45 (50)
Tobramycin	2.8 (1.7)	6.0 (2.7)	2.0 (1.2)	11 (2.4)	1.9 (1.6)	27 (13)	0.8 (0.4)	3.2 (1.5)	0.3 (0.21)	1.6 (1.2)	15 (13)	48 (54)
Oxacillin	—[c]	—	—	—	—	—	—	—	0.2 (0.13)	0.4 (0.13)[b]	—	—
Cefamandole	0.2 (0.1)	1.1 (0.4)	—	—	0.5 (0.18)	—	—	—	0.4 (0.21)	1.1 (0.66)	24 (12)	29 (18)[b]
Ampicillin	1.6 (0.5)	5.4 (1.3)	—	—	1.7 (1.1)	—	—	—	—	—	0.6 (0.3)	0.7 (0.6)[b]
Amoxicillin	—	—	—	—	0.5 (0.2)	1.8 (0.9)	—	—	—	—	—	—
Carbenicillin	—	—	—	—	0.9 (1.1)	1.4 (0.94)[b]	—	64 (26)	—	—	—	—
Ticarcillin	—	—	—	—	—	—	—	18 (8.5)	—	—	—	—

[a] The 24-h MICs were higher than the 5-h MICs ($P < 0.05$ to < 0.001).
[b] $P < 0.1$.
[c] —, Test not performed.

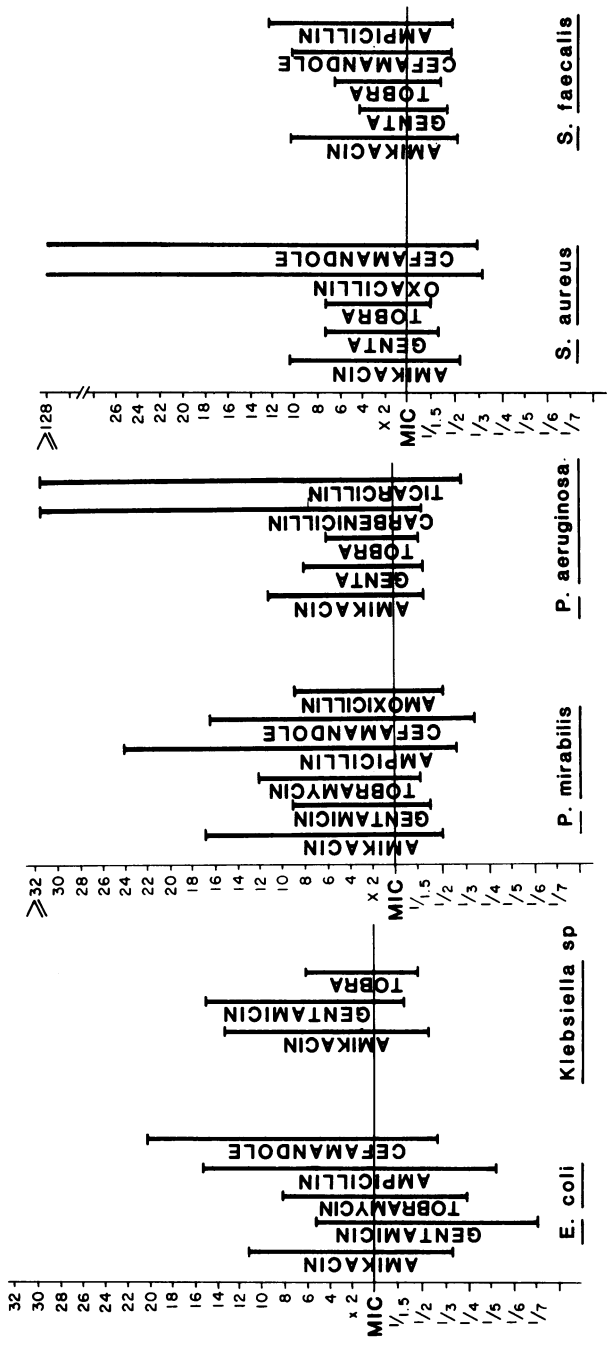

FIG. 1. Ranges of antibacterial activity. The bars above the MIC line represent multiples of MIC required to achieve a bactericidal effect after 5 h of incubation. The bars below the MIC represent fractions of the MIC required to achieve a 90% inhibitory effect (MAC).

