ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1986, p. 927–932
0066-4804/86/120927-06$02.00/0

Vol. 30, No. 6

Ethambutol MICs and MBCs for *Mycobacterium avium* Complex and *Mycobacterium tuberculosis*

LEONID B. HEIFETS,[1,2,3]* MICHAEL D. ISEMAN,[1,2] AND PAMELA J. LINDHOLM-LEVY[1]

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206,[1] and Department of Medicine[2] and Department of Microbiology,[3] University of Colorado Health Sciences Center, Denver, Colorado 80262

Received 9 May 1986/Accepted 23 September 1986

We determined the MICs of ethambutol for both *Mycobacterium avium* and *Mycobacterium tuberculosis* strains by using broth dilution (7H12 broth, radiometric method) and agar dilution (7H11 agar) methods. We found the MICs to be much lower in liquid than in solid medium. The broth-determined MICs for susceptible *M. tuberculosis* and most of the *M. avium* strains were comparable to the levels in blood of patients, being lower than the peak levels. We propose that the MICs, determined radiometrically in 7H12 broth, be considered as tentative criteria for susceptibility testing of *M. avium* isolates in future clinical trials. The use of these values instead of critical concentrations should also be considered as an alternative to the conventional susceptibility testing method in chemotherapy of tuberculosis. Ethambutol produced bactericidal effects against both *M. tuberculosis* and *M. avium*, and the MIC/MBC ratios were in the same range for both species when MICs and MBCs were tested in 7H12 broth by conventional sampling and plating.

Ethambutol (EMB) was introduced by Lederle Laboratories in 1961 (16, 22, 26) and recommended in a leading article in *Tubercle* for practical use in 1966 (Tubercle 47:292–295). Controlled clinical trials (1, 8) have shown its effectiveness in the treatment of tuberculosis, especially in combination with other antituberculosis drugs (2, 21). There have been no clinical trials with EMB in the treatment of mycobacterial infections other than tuberculosis and only a few reports about the usefulness of EMB in the treatment of infections caused by *Mycobacterium avium* (10, 28) and *Mycobacterium marinum* (29). Nevertheless, the Committee on Therapy of the National Tuberculosis and Respiratory Disease Association (under the American Thoracic Society) issued a statement in 1968 that EMB "is useful in combination with three or four other antimicrobial agents, for the treatment of the atypical group of mycobacteria," indicating specifically *Mycobacterium kanasasii* and *M. avium* complex (4). EMB is widely used in combination with other drugs in the therapy of both tuberculosis and *M. avium* infection. Whereas the use of EMB for treatment of tuberculosis is based on the results of controlled clinical trials, the clinical efficacy of EMB in *M. avium* infection has been less well documented and is mainly based on retrospective analysis of multidrug chemotherapy (6, 9).

Administration of EMB alone in a dose of 25 mg/kg daily has led to a sputum conversion within 1 to 3 months in most of the patients with tuberculosis, but "bacteriologic relapse soon occurred with loss of organism susceptibility" (4). The criteria for in vitro susceptibility of *Mycobacterium tuberculosis* to EMB were derived from comparison of the in vitro tests and the response to treatment, regardless of the concentrations of EMB in sera of patients. Peak levels of 3 to 5 μg/ml have been achieved 2 to 4 h after oral administration of 20 to 30 mg of EMB per kg (20). Most *M. tuberculosis* strains are inhibited by 1 and 2 μg of EMB per ml incorporated into Lowenstein-Jensen medium (L. B. Robinson and R. H. Wichelhausen, Trans. 21st Conf. Pulm. Dis., p. 351, 1962), and the so-called critical concentration of 2 μg/ml in Lowenstein-Jensen medium has been suggested as a breakpoint to differentiate susceptible and resistant isolates (3). It has been found that the MIC of EMB is different in different media for the same strains of *M. tuberculosis*, and equivalents of critical concentrations have been suggested as 5 μg/ml in 7H10 agar (5, 12) and 7.5 μg/ml in 7H11 agar (18).

These criteria of susceptibility developed for *M. tuberculosis* have often been applied to *M. avium* in the absence of any data about the correlation between the in vitro tests and clinical or bacteriological response to treatment with EMB. Nor has there been any clear evidence of the emergence of drug resistance as a result of treatment with EMB and as a marker of drug effect. Moreover, a substantial percentage of *M. avium* strains isolated before treatment have been resistant in vitro to the so-called critical concentration (6, 9).

