MIC as a Quantitative Measurement of the Susceptibility of
Mycobacterium avium Strains to Seven Antituberculosis Drugs

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Received 22 January 1988/Accepted 10 May 1988

MICs of isoniazid, rifampin, ethionamide, streptomycin, amikacin, kanamycin, and capreomycin were
determined for Mycobacterium avium complex strains by two methods: broth dilution in 7H12 medium
radiometrically and agar dilution on 7H10 agar plates. The broth-determined MICs of all drugs with the
exception of isoniazid were two to eight times lower than the agar-determined MICs for most of the tested M.
avium strains, which is probably due to the higher absorption and degradation of the drugs in solid media. The
MICs, especially those determined in broth, are suggested as quantitative measurements of the degree of
susceptibility of M. avium complex strains. For a certain percentage of the M. avium strains the broth-
determined MICs were within the limits of MICs found for wild susceptible Mycobacterium tuberculosis strains.
These M. avium strains were classified as presumably susceptible. In contrast to M. tuberculosis, the MICs for
M. avium strains had a wide range. When the MICs for M. avium strains were only one dilution higher than
those for M. tuberculosis, they were tentatively classified as moderately susceptible. The designation moderately
resistant or resistant, respectively, is suggested for those M. avium strains for which the MICs were at or above
the concentrations achievable in blood. The quantitation of the degree of susceptibility by the MICs and the
tentative interpretation of the MICs are suggested for future use in clinical trials as a means of evaluating the
patients’ responses to chemotherapy compared with the degree of susceptibility of the initial strain isolated
before treatment.

Critical concentrations of the conventional antituberculosis drugs are well-established qualitative criteria to determine whether a Mycobacterium tuberculosis clinical isolate is resistant or susceptible in the light of the probable response of the patient to chemotherapy (2, 3). A diminished clinical response may occur when the isolate is resistant to the critical concentrations of the administered drugs. The Mycobacterium avium complex (M. avium and M. intracellulare) clinical isolates are usually resistant to almost all antituberculosis drugs when tested against the critical concentrations developed for M. tuberculosis (19). Therefore the conventional qualitative tests, proven to be very useful in monitoring the chemotherapy of tuberculosis, are useless in the management of M. avium complex infection. (For the sake of simplicity, from this point on, the term M. avium will represent M. avium complex.) The susceptible M. tuberculosis wild strains are remarkably uniform in their susceptibility within narrow ranges of concentrations of antituberculosis drugs (2, 3, 14). M. avium strains, whether classified as resistant or susceptible by the conventional test against the critical concentrations, present a wide range in the degree of their susceptibility to most of the antituberculosis drugs (7–9). An alternative to this approach is to quantitate the degree of susceptibility of M. avium strains. We reported previously the application of the MIC as a quantitative measurement of susceptibility of M. avium strains to rifabutin (9), ethambutol (7), ciprofloxacin and ofloxacin (10), and clofazimine (P. J. Lindholm-Levy and L. B. Heifets, Tubercle, in press) and preliminary data about some experimental drugs (8).

The aim of this study was to evaluate the MICs of seven conventional antituberculosis drugs: isoniazid, rifampin, ethionamide, streptomycin, capreomycin, amikacin, kanamycin. The MICs of these drugs against M. avium strains were compared with the MICs against M. tuberculosis.

MATERIALS AND METHODS

Test strains. From a total of 31 M. avium strains included in this study, 18 were isolated from the blood of patients with acquired immunodeficiency syndrome. The remaining cultures were isolated from the sputum of patients with pulmonary disease. Subcultures obtained from smooth transparent type colonies were subcultivated in 7H9 broth (Difco Laboratories, Detroit, Mich.) for 3 to 5 days, and frozen samples of these cultures were kept at −70°C. Seventeen M. tuberculosis wild strains, susceptible to all drugs, were also preserved in frozen samples.