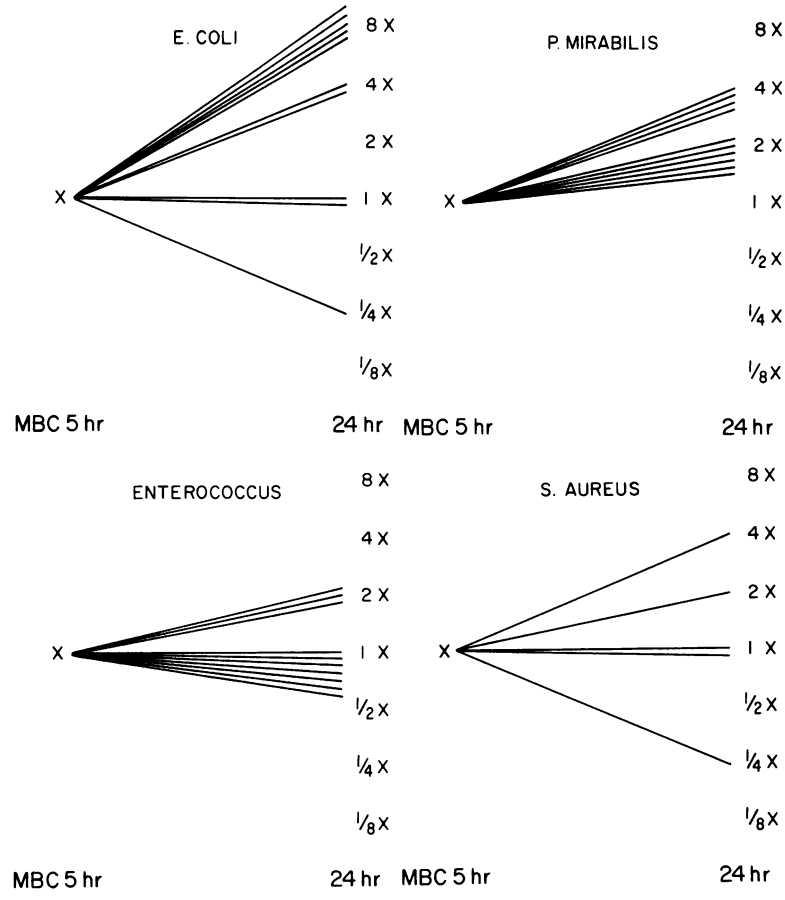

FIG. 2. Ratios of MBCs at 5 and 24 h of incubation with gentamicin.

14.2 times lower for *Proteus mirabilis* with amikacin and tobramycin, respectively). In most cases, however, the MICs at 5 h were three to five times lower than the MICs determined at 24 h ($P < 0.05$ to <0.001). Statistics were done by the modified two-sample t test (10).

The ranges of activity are shown in Fig. 1. The bactericidal effect of aminoglycoside antibiotics after 5 h of exposure was closer to the MIC than was the concentration of beta-lactam antibiotics, which required a higher multiple of the MIC to show a bactericidal effect. Oxacillin and cefamandole had practically no bactericidal effect on *S. aureus* after 5 h of incubation (the MBCs were

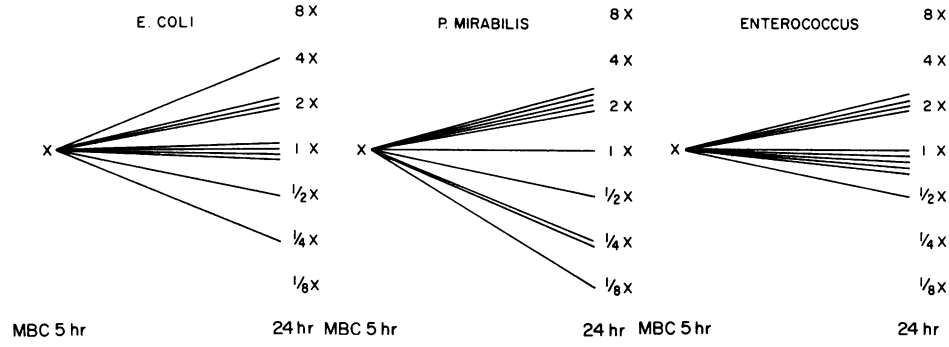

FIG. 3. Ratios of MBCs at 5 and 24 h of incubation with ampicillin.