M. avium complex (*M. avium* and *Mycobacterium intracellulare*) has recently become a major opportunistic pathogen causing disseminated life-threatening disease among an increasing number of patients with acquired immunodeficiency syndrome (AIDS) and other immunodeficiencies. The chemotherapy of both disseminated and localized *M. avium* infections is still largely based on empirical observations, and there is an obvious need for the development of more rational criteria of susceptibility for in vitro tests with EMB and other drugs. Clinical trials, which are now under consideration, may provide an opportunity to solve this problem. One issue that should be answered in anticipation of such clinical trials is the kind of in vitro data that should be compared with the observed clinical responses to develop more accurate criteria of susceptibility. Unlike the previous situation when criteria of susceptibility for *M. tuberculosis* were developed, it is now possible to use not only solid media, but a liquid medium as well, in determining the MIC. The use of 7H12 broth offers an opportunity for radiometric MIC determination in the automated BACTEC system, a faster, more convenient, and less labor intensive method than conventional (with sampling and plating) determination in liquid medium (13, 14). The use of the 7H12 liquid medium helps to avoid some of the problems inherent in the use of various solid media, such as absorption and binding of the drug or partial inactivation during preparation of the medium and during long periods of incubation at 37°C. The 7H12

* Corresponding author.

broth does not contain any significant amounts of substrates which could absorb or bind the drug, does not contain Tween 80, which can affect the results of susceptibility testing (30), and requires a relatively short period of incubation, which decreases the length of exposure of the drug to 37°C. These advantages seem to be especially important for testing with EMB, for which in vitro activity is highly dependent on the method of cultivation (12). In addition, the use of 7H12 broth allows the MBC and MIC to be determined under the same conditions, making the MIC/MBC ratio determination more precise and more reliable.

The aims of this study were to determine the range of MICs of EMB for *M. avium* clinical isolates in liquid medium (7H12 broth), to compare the results obtained in this liquid medium and by an agar dilution method (7H11 agar), to determine the MIC/MBC ratios in 7H12 broth, and to compare the results of MIC and MBC determinations for *M. avium* and *M. tuberculosis* clinical isolates.

MATERIALS AND METHODS

Antimicrobial agent. EMB hydrochloride was obtained from Lederle Laboratories, Pearl River, N.Y. Stock solutions (10,000 μg/ml) were made in distilled water, sterilized by filtration through a membrane filter (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.), and kept as frozen aliquots at −70°C. Working solutions were made from stock solutions in sterile distilled water.

Cultures. Clinical isolates of *M. avium* complex strains isolated from 52 patients with disseminated AIDS and 51 patients with pulmonary disease before chemotherapy were referred by different laboratories to our institution for identification. Subcultures from transparent-type colonies were preserved in 7H9 broth at −70°C. Two kinds of *M. tuberculosis* clinical isolates were used in this study: (i) 28 strains obtained from patients before chemotherapy and referred to our laboratory for identification and drug susceptibility testing and (ii) 22 isolates from National Jewish Center for Immunology and Respiratory Medicine inpatients who previously had been treated with different drugs, including EMB, and whose strains were resistant to 7.5 or 15 μg of EMB per ml when tested by conventional tests in 7H11 agar plates. *M. tuberculosis* and *M. avium* 7H9 broth cultures were preserved as aliquots at −70°C. Before an experiment, the *M. avium* strains were subcultivated in 7H9 broth for 2 days, and the *M. tuberculosis* strains were subcultivated for 5 to 7 days.

MIC determination in 7H12 broth by radiometric method. 7H12 medium (19), which contains ^{14}C-labeled substrate (palmitic acid) as a source of carbon, was used. Growth leads to the consumption of the substrate, with subsequent release of $^{14}CO_2$ into the atmosphere above the medium in the sealed vial, and the BACTEC TB-460 instrument (Johnston Laboratories, Inc., Towson, Md.) detects the amount of $^{14}CO_2$ and records it as GI (growth index) on a scale of 0 to 999. The GI was recorded daily to produce a GI curve.

For *M. avium* testing, an initial vial of 7H12 medium was inoculated with a 1:50 dilution of a 7H9 broth culture adjusted to the optical density of a no. 1 McFarland standard. When the GI of the vial reached the maximum (999), the culture was diluted 1:100 and was used to inoculate a set of vials (0.1 ml per vial). Our previous study (14) showed that this inoculum provides an initial bacterial concentration of 10^4 to 10^5 CFU/ml.

In preparing *M. tuberculosis* strains for MIC determination, a 7H12 vial was inoculated with 0.1 ml of a 1:2 dilution of a fresh 7H9 culture which equaled the optical density of a no. 1 McFarland standard. When growth in this vial reached a GI reading of 400 to 500, 0.1 ml of the 7H12 broth culture was used undiluted to inoculate a set of vials, yielding 10^4 to 10^5 CFU/ml (13).

