Activities of Antimicrobial Agents against Clinical Isolates of 
Mycobacterium haemophilum

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Mycobacterium haemophilum, first described in 1978, can cause severe infections of skin, respiratory tract,
bone, and other organs of immunocompromised patients. There is no standardized antimicrobial susceptibility
test, and for the 27 reported cases, a variety of test methods have been used. This paper reports the in vitro
test results for 17 isolates of M. haemophilum recovered from 12 patients in the New York City area. MICs of
16 antimicrobial agents were determined in microtiter trays containing Middlebrook 7H9 broth plus 60 μM
heparin, inoculated with 10⁶ CFU of the organism per ml and incubated at 30°C for 10 days. Ethambutol,
etionamide, tetracycline, cefoxitin, and trimethoprim-sulfamethoxazole were inactive against initial isolates
from the 12 patients. Isoniazid was weakly active with a MIC for 50% of strains tested (MIC₅₀) of 8 μg/ml and a
MIC₉₀ of >32 μg/ml. Three quinolones, ciprofloxacin, ofloxacin, and sparfloxacin, were moderately active
with MIC₅₀s of 2 to 4 μg/ml and MIC₉₀s of 4 to 8 μg/ml. Amikacin and cefazolin were active with MIC₅₀s
of 4 and 2 μg/ml, respectively. Clarithromycin was the most active macrolide with a MIC₅₀ of ≤0.25 μg/ml.
The MIC₉₀ of azithromycin was 8 μg/ml, and the MIC₉₀ of erythromycin was 4 μg/ml. The rifamycins were
active with a MIC₅₀ of 1 μg/ml for rifampin and one of ≤0.03 μg/ml for rifabutin. For a second isolate from
the skin of one patient and an isolate from an autopsy culture of the spleen of a second patient, MICs of
rifampin and rifabutin were >16 μg/ml, whereas initial isolates were inactivated by low concentrations of
the rifamycins. Both patients had been treated for several months with several antimicrobial agents, including a
rifamycin.

Mycobacterium haemophilum was first reported as a human pathogen in 1978 (19). Since then, 26 cases of
infection have been described (1, 2, 4–7, 9–18, 20, 21, 23). Patients were usually adults who were immunosuppressed
either after renal or bone marrow transplantation or with AIDS or lymphoma, or were children with apparently nor-
nal immune function. One patient had coronary bypass surgery (14). Clinical presentation usually included either
Cutaneous lesions or abscesses, septic arthritis, adenitis, pneumonia, and/or bacteremia. Cases have been reported
from the United States, Australia, Canada, France, Israel, and the United Kingdom. M. haemophilum is a slowly
Growing organism in vitro, and media for culture should contain an iron source such as hemin or ferric ammonium
citrate, and agar or broth should be incubated at 30°C for 6 weeks.

A cluster of 13 cases of M. haemophilum infection that occurred in the New York City metropolitan area in 1990 to
1991 was reported by the Centers for Disease Control (3). Four of the 13 cases (11) and one additional case in 1992
were seen at Memorial Sloan-Kettering Cancer Center (MSKCC) in two patients with AIDS, two patients who had
either aplastic anemia or leukemia and had received bone marrow transplants, and one patient with non-Hodgkins
lymphoma. No source of the cluster of cases in the New York City area was found; additional cases from the United
States, including more from New York City, have been

reported to the Centers for Disease Control; and the natural
habitat of M. haemophilum is still not known.

There is no standardized antimicrobial susceptibility test
for M. haemophilum. A variety of test methods using both
agar and broth media have been described in the case
reports. The purpose of the present study was to test a larger
number of isolates by one method and to identify agents that
might merit clinical evaluation. A modification of a microdil-
ution broth method developed for testing slow-growing
mycobacteria (22) was used to test 17 strains of M. haemoph-
ilum recovered from 12 patients with AIDS or neoplastic
disease in the New York City area. Successive isolates from
two patients with recurrent or progressive infection despite
aggressive therapy were tested for the possibility of devel-
oping resistance.