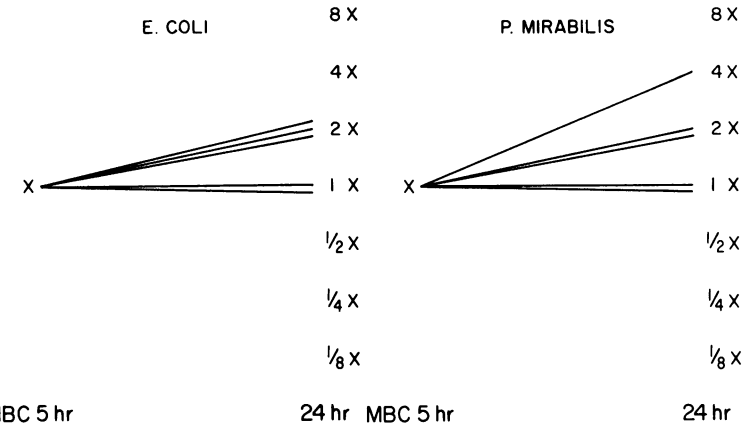

FIG. 4. Ratios of MBCs at 5 and 24 h of incubation with cefamandole.

more than 128 times the MIC). Carbenicillin and ticarcillin were bactericidal for *Pseudomonas aeruginosa* only at or above 32 times the MIC. The MAC (activity below the MIC) ranged from 1/1.5 to 1/7 of the MIC. The ratios of MBCs at 5 h to MBCs at 24 h are shown in Fig. 2 through 5. The MBCs at 5 and 24 h were identical in 32% of the tests; another 32% were twice the MBC at 5 h, and 7% were one-half of the MBC at 5 h. Therefore, 71% of the MBCs at 24 h were equal, twice, or one-half of the MBCs at 5 h.

When enterococci were incubated in broth for 5 h with ampicillin, 7 of 10 strains showed a 1- to 2-log increase in CFU at concentrations two to eight times the MBC. Two strains also showed this paradoxical effect after 24 h of incubation (data not shown).

DISCUSSION

The inoculum of 10^5 organisms used is slightly higher than that recommended for routine use in current textbooks (3, 14). The dilution in saline of antibiotic-exposed bacteria precluded, however, the carry-over of inhibitory amounts of the drug. A bacterial population of at least 10^5 organisms per ml in a specimen is required before it is considered significant in urine (9, 12), lung (16), or wound (17) infections. Thus, the inoculum used in this study was selected to be comparable to significant minimal bacterial populations encountered in infections in humans. The MICs obtained on agar after 24 h of incubation were comparable to those published in the literature (1). They were, however, higher than those obtained in broth by earlier readings. Sherris et al. showed that agar versus broth MICs, when read after overnight incubation, produced variable results ranging from one-fourth to 4 times the MIC (20). In a study in which broth dilutions were used, one-half of the tests showed a fourfold or greater increase in MICs after 18 h of incubation, as compared with the 3-h readings (13).

The relatively large increases in the MIC from 5 to 24 h, such as that observed with *Proteus mirabilis* grown with amikacin or tobramycin, could be due to the selection of a subpopulation of bacteria with a higher MIC. Such a phenomenon has been reported with aminoglycosides (18). It is possible that such a small bacterial subpopulation which did not manifest itself after 5 h of incubation would be dealt with by phagocytosis, and therefore the 5-h MIC appears to be more relevant than the 24-h MIC for therapeutic guidance in an immunocompetent patient. In a leukopenic patient, however, the subpopulation with a higher MIC could survive the local achievable concentration resultant from a dose selected according to the 5-h MIC; therefore, in this special situation, the MIC determined after 24 h of incubation would become more meaningful.

Many antibacterial agents, when administered at recommended dosages, will produce concentrations in tissues and organs that are lower than the MIC. Antibiotic concentrations below the MIC affect bacterial adherence to epithelial cells (19), alter the synthesis of toxins (6), and partially inhibit the growth rate (15). The MAC points to the lowest concentration which affects such activities.

With the exception of *S. aureus*, which showed no bactericidal effect at 5 h with beta-lactam antibiotics, and *Pseudomonas aeruginosa* exposed to carbenicillin, most MBCs were comparable when tested after 5 or 24 h of incubation. For practical purposes, the MBC for the species and drugs indicated can be reported at 24 h instead of 48 h after the isolation of the organism.