Antimicrobial agents. The drugs were obtained from the following suppliers: isoniazid from Sigma Chemical Co. (St. Louis, Mo.), rifampin from Merrell Dow (Zionsville, Ind.), ethionamide from Ives Laboratories (New York, N.Y.), streptomycin from GIBCO Laboratories (Grand Island, N.Y.), capreomycin from Eli Lilly & Co. (Indianapolis, Ind.), and amikacin and kanamycin from Bristol Myers (Syracuse, N.Y.). The stock solutions of each drug were prepared in accordance with manufacturer’s instructions and kept in aliquots at −70°C. From these stock solutions, working solutions were made in distilled water to be incorporated into the media used.

MIC determination by the agar dilution method. Quadrant petri dishes were prepared with 7H10 agar (made from Middlebrook and Cohn 7H10 agar; BBL Microbiology Systems, Cockeysville, Md.) so as to have different concentrations of drug in each of three quadrants; the fourth quadrant was the drug-free control. Each quadrant was inoculated with 0.1 ml of a bacterial suspension made in a 10−4 dilution from 3- to 5-day-old 7H9 broth culture adjusted to the optical density of a no. 1 McFarland standard. This inoculum usually provided 200 to 300 CFU in the drug-free quadrants. The plates were incubated at 37°C in the presence of 5% CO2 for 12 to 14 days, and the colonies were counted. The lowest
concentration of drug that inhibited more than 99% of the bacterial population was considered to be the MIC.

**MIC determination by the broth dilution method radiometrically.** Appropriate solutions of drugs were added in a volume of 0.1 ml to vials containing 7H12 (Johnston Laboratories, Towson, Md.) to achieve the desired final concentrations. The range of concentrations used for each drug is shown below. For *M. avium* an inoculum was made from an initial 7H12 broth culture after it had reached the maximum growth detected radiometrically (growth index [GI] 999). This culture was diluted 1:100, and 0.1 ml of this dilution was inoculated into each vial, providing, as in our previous observations (4, 5), an initial concentration of 10^6 to 10^7 CFU/ml. If the inoculum is correct, the growth in the drug-free vial reaches GI 999 not sooner than on day 4 and not later than on day 8 of cultivation. For *M. tuberculosis* a 7H12 both culture that had reached GI 400 to 500 was used as an inoculum; 0.1 ml of this broth per test vial was added undiluted, as contrasted with the *M. avium* inocula. With both organisms two drug-free controls were used: one vial was inoculated to match the drug-containing test vials, and the second was inoculated with a 1:100 dilution of the inoculum to represent 1% of the bacterial population (10^6 to 10^7 CFU/ml). The vials were incubated at 37°C, and the GI readings were recorded daily in the BACTEC-460TB instrument (Johnston Laboratories). The observation was conducted until the GI in the 1:100 control had been positive (>10) for 3 days. The concentration of drug producing daily GI increases and final GI reading lower than those in the 1:100 control was considered to have inhibited more than 99% of the bacterial population (15, 16) and was therefore defined as the MIC.

**RESULTS**

**Isoniazid.** The MICs of isoniazid ranged, for *M. avium* strains, from 1.25 to 10.0 µg/ml by either broth or agar dilution (Fig. 1A). No difference was found between the broth- and agar-determined MICs for 16 of 31 strains. For 12 strains the broth-determined MICs were two times lower (the difference of one dilution) than the agar-determined MICs. Generally, even the lowest MIC found by either method, 1.25 µg/ml, was significantly higher than the highest MIC of this drug found in our previous studies (14) with susceptible wild strains of *M. tuberculosis*: 0.05 µg/ml in broth and 0.2 µg/ml in agar (Fig. 1B). These data indicate that none of the tested *M. avium* strains was even close in its degree of susceptibility to isoniazid to the susceptible *M. tuberculosis* strains. If the highest MIC of the drug found for a susceptible *M. tuberculosis* strain is considered to be a breakpoint, then none of the tested *M. avium* strains would fall into the category of presumably susceptible strains. If the achievable concentration in blood (C_max) is taken as the next breakpoint, then 10 strains (32%) for which the broth-determined MICs were significantly lower than the C_max of 3.0 µg/ml (12) could have been classified as presumably moderately susceptible. By the same criterion, the next group of 12 strains (38.7%) for which the broth-determined MICs were 2.5 µg/ml, e.g., close to the C_max for this drug, can be called resistant, and the remaining 9 strains (29%), for which the MIC was 5.0 µg/ml and higher, can be called very resistant to isoniazid.