MATERIALS AND METHODS

Test organisms. Isolates of M. haemophilum were col-
lected from patients at MSKCC, Lenox Hill Hospital, St.
Vincent’s Hospital, and New York Hospital in New York
City; St. John’s Hospital in Yonkers, New York; and North
Shore Hospital in Manhasset, New York. Isolates were
identified as M. haemophilum when they were strongly
acid fast, had a growth requirement for hemin, grew at 30°C
but not at 37°C, and had a positive conventional biochemical
test only for the production of pyrazinamidase. The identity
of several of the isolates was confirmed at the Centers for
Disease Control, Atlanta, Ga.

Antimycobacterial agents. Analytical-grade samples of
ethambutol (Lederle Laboratories, Pearl River, N.Y.), cip-

crofloxacin (Miles Inc., New Haven, Conn.), ofloxacin (R. W.
Johnson Pharmaceutical Research, New Brunswick, N.J.),

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sparfloxacin (Parke-Davis Pharmaceutical Research Division, Ann Arbor, Mich.), clofazamine (CIBA-GEIGY, Basel, Switzerland), rifabutin (Adria Laboratories, Dublin, Ohio), azithromycin (Pfizer Central Research, Groton, Conn.), and clarithromycin (Abbott Laboratories, North Chicago, Ill.) were provided by the manufacturers. Isoniazid and ethionamide were purchased from Sigma Chemical Co., St. Louis, Mo. Rifampin (Marion Merrell Dow, Kansas City, Mo.), amikacin and ethromycin (Elkins-Sinn, Cherry Hill, N.J.), tetracycline (Lederle Laboratories), cefoxitin (Merck & Dohme, West Point, Pa.), and trimethoprim-sulfamethoxazole (Roche Laboratories, Nutley, N.J.) were purchased from the MSKCC pharmacy.

Stock solutions of azithromycin and clarithromycin were prepared in buffered saline (pH 7.4). Stock solutions of rifabutin and clofazamine were prepared in absolute ethanol. Ethionamide was dissolved in ethylene glycol. Sterile distilled water was used as the solvent for preparing stock solutions of the other agents. Stock solutions were stored at −20°C.

**Susceptibility testing procedure.** Susceptibility tests were performed with a microdilution broth method for slowly growing mycobacteria (22); hemin was added to the broth for the growth of *M. haemophilum*. Inocula were harvested from 2-week-old subcultures of the organism from chocolate agar plates (Becton-Dickinson, Cockeysville, Md.). A loopful of the organism was suspended in sterile saline, the tube was vortexed and centrifuged, and the supernatant was discarded. Suspensions were diluted with saline to the turbidity of a 0.5 McFarland nephelometry standard. A further 100-fold dilution in sterile 2x broth (see below) gave a final concentration of 2 x 10^6 CFU/ml as determined by quantitative cultures. This concentration facilitated microtiter endpoint determinations. Lower inocula of the organism delayed reading of the results by several days, and antimicrobial degradation became a concern.

Doubling dilutions of the antimycobacterial agents in sterile water were placed in wells of microtiter trays (0.1 ml of drug solution per well), and the wells were inoculated with equal volumes of the test strains suspended in 2x strength sterile broth. The broth, at 1x strength, consisted of complete Middlebrook 7H9 broth supplemented with glycerol and Middlebrook ADC enrichment per manufacturer's instructions (Difco Laboratories, Detroit, Mich.) plus 60 µM hemin (Eastman Kodak, Rochester, N.Y.). The broth was sterilized and cleared of precipitates by filtration through a 0.45-µm-pore-size filter, and wells were inoculated with 10^6 CFU of organism per ml. The final pH was approximately 7.0 for wells containing azithromycin and clarithromycin and 6.8 for the other wells. Plates were placed in a moist chamber to limit evaporation and were incubated for 10 days at 30°C. The minimum concentration of the drug that prevented visible growth of the test strains suspended in 2x strength sterile broth.