For experiments with both *M. avium* and *M. tuberculosis*, two drug-free vials per set of drug-containing vials were used as controls; one vial was inoculated in the same way as the test vials, and the other was inoculated with a 1:100 dilution of the inoculum (1:100 control) to produce an initial concentration representing 1% of the bacterial population (10^2 to 10^3 CFU/ml) found in the drug-containing vials. Appropriate working solutions of EMB were added in a volume of 0.1 ml per vial. Final concentrations of 3.8, 1.9, and 0.95 μg of EMB per ml were used for primary titrations, followed by another set of higher or lower concentrations if the first experiment was out of scale.

The principles of the so-called radiometric proportion method (23, 24) state that the concentration of the drug which produces a daily GI increase and final GI reading lower than that in the 1:100 control can be considered the concentration inhibiting more than 99% of the bacterial population. The lowest concentration meeting these criteria was defined as the MIC. The period of observation was no less than 4 days and no more than 8 days for *M. avium* or 10 days for *M. tuberculosis*. The endpoint of the experiment with *M. avium* was the day on which the GI of the undiluted control reached 999, but for *M. tuberculosis* the experiment ended when growth in the 1:100 control reached a GI of 30 or more for two consecutive days.

MIC determination in 7H12 broth by plating. The experiments for MIC determination in 7H12 broth by plating were conducted as described above for radiometric MIC determinations, but duplicates were used for each concentration and for both controls. Samples from alternate vials were removed on various days (see Fig. 1 and 3) for plating. An allergist syringe with a fixed 27-gauge, 1.27-cm needle (Becton Dickinson and Co., Paramus, N.J.) was used to draw up 0.7 to 0.8 ml of the culture; the plunger was then given a rapid push so that the medium was forced back into the vial. Repetition of this procedure two more times was sufficient to break up most of the clumps of *M. tuberculosis*. Two or three 10-fold dilutions of each sample were used for plating (based on preliminary studies) so as to have a range of 50 to 500 CFU per plate. Four to six 7H11 agar plates were used for each sample; each dilution was inoculated at a volume of 0.5 ml, which was distributed by tilting the plate (not by use of a spreader). Incubation was at 37°C for 12 to 14 days; the colonies were then counted. The MIC was the lowest concentration that inhibited more than 99% of the bacterial population for at least 4 days of cultivation in 7H12 broth.

MIC determination by agar dilution method. Different concentrations of EMB (0.95 to 30.0 μg/ml) were incorporated into 7H11 agar. Two sets of petri dishes (one quadrant in each plate contained drug-free medium, and the other three held different drug concentrations) were inoculated with each strain, one with a 10^{-3} dilution and the other with a 10^{-5} dilution made from either a 7H9 broth culture adjusted to the optical density of a no. 1 McFarland standard or from the 7H12 broth culture which had achieved a GI of 999. Each quadrant was inoculated with 0.1 ml of the bacterial suspension, and the inoculum was distributed by tilting the plate. The higher inoculum consisted of 1×10^4 to 3×10^4 CFU; the lower inoculum contained 100 to 300 CFU. Incubation was at 37°C in the presence of 5% CO_2 for 12 to

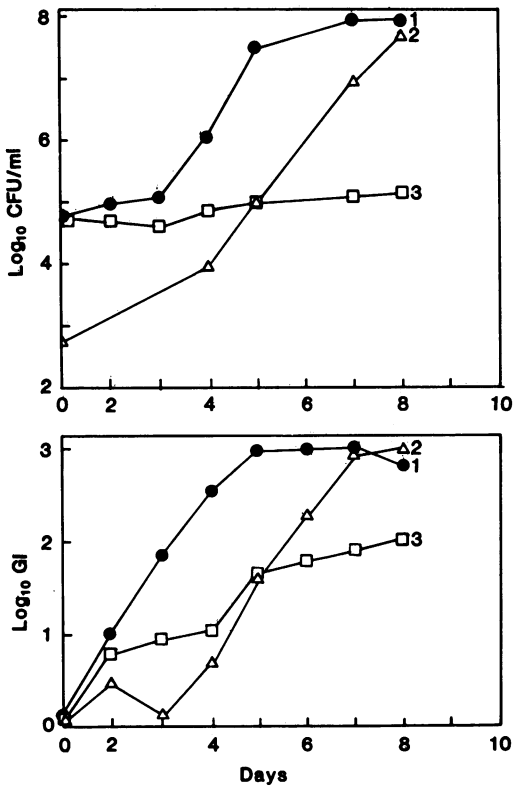

FIG. 1. Example of MIC determination for *M. avium* by two methods and correlation between growth curves (CFU per milliliter) and daily GI reading curves. 1, Undiluted control; 2, 1:100 control; 3, 1.9 μg of EMB per ml (MIC for the strain).

14 days. The lowest concentration of EMB that inhibited more than 99% of the bacterial population was considered the MIC.