The MBC represents the death of bacteria,

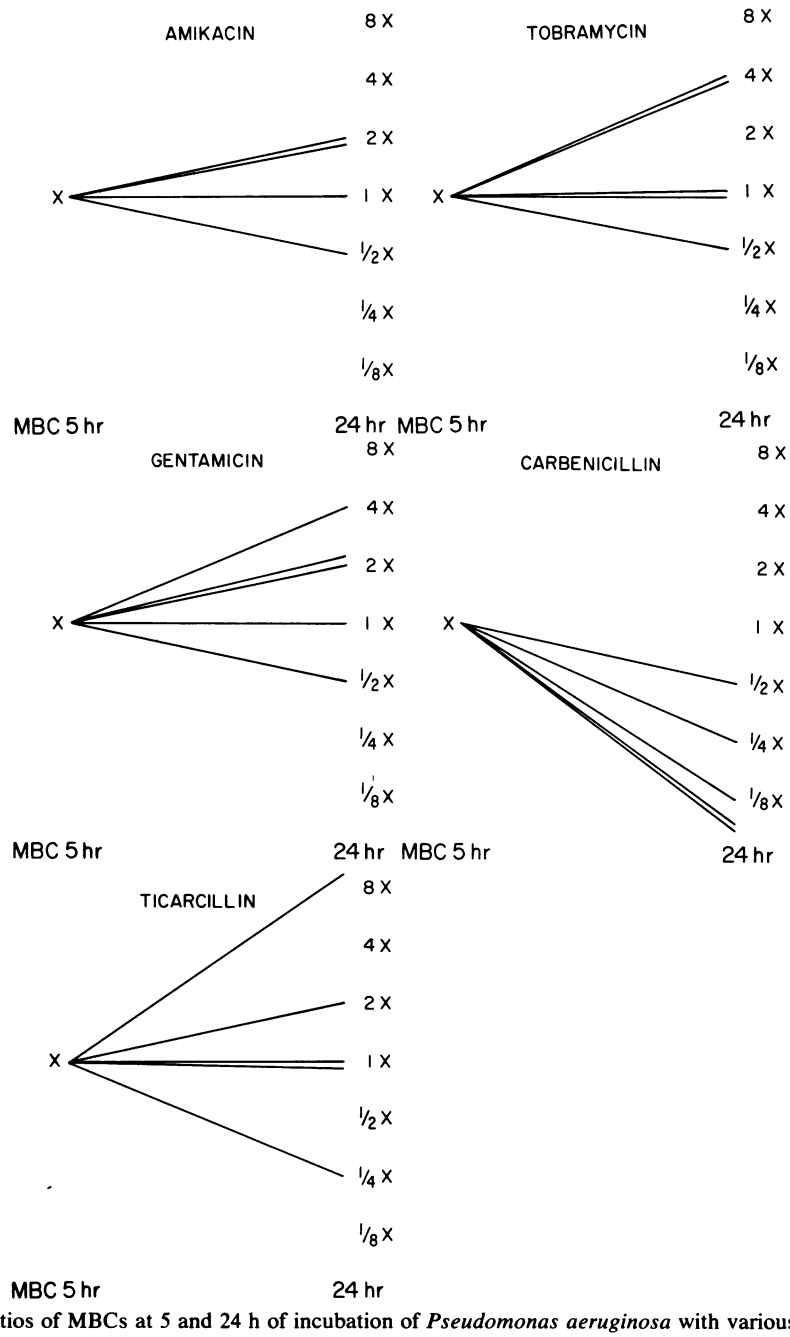

FIG. 5. Ratios of MBCs at 5 and 24 h of incubation of *Pseudomonas aeruginosa* with various antibiotics.

whereas the MIC indicates that multiplication has stopped. The drug concentrations between the MIC and the MAC encompass the other antibiotic effects which were described. These three endpoints, which were obtained by the direct determination of the number of live organisms in the drug-exposed bacterial population, indicate the antibacterial range of an antibiotic in terms of growth inhibition and bacterial survival of a given strain. The data suggest that the antibacterial range is similar for most strains of a given species and is, to some extent, a characteristic of similar antibiotics.

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