### RESULTS
Table 1 notes the sources of the isolates of *M. haemophilum*. Seventeen strains were recovered from 12 immunocompromised patients. The organisms were recovered ante-mortem from skin, lung, sputum, synovial fluid, and bone and at autopsy from a lymph node, adrenals, and the spleen of one patient. Strain numbers with the suffix "A" were the first isolates and were recovered before or shortly after therapy was initiated. Additional clinical and laboratory information regarding patients 1 to 4, 6 to 8, 11, and 12 has been published (3, 11, 23).

Final MICs were noted after the microtiter trays had been incubated at 30°C for 10 days. Prior to 10 days, a small amount of precipitate in some wells obscured interpretation of the results. The *M. tuberculosis* control strain was susceptible to all standard antimycobacterial agents. MIC results for the *M. haemophilum* reproducibility control strain did not vary by more than one doubling dilution of the antimicrobial agents in all tests.

| Patient | Hospital | Underlying disease | Strain | Site |
|---------|----------|-------------------|--------|------|
| 1       | MSKCC    | AIDS              | 1A     | Skin |
|         |          |                   | 1B     | Lymph node (autopsy) |
| 2       | MSKCC    | Aplastic anemia,  | 2A     | Lung |
|         |          | BMT               |        |      |
| 3       | MSKCC    | AIDS              | 3A     | Sputum |
| 4       | MSKCC    | Leukemia, BMT     | 4A     | Skin |
| 5       | MSKCC    | Lymphoma          | 5A     |      |
| 6       | Lenox Hill| AIDS              | 6A     | Bone |
| 7       | St. Vincent's| AIDS        | 7A     |      |
| 8       | New York | AIDS              | 8A     | Skin |
| 9       | New York | AIDS              | 9A     |      |
| 10      | New York | AIDS              | 10A    | Skin |
| 11      | New York | AIDS              | 11A    | Skin |
| 12      | St. John's| AIDS             | 12A    | Skin |
|         |          |                   | 12B    |      |

* BMT, bone marrow transplant.

**TABLE 2. In vitro activity of antimicrobial agents showing some activity against initially isolated strains of *M. haemophilum***

| Antimicrobial agent | MIC (µg/ml) |
|---------------------|-------------|
| Rifabutin           | ≤0.03-0.06  |
| Rifampin            | 0.5-2       |
| Clarithromycin      | ≤0.25-0.5   |
| Erythromycin        | 2-4         |
| Azithromycin        | 2-16        |
| Clofazamine         | 0.5-4       |
| Amikacin            | 2-8         |
| Ciprofloxacin       | 1-8         |
| Ofloxacin           | 2-8         |
| Sparfloxacin        | 0.5-4       |
| Isoniazid           | 4-32        |

* Ethambutol, ethionamide, tetracycline, cefoxitin, and trimethoprim-sulfamethoxazole were inactive against all strains.
variable MICs among the three agents. While the MIC₅₀ and MIC₉₀ were similar, the MIC of ciprofloxacin was lower than those of ofloxacin and sparfloxacin for 6 of 12 strains and higher for 2 of 12 strains. Amikacin and clofazamine were active against all strains. Clarithromycin was the most active macrolide, followed by erythromycin and azithromycin. Between the two rifamycins, rifabutin was more active than rifampin. All strains were inhibited by low concentrations of rifampin and very low concentrations of rifabutin.

Table 3 notes the MIC results for serial strains of Micrococcus haemophilum isolated from two patients (patients 1 and 12 in Table 1) whose infections progressed despite aggressive therapy. Both patients were severely immunosuppressed as a result of CD4 lymphocyte depletion due to human immunodeficiency virus infection, and Micrococcus haemophilum was their first serious opportunistic infection.