MBC determination. The 7H12 vials were inoculated as described above for MIC determination in this medium, providing an initial concentration of bacteria in the range of 10^4 to 10^5 CFU/ml. Different drug solutions were added when the daily radiometric GI was between 20 and 80 for *M. avium* and 400 and 500 for *M. tuberculosis*, indicating that the bacteria were actively multiplying and had reached, as in previous findings, 10^5 to 10^6 CFU/ml. Samples from alternate vials containing either drug-free medium (undiluted control) or different drug concentrations were taken on various days of cultivation starting with day 1 at intervals of 1 to 4 days depending on the changes in the GI. The samples were diluted and plated so as to have no more than 500 CFU per plate; 0.5-ml portions of the diluted samples were inoculated onto 7H11 agar plates. Incubation was at 37°C in 5% CO_2 for 12 to 14 days; the colonies were then counted.

Initially we tried to apply to these studies the widely accepted definition that the MBC is the lowest concentration which kills 99.9% of the bacterial population (25). Our preliminary studies with several *M. avium* strains indicated that the dose-dependent bactericidal effect of EMB on these organisms has a limit: with some strains it did not kill more than 99% of the population. Therefore, for comparisons between *M. avium* and *M. tuberculosis*, we defined the MBC of EMB in this study as the lowest concentration that killed 99% of the population of *M. avium* or *M. tuberculosis* within 15 days of cultivation in 7H12 broth. Such an approach corresponds with other mycobacteriology studies in which 99% or even lower percentages have been used (7, 27), but it does not exclude the use of 99.9% as the criterion for other drugs.

MIC/MBC ratio determination. A limited number of *M. avium* and *M. tuberculosis* strains (5 of each) with low and high MICs were included in the determination of MIC/MBC ratios. Most experiments covered simultaneous determination of both the MIC and MBC in 7H12 broth by plating as described above.

RESULTS

MICs for *M. avium*. The initial concentration of bacteria in 7H12 broth, incubated by the procedure described above (see Materials and Methods), was between 10^4 and 10^5 CFU/ml. Within 4 and 8 days in drug-free medium (undiluted control) the *M. avium* population reached the maximum of about 10^8 CFU/ml. After a period of stabilization (2 to 4 days), the number of CFU per milliliter began to decline. The increase in daily GI readings correlated well with the growth curve during the period of active growth (Fig. 1). This course of events corresponds with our previous observations (13, 14). The growth curves and daily GI curves also correlated in the presence of EMB (Fig. 1). These experiments confirmed that the inhibition of GI in drug-containing vials reflects actual inhibition of growth by the drug. The MICs determined by both CFU per milliliter and GI readings were the same in almost all experiments, with a possible difference of one dilution (Table 1). These data correspond with our previous finding in regard to determination of the ansamycin LM427 (rifabutine) MICs (14) and justified the use of the radiometric method for determination of MICs of EMB in liquid medium for the remaining 98 strains of *M. avium*. Replicate studies showed a high consistency in results by both broth and agar dilution methods; the MICs never varied by more than one dilution.

The MICs of EMB determined in liquid medium were between 0.95 and 15.0 μg/ml, with no significant differences between the strains of *M. avium* isolated from AIDS and non-AIDS patients. The broth-determined MIC (per milliliter) was 1.9 μg or less for 63.1% of the *M. avium* strains, 3.8 μg for 28.2% of the strains, 7.5 μg for 5.8% of the strains, and 15.0 μg for 2.9% of the strains. There were also no differences between AIDS and non-AIDS isolates in the MICs determined by the agar dilution method. The MICs determined by this method were between 7.5 and 30.0 μg/ml; the agar plate-determined MICs (per milliliter) were 7.5 μg for 3.8% of the strains, 15.0 μg for 54.4% of the strains, and 30.0 μg or more for 41.8% of the strains. Most of the *M. avium* isolates appeared much more susceptible to EMB in 7H12 broth than in 7H11 agar plates (with a median difference of eightfold); the strains most susceptible by the broth method were found to be the most susceptible by the agar

TABLE 1. MICs and MBCs of EMB determined in 7H12 broth by sampling and plating for five *M. avium* strains

Strain	MIC (μg/ml) determined:		Bactericidal effect		MIC/MBC ratio
	By CFU/ml	Radiometrically	CFU/ml when drug added	MBC (μg/ml)	
1339-3	1.9	1.9	228,000	15.0	1/8
101-5	1.9	1.9	126,000	15.0	1/8
211-5	3.8	1.9	352,000	15.0	1/8
3350-4	7.5	7.5	215,000	30.0	1/8
168-4	7.5	7.5	51,800	30.0	1/4