**Rifampin.** The broth-determined MICs for 30 of 31 *M. avium* strains were two, four, and eight times lower than the agar-determined MICs (Fig. 1A), which data are in agreement with other studies of the rifamycins (4, 9), indicating that the difference was due to the degradation of the drug during a prolonged period of incubation required for cultivation of the agar cultures. We assumed, due to this fact as well as to the absorption of drugs by the agar medium, that the broth-determined MICs may be a more accurate measurement of the degree of susceptibility than the agar-determined MICs (7, 9). The highest broth-determined MICs of this drug found for the susceptible *M. tuberculosis* strains was 0.25 µg/ml (Fig. 1B). Even taking into account the possible one-dilution error and that for some susceptible *M. tuberculosis* strains the MIC may be as high as 0.5 µg/ml, there were only six *M. avium* strains (19.4%) with this degree of susceptibility to rifampin. When the MICs of the tested *M. avium* strains were compared with the lowest reported C_max of 4.0 µg/ml (12), 22 *M. avium* strains (70.9%) could be considered as presumably moderately susceptible and only 3 strains, for which the broth-determined MIC was 8.0 and 16.0 µg/ml, could be classified as resistant or very resistant.

**Ethionamide.** The broth-determined MICs of ethionamide for *M. avium* strains were in the widest range (0.3 to >15.0 µg/ml) compared with those of the other drugs tested in this study (Fig. 1A). The broth-determined MICs were lower than the agar-determined MICs for 18 strains, were higher for 5 strains, and were the same by both methods for the remaining 8 strains. By comparison with the previously reported broth-determined MICs for the susceptible *M. tuberculosis* strains, 10 *M. avium* strains (32.2%) could be classified as presumably susceptible (MIC, ≥1.25 µg/ml). The next group of five strains, for which the MIC (2.5 µg/ml) still was less than the C_max (12), could be classified as moderately susceptible. In fact, the MIC for one of the 17 susceptible *M. tuberculosis* strains was also 2.5 µg/ml (Fig. 1B). The next two groups of 6 and 10 *M. avium* strains could be classified by this criterion as moderately resistant (MIC, 5.0 µg/ml) and resistant (MIC, ≥10.0 µg/ml).

**Streptomycin.** The broth-determined MICs for most strains were two and four times lower than the agar-determined MICs (Fig. 1A). The broth-determined MICs for 11 *M. avium* strains (35.5%) were within the range (≥2.0 µg/ml) found for susceptible *M. tuberculosis* strains (Fig. 1B) and by this criterion could be considered as presumably susceptible. Probably the *M. avium* strains for which the broth-determined MICs were one dilution higher (4.0 µg/ml) could be tentatively classified as moderately susceptible (3B, 15). The remaining strains for which the MICs were four or more times above the breakpoint found for susceptible *M. tuberculosis* strains could be tentatively classified as resistant.

**Amikacin.** The broth-determined MICs for *M. avium* strains ranged from 1.0 to 16.0 µg/ml, and the agar-determined MICs ranged from 4.0 to >16.0 µg/ml (Fig. 1A), whereas the MICs for susceptible *M. tuberculosis* strains were 1.0 µg/ml or less by both methods (Fig. 1B). Only one *M. avium* strain was in this category. If the same approach is used as for streptomycin, then 20 strains could be classified as moderately susceptible (MICs, 2.0 and 4.0 µg/ml), six strains could be classified as resistant (MIC, 8.0 µg/ml), and four strains could be classified as very resistant (MIC, ≥16.0 µg/ml).