Patient 1 presented with skin lesions and received treatment with multiple agents including isoniazid, ethambutol, rifampin, doxycycline, ciprofloxacin, and amikacin. During the next 11 months, the patient improved but later developed disseminated Micrococcus haemophilum infection while therapy was stopped because of abnormal liver function tests. Growth of isolates recovered at autopsy from a lymph node (strain 1B) and the adrenals (strain 1C) was inhibited in vitro by low concentrations of the rifamycins, as was that of the initial isolate (strain 1A), while for an isolate from the spleen at autopsy (strain 1D), MICs of both rifampin and rifabutin were >16 μg/ml.

Patient 12 presented with a gluteal abscess that yielded positive acid-fast smears and was culture negative when conventional methods for mycobacterial culture were used. Subsequently, Micrococcus haemophilum was recovered from cheek lesions (strain 12A). The patient was initially treated with isoniazid, ethambutol, and rifampin; this was followed by several months of treatment with rifabutin, clofazamine, ciprofloxacin, and amikacin. The patient was also briefly treated with clarithromycin. Strain 12B was recovered from a specimen taken 18 months after the specimen that yielded strain 12A. Rifampin and rifabutin were inactive, and clofazamine and clarithromycin were only moderately active against strain 12B, whereas the four antimicrobial agents were very active against the first isolate, 12A.

**DISCUSSION**

Although there are no standardized methods for antimicrobial susceptibility testing of Micrococcus haemophilum, several of the case reports have included test results. The most detailed descriptions of test procedures used were noted in six papers reporting the results of 18 isolates. A variety of agar and broth methods were described in five papers (3, 7, 11, 13, 14), and a broth microdilution method was used for the sixth paper (20). Media were supplemented with hemin, and results were noted after 1 to 2 weeks. Not all strains were tested against the same antimicrobial agents; however, a summary of results indicates that amikacin, ciprofloxacin, and rifampin were usually active and doxycycline, ethambutol, ethionamide, isoniazid, and streptomycin were relatively inactive against strains tested.

The results in this paper generally agree with previous test results. The most active antimicrobial agents in the present study were the rifamycins, macrolides, and quinolones, clofazamine, and amikacin. Ethambutol, ethionamide, tetracycline, cefoxitin, and trimethoprim-sulfamethoxazole were inactive, and isoniazid showed some activity. Isoniazid may be more active than the in vitro test results indicate, since hemin, used as a broth supplement, can antagonize the in vitro activity of isoniazid (8). The new macrolides, clarithromycin and azithromycin, may even be more active than indicated. Although the pH of the test broth was approximately 7.0, macrolide activity has been shown to increase at a pH of 7.2 to 7.4. However, increasing the pH may slow the growth of Micrococcus haemophilum; thus, results may suggest inhibition that is actually not due to antimicrobial action. Trimethoprim-sulfamethoxazole may also be more active than these results indicate. Sulfonamide susceptibility has been defined as ≥80% inhibition of growth. Use of a high inoculum and an endpoint of no visible growth may have obscured some activity of the antimicrobial agent.

This is the first report of the emergence of resistance in Micrococcus haemophilum. In vitro growth of isolates from two patients was initially inhibited by low concentrations of the rifamycins. After the patients were treated with several antimicrobial agents, including a rifamycin, for prolonged periods, subsequent isolates were not inactivated by low concentrations of the rifamycins. Results indicate the need to culture another specimen from the patient if therapy appears to be failing and to test the new isolates against the appropriate antimicrobial agents.

**M. haemophilum** has a markedly different pattern of susceptibility to antimicrobial agents than do other mycobacterial species. Patients with Micrococcus haemophilum infection will receive optimal therapy only if there is prompt recognition of the causative agent. Recovery of the organism from clinical specimens requires the use of media supplemented with hemin or other iron sources and incubation of cultures at 30°C. Awareness of the clinical presentation and good communication with the clinical laboratory are essential for detection of this emerging pathogen.

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