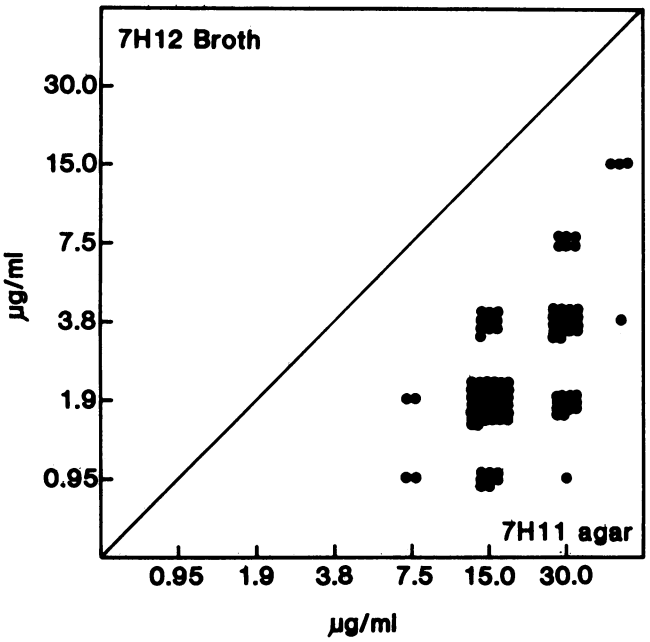

FIG. 2. MICs of EMB determined by 7H12 broth and 7H11 agar plate methods for 103 *M. avium* strains.

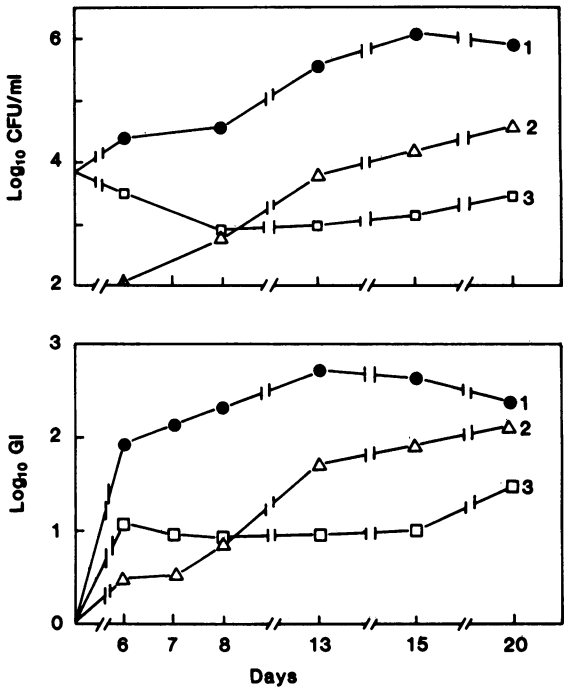

FIG. 3. Example of MIC determination for *M. tuberculosis* by two methods and correlation between growth curves (CFU per milliliter) and daily GI reading curves. 1, Undiluted control; 2, 1:100 control; 3, 1.9 μg of EMB per ml (MIC for the strain).

method, and the most resistant strains by one of the methods were also the most resistant by the other method (Fig. 2).

MICs for *M. tuberculosis*. In this study we confirmed our previous observations (13) that the increase in daily GI readings correlates well with the growth curve during the period of active growth of *M. tuberculosis* (Fig. 3) and that the maximum number of CFU of *M. tuberculosis* per milliliter in 7H12 broth is about 10^6, approximately 100 times less than for *M. avium*. The inhibition of daily GI by EMB reflected a true inhibition of growth (Fig. 3), and the MICs determined in 7H12 broth by the radiometric method and by sampling and plating were the same when the comparison experiments were conducted with four strains of *M. tuberculosis* (Table 2). The MICs for strains considered susceptible (to 7.5 μg of EMB per ml of 7H11 agar) were in a narrow range of 1.9 to 7.5 μg/ml when tested in agar plates: 1.9 μg/ml for 25.0%, 3.8 μg/ml for 57.0%, and 7.5 μg/ml 18.0%. The broth-determined MICs for these susceptible strains were 0.48 μg/ml for 7.1%, 0.95 μg/ml for 39.3%, 1.9 μg/ml for 50%, and 3.8 μg/ml for 3.6%. These results correspond with previous observations for strain H37Rv, for which the MIC of EMB in liquid medium containing Tween 80 was less than 5.0 μg/ml (15). The MICs of EMB against *M. tuberculosis* strains resistant to EMB by conventional testing were 15.0 or 30.0 μg/ml in agar plates (59.1 and 40.9% of the strains, respectively). The broth-determined MICs (per milliliter) were 0.95 μg or less for 18.2% of the strains, 3.8 μg for 13.6% of the strains, 7.5 μg for 45.5% of the strains, and 15.0 μg for 22.7% of the strains. It can be concluded that both susceptible and resistant *M. tuberculosis* strains required significantly lower concentrations of EMB in 7H12 broth than in 7H11 agar plates (with a median difference of twofold) to produce the same inhibitory effect (Fig. 4).