**Kanamycin.** The MICs of kanamycin for *M. avium* strains ranged from 3.0 to 24.0 µg/ml by both methods (Fig. 1A). For three strains, the agar-determined MICs were even lower than 3.0 µg/ml. For 9 strains the MICs determined by two methods were the same, and for 18 strains the broth-determined MICs were two- or four-times lower than the agar-determined MICs (Fig. 1). The broth-determined MICs of kanamycin for susceptible *M. tuberculosis* strains were
3.0 μg/ml and less (Fig. 1B). Only eight M. avium strains (25.8%) were within these limits and could be classified by this comparison as presumably susceptible. The 13 M. avium strains (41.9%) for which the MICs were one dilution higher than these limits could be tentatively classified as moderately susceptible, if the same principle is applied as above for streptomycin and amikacin. By the same principle the remaining nine M. avium strains could be classified as presum-ably resistant (MIC, 12.0 μg/ml) and very resistant (MIC, 24.0 μg/ml).

Capreomycin. The broth-determined MICs for M. avium strains ranged from 2.5 to 40.0 μg/ml, and the agar-determined MICs were four to eight times higher for most strains (Fig. 1A). The MICs of this drug for susceptible M. tuberc-ulosis strains were 2.5 μg/ml or lower by either method (Fig. 1B). Only one M. avium strain was within this range (Fig. 1) and could be classified by this criterion as presumably susceptible. For eight M. avium strains the broth-determined MICs were only one dilution higher (5.0 μg/ml) and could be considered as moderately susceptible, whereas the remaining strains (71%) were presumably moderately resistant (MIC, 10.0 μg/ml) or very resistant (MIC, ≥20.0 μg/ml).

Table 1 represents a summary of my findings with the seven drugs described above and the results obtained with four other drugs, presented in previous reports (7, 9–11). The M. avium strains for which the MICs were within the range found for the susceptible wild M. tuberculosis strains were considered presumably susceptible. Moderately susceptible strains, as a rule, were M. avium strains for which the MICs were one dilution higher than in the susceptible category (one twofold dilution difference). The remaining strains,
inhibited by concentrations higher than MICs for moderately susceptible strains, were considered presumably resistant or very resistant.

**DISCUSSION**

The broth-determined MICs of seven antituberculosis drugs were usually lower than the agar-determined MICs in experiments with *M. avium*, which data are in agreement with other reports (4, 7–9, 13). These differences may have been caused, as suggested in these and other reports, by such factors as (i) higher absorption and degradation of drug in solid medium (17); (ii) the longer period of incubation required when using agar medium, which may lead to greater degradation of the drug (5, 9); and (iii) low bactericidal effect of some drugs against *M. avium*, in comparison with the effect against *M. tuberculosis* (11), which may result in secondary growth of the temporarily suppressed bacterial population after the inhibitory concentration of a drug has diminished during the long period of cultivation, particularly on agar plates. In the current study, a significant difference between broth- and agar-determined MICs for susceptible *M. tuberculosis* strains was found only in experiments with isoniazid and ethionamide. For the other five tested drugs either the broth- and agar-determined MICs were the same, or the difference was within a permissible one-dilution limit. It is possible that this correlation of broth- and agar-determined MICs of rifampin, streptomycin, amikacin, kanamycin, and capreomycin is due to the fact that these drugs are more bactericidal for *M. tuberculosis* than for *M. avium*. The role of the degree of bactericidal activity of a drug on the MICs was shown for ciprofloxacin and ofloxacin (10). The broth- and agar-determined MICs of these two quinolones were the same in experiments with both *M. tuberculosis* and *M. avium*. That was due to the fact that the MICs and MBCs were very close to each other for both species: the MIC/MBC ratios for most strains were 1:1 to 1:4. It is obvious that due to the variety of factors involved one should not necessarily anticipate correlations between the MICs determined in broth and agar dilution tests. It is well known that the composition of the medium, particularly the contents of metallic cations, phosphates, and some other salts, can affect the in vitro activity of some antimicrobial agents (1, 6, 18). This factor was not involved in our study, because both 7H10 agar and 7H12 broth contain the same seven salts incorporated into either of the two media prepared from 7H9 Middlebrook-Cohn base. Therefore, the differences in MICs in 7H10 agar and 7H12 broth dilution tests could have been caused by elements i, ii, and iii described above. The specific effect of these elements on the activity of different antimicrobial agents needs further clarification in experiments especially designed for such studies.