MIC/MBC ratios for *M. avium* and *M. tuberculosis*. When different concentrations of EMB were added to the growing 7H12 broth cultures, as described above, it was found that increasing concentrations of EMB produced a greater lethal effect on both *M. tuberculosis* and *M. avium*. We achieved 99.9% maximum killing of the bacterial populations when four *M. tuberculosis* strains were tested, but the same effect occurred with only three of five *M. avium* strains tested. To compare the MIC/MBC ratios for the two species, we chose to consider the MBC the lowest concentration of EMB that killed 99% (not 99.9%) of the bacterial population. By using this criterion, we found that the MBC for five tested *M. avium* strains with different MICs was 15.0 or 30.0 μg/ml and the MIC/MBC ratio was 1/4 to 1/8 (Table 1). The MBCs for the *M. tuberculosis* strains were lower for strains with low MICs and higher for strains with high MICs, ranging from 3.8 to 60.0 μg/ml (Table 2), and the MIC/MBC ratio was 1/8 for all tested strains, not significantly different from the results obtained with *M. avium* (Table 1).

TABLE 2. MICs and MBCs of EMB determined in 7H12 broth by sampling and plating for *M. tuberculosis* strains

Strain[a]	MIC (μg/ml) determined:		Bactericidal effect		MIC/MBC ratio
	By CFU/ml	Radiometrically	CFU/ml when drug added	MBC (μg/ml)	
S-H37Rv	1.9	1.9	570,000	15.0	1/8
S-70308	ND[b]	0.95	1,110,000	3.8	
S-261-8	1.9	1.9	310,000	15.0	1/8
R-2923-5	7.5	7.5	446,000	60.0	1/8
R-2229-5	7.5	7.5	733,000	60.0	1/8

[a] R strains were resistant to 7.5 μg of EMB per ml in 7H11 agar plate test; S strains were susceptible under the same conditions.
[b] ND, Not determined.

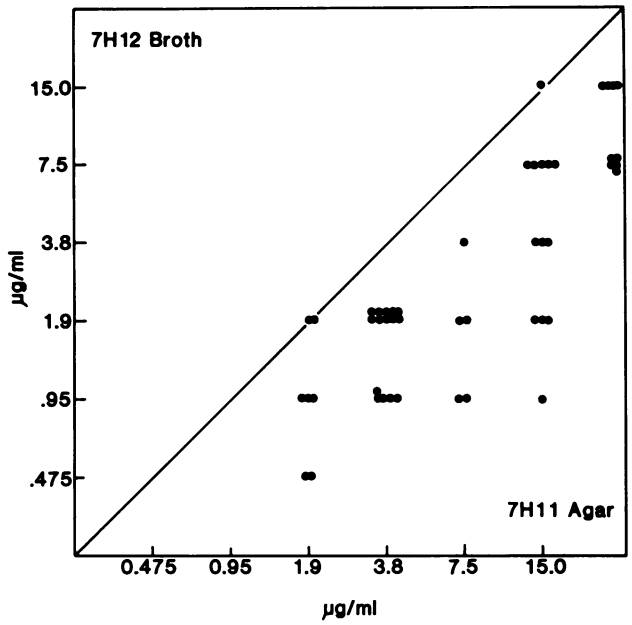

FIG. 4. MICs of EMB determined by 7H12 broth and 7H11 agar plate methods for 50 *M. tuberculosis* strains.

DISCUSSION

The MICs of EMB determined by the agar dilution method were in quite a narrow range for most of the strains of both *M. avium* and *M. tuberculosis*. The range of MICs for 22 resistant strains of *M. tuberculosis* and for *M. avium* strains was wider when determined in 7H12 broth than in 7H11 agar. We believe that this indicates that the broth dilution method can detect different levels of susceptibility or resistance and therefore can be considered more sensitive than the agar dilution method in determining the MIC. The MICs determined by the broth dilution (radiometric) method were four to eight times lower for most of the *M. avium* and *M. tuberculosis* strains than the MICs determined by the agar plate method. It is well known that the activity of EMB is quite different in various media due to variable absorption and inactivation (12). 7H12 broth is almost free of agents that would absorb or inactivate EMB and does not contain Tween 80, and the period of cultivation to determine MICs by radiometric reading is much shorter than in any other method. Except for one, the MICs of EMB determined by this method for strains of *M. tuberculosis* recovered from untreated patients (susceptible to 7.5 μg/ml in 7H11 agar plates) were all in a range of 0.48 to 1.9 μg/ml, a concentration which is lower than the usual peak level achievable in the blood of patients after a dose of 15 to 20 mg/kg (3.0 μg/ml). At the same time, only 4 of 22 of the resistant *M. tuberculosis* strains had an MIC lower than 3.0 μg/ml. These data suggest that determination of EMB MICs in 7H12 broth by radiometric reading would allow the monitoring of chemotherapy with this drug in a more sensitive and reasonable way than with the currently used critical concentration, especially in solid media. We suggest that instead of one critical concentration as a qualitative criterion of susceptibility, quantitative criteria should be used to evaluate the results. The criteria for the radiometric determination in 7H12 broth, in accordance with our results, can be established as follows: susceptible, MIC $\leq$ 1.9 μg/ml; moderately susceptible, MIC = 3.8 μg/ml; moderately resistant, MIC = 7.5 μg/ml; resistant, MIC $\geq$ 15.0 μg/ml.

For *M. avium* infection no critical concentration has been established, and our findings indicate that 63.1% of the strains had a broth-determined MIC of 1.9 μg/ml or lower, as did most of the susceptible *M. tuberculosis* strains, whereas only 3.9% of the *M. avium* strains could have been considered susceptible to the critical concentration of 7.5 μg/ml in 7H11 agar. It is possible that the difference in clinical response among patients with *M. avium* infection depends on the MIC of EMB against an individual strain. Thus, testing of the MICs for *M. avium* isolates in broth should provide, in our opinion, better predictive data for chemotherapy and possibly could become a tool to monitor chemotherapy with EMB in *M. avium* infection.

We confirmed the bactericidal activity of EMB against *M. tuberculosis* (7, 17) and found that EMB is also bactericidal against *M. avium*. We observed almost the same delayed bactericidal activity against *M. avium* as against *M. tuberculosis*, which is thought to be associated with the inability of EMB to affect the nonproliferating cells (11, 17). The MIC/MBC ratios of EMB were in the same range for both *M. tuberculosis* and *M. avium* when the 99% MBC criterion was applied; however, high-level bactericidal effects (killing of 99.9%) were more common among *M. tuberculosis* strains.

Comparison of the activity of EMB in vitro against *M. tuberculosis* and *M. avium* indicates that for most of the *M. avium* isolates the drug had the same bacteriostatic (MIC) and bactericidal (MBC) effect as for susceptible *M. tuberculosis* strains. Therefore, the in vitro findings of EMB activity against *M. avium* support the utility of EMB in treatment of *M. avium* infection and justify its present use for this purpose, a use which has thus far been based only on empirical evidence.

ACKNOWLEDGMENTS

This work was supported by contract NO1-A1-42544 with the National Institute of Allergy and Infectious Diseases.

We thank the Lederle Laboratories for supplying the EMB, B. Silverstein for the artwork, N. Eig for editorial help, and C. J. Queen for preparation of the manuscript.

LITERATURE CITED

1. **Bobrowitz, I. D.** 1966. Comparison of ethambutol-inh versus inh-pas in original treatment of pulmonary tuberculosis. Ann. N.Y. Acad. Sci. **135**:921–939.
2. **Bobrowitz, I. D., and D. E. Robbins.** 1967. Ethambutol-isoniazid versus PAS-isoniazid in original treatment of pulmonary tuberculosis. Am. Rev. Respir. Dis. **96**:428–438.
3. **Canetti, G., W. Fox, A. Khomenko, H. T. Mahler, N. K. Menon, D. A. Mitchison, N. Rist, and N. A. Smelev.** 1969. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis programmes. Bull. W.H.O. **41**:21–43.
4. **Committee on Therapy of the National Tuberculosis and Respiratory Disease Association.** 1968. Ethambutol in the treatment of tuberculosis. Am. Rev. Respir. Dis. **98**:320–321.
5. **David, H. L.** 1976. Bacteriology of mycobacterioses. U.S. Department of Health, Education, and Welfare publication no. (CDC) 76-8316. Center for Disease Control, Atlanta.
6. **Davidson, P. T., V. Khanijo, M. Goble, and T. S. Moulding.** 1981. Treatment of disease due to *Mycobacterium intracellulare*. Rev. Infect. Dis. **3**:1052–1059.
7. **Dickinson, J. M., V. R. Aber, and D. A. Mitchison.** 1977. Bactericidal activity of streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide alone and in combination against *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **116**:627–635.