In accordance with our previous studies (7, 9–11) the MICs determined in 7H12 broth are more accurate and closer to the true MICs than those determined in agar medium, due to the shorter period of incubation required in broth, which diminishes degradation of the drugs; moreover, 7H12 broth does not contain significant amounts of substances that could absorb the drugs or change their potency for mycobacteria. Therefore, in the current study with seven antituberculosis drugs the major emphasis is made on MICs determined in 7H12 broth radiometrically. Our MIC definition, the lowest concentration inhibiting more than 99% of the bacterial population, agrees with that generally accepted in clinical microbiology. To determine this value radiometrically (in the BACTEC system) a comparison is made between the daily GI curves observed for cultures in drug-free medium and in vials containing doubling dilutions of a drug (Fig. 2). The MIC is the lowest concentration in the presence of which the daily increase in GI (ΔGI) and final GI reading are lower than that in the drug-free medium that initially contained 1% of the inoculum of the drug-containing vials.

Another method of analyzing radiometric readings is based on determination of the time in hours required to achieve a cumulative GI reading of 100 (T100 value) (13), but we did not use this method to analyze our data because we could not find the advantages in using graphic extrapolation of GI values rather than the actual readings.

Only clinical trials will show whether the clinical response to chemotherapy depends on the degree of susceptibility of the patient’s strain. In anticipation of such clinical trials, it is important to develop some tentative criteria for expressing quantitatively the differences in the degree of susceptibility of *M. avium* clinical isolates. We suggest four categories for this purpose depending on the broth-determined MIC values: susceptible, moderately susceptible, moderately resistant, and resistant. This approach will make it possible to analyze the clinical response to chemotherapy of different groups of patients, depending on the MICs of the administered drugs for the strain isolated from the patient before treatment. The first category, susceptible, is suggested on the basis of comparison with the MICs for the wild *M. tuberculosis* strains, but only clinical trials will show whether the presumably susceptible *M. avium* clinical isolates are really susceptible in regard to the patient’s response.
to chemotherapy, and whether chemotherapy will be less successful for the patients with presumably resistant or very resistant strains. If the patient's response to chemotherapy indeed depends on the degree of susceptibility of the clinical isolate to the drugs selected for chemotherapy, then the correlation between the clinical data and the actual MICs against the strain isolated from the patient will provide a firm basis for interpretation of the MICs. In the absence of such data, we are suggesting tentative interpretations (Table 1) as a necessary step for future studies. Taking into account the wide range of MICs of most drugs against M. avium strains, the categorization of the degree of susceptibility of the clinical isolate based on MIC will eventually provide a scientific basis for better selection of drugs for chemotherapy.

Unlike tuberculosis, it is impossible in the management of M. avium infection to detect the emergence of drug resistance during the course of chemotherapy if the drug susceptibility test is performed with critical concentrations only. There is hope that quantitative measurement may help detect changes in the degree of susceptibility of the bacterial population of the patient as a result of chemotherapy, which data can in turn be used to improve the management of the infection. An increase in MIC during the course of chemotherapy may be an indirect marker that the drug is indeed affecting the bacterial population (17). If the new isolate is in a higher susceptibility category than the strain isolated before treatment, this may show that the measurement of susceptibility by MIC during the course of treatment is a helpful tool in monitoring the chemotherapy of M. avium infection.

ACKNOWLEDGMENTS

This work was supported by Public Health Service contract 1-Al-72636 with the National Institute of Allergy and Infectious Diseases. I thank P. Lindholm-Levy and M. Flory for excellent technical assistance, L. Landskroner for artwork, N. Eig for editorial help, and C. J. Queen for the preparation of the manuscript.

LITERATURE CITED