8. **Donomae, L., and K. Yamamoto.** 1966. Clinical evaluation of ethambutol in pulmonary tuberculosis. Ann. N.Y. Acad. Sci. **135**:849–881.
9. **Dutt, A. R., and W. W. Stead.** 1979. Long-term results of medical treatment in *Mycobacterium intracellulare* infection. Am. J. Med. **67**:449–453.
10. **Evans, C., and K. L. Thong.** 1969. A clinical trial of ethambutol plus capreomycin in the treatment of atypical tuberculosis. Med. J. Aust. **2(15)**:744–748.
11. **Forbes, M., E. A. Peets, and N. A. Kuck.** 1966. Effect of ethambutol on mycobacteria. Ann. N.Y. Acad. Sci. **135**:726–731.
12. **Gangadharam, P. R., and E. R. Gonzales.** 1970. Influence of the medium on the *in vitro* susceptibility of *Mycobacterium tuberculosis* to ethambutol. Am. Rev. Respir. Dis. **102**:653–655.
13. **Heifets, L. B., M. D. Iseman, J. L. Cook, P. J. Lindholm-Levy, and I. Drupa.** 1985. Determination of in vitro susceptibility of *Mycobacterium tuberculosis* to cephalosporins by radiometric and conventional methods. Antimicrob. Agents Chemother. **27**:11–15.
14. **Heifets, L. B., M. D. Iseman, P. J. Lindholm-Levy, and W. Kanes.** 1985. Determination of ansamycin MICs for *Mycobacterium avium* complex in liquid medium by radiometric and conventional methods. Antimicrob. Agents Chemother. **28**:570–575.
15. **Hobby, G. L., and T. F. Lenert.** 1972. Observations on the action of rifampin and ethambutol alone and in combination with other antituberculous drugs. Am. Rev. Respir. Dis. **195**: 292–295.
16. **Karlson, A. G.** 1961. Therapeutic effect of ethambutol (dextro-2,2'-(ethylenediamino)-di-1-butanol) on experimental tuberculosis in guinea pigs. Am. Rev. Respir. Dis. **84**:902–904.
17. **Kuck, N. A., E. A. Peets, and M. Forbes.** 1963. Mode of action of ethambutol on *Mycobacterium tuberculosis*, strain H37Rv. Am. Rev. Respir. Dis. **87**:905–906.
18. **McClatchy, J. K.** 1978. Susceptibility testing of mycobacteria. Lab. Med. **9**:47–52.
19. **Middlebrook, G., Z. Reggiardo, and W. D. Tigertt.** 1977. Automatable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. Am. Rev. Respir. Dis. **15**:1067–1069.
20. **Place, V. A., E. A. Peets, D. A. Buyske, and R. R. Little.** 1966. Metabolic and special studies of ethambutol in normal volunteers and tuberculous patients. Ann. N.Y. Acad. Sci. **135**:775–795.
21. **Pyle, M. M., K. H. Pfuetze, M. D. Pearlman, J. de la Huerga, and R. H. Hubble.** 1966. A four-year clinical investigation of ethambutol in initial and retreatment cases of tuberculosis. Efficacy, toxicity, and bacterial resistance. Am. Rev. Respir. Dis. **93**:428–441.
22. **Schmidt, L. H.** 1966. Studies of the antituberculosis activity of ethambutol in monkeys. Ann. N.Y. Acad. Sci. **135**:747–758.
23. **Snider, D. E., R. C. Good, J. O. Kilburn, L. F. Laskowski, R. H. Lusk, J. J. Marr, Z. Reggiardo, and G. Middlebrook.** 1981. Rapid susceptibility testing of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **123**:402–406.
24. **Tarrand, J. J., and D. H. M. Gröschel.** 1985. Evaluation of the BACTEC radiometric method for detection of 1% resistant populations of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **21**:941–946.
25. **Taylor, P. C., F. D. Schoenknecht, J. C. Sherris, and E. C. Linner.** 1983. Determination of minimum bactericidal concentrations of oxacillin for *Staphylococcus aureus*: influence and significance of technical factors. Antimicrob. Agents Chemother. **23**:142–150.
26. **Thomas, J. P., C. O. Baughn, R. G. Wilkinson, and R. G. Shepherd.** 1961. A new synthetic compound with antituberculous activity in mice: ethambutol. Am. Rev. Respir. Dis. **83**:891–893.
27. **Tsukamura, M.** 1985. *In vitro* antituberculosis activity of a new antibacterial substance ofloxacin (DL 8280). Am. Rev. Respir. Dis. **131**:348–351.
28. **Watson, B. M., and J. T. Smyth.** 1968. B663 and ethambutol in the treatment of Battey disease. Med. J. Aust. **1(7)**:261–263.
29. **Wolinsky, E., F. Gomez, and F. Zimpfer.** 1972. Sporotrichoid *Mycobacterium marinum* infection treated with rifampin-ethambutol. Am. Rev. Respir. Dis. **105**:964–967.
30. **Youmans, A. S., and G. P. Youmans.** 1948. The effect of "Tween 80" in vitro on the bacteriostatic activity of twenty compounds for *Mycobacterium tuberculosis*. J. Bacteriol. **56**:245–252.