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Mycobacterium haemophilum: Microbiology and Expanding Clinical and Geographic Spectra of Disease in Humans

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INTRODUCTION

New diseases caused by the nontuberculous mycobacteria have been reported in recent years. Organisms identified as human pathogens include newly recognized and identified mycobacterial species, as well as species that have been known for some time but whose virulence and spectrum of clinical presentations only recently have been appreciated. The sudden emergence and proliferation of disease caused by the nontuberculous mycobacteria can be attributed both to an increase in the number of immunocompromised patients and to improved technical capabilities of clinical laboratories.

Two newly emerged mycobacterial pathogens requiring special conditions for laboratory culture are *Mycobacterium genavense* and *Mycobacterium haemophilum*. The latter, which causes cutaneous, joint, or pulmonary infections in immunocompromised patients and lymphadenitis in children, is the subject of this review.

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HISTORICAL PERSPECTIVE

M. haemophilum was first described and named in 1978 in Israel by Sompolinsky, who recovered the organism from sub-

TABLE 1. Patient group or underlying disease associated with 64 reported cases of *M. haemophilum* infections in humans[a]

Patient group or underlying disease	No. of cases	Yr of first underlying report (reference)
Lymphoma	4	1978 (42)
Renal transplant recipients	8	1976, 1980 (17, 37, 50)
AIDS	29	1987 (33)
Children	9	1981 (16)
Bone marrow transplant recipients	5	1991 (11)
Rheumatoid arthritis	3	1994 (14)
Coronary artery bypass surgery	1	1991 (35)
Cardiac transplant recipient	2	1983, 1994 (12, 30)
Marrow hypoplasia	1	1994 (3)
Crohn's disease	1	This work
AIDS and renal transplant recipient	1	This work

[a] As of September 1995.

cutaneous lesions of a woman with Hodgkin's disease (42, 43) (Table 1).

Before that, the organism was possibly the cause of infections in which acid-fast bacilli (AFB) were visualized in specimens but cultures failed to yield an etiologic agent. For example, skin biopsy specimens from patients in Virginia (32) and the midwestern United States and southern Manitoba, Canada (19), contained AFB, but routine mycobacterial cultures remained negative.

In 1980, Dawson and Jennis retrospectively identified three isolates as *M. haemophilum* (17). The first, initially reported in a 1976 review of cutaneous complications of renal transplantation (50), was a nontuberculous mycobacterium that grew only on Lowenstein-Jensen agar slants enriched with ferric ammonium citrate or mycobactin and incubated at 30°C (50). The other two isolates, originally noted in a paper in 1979, were isolated from skin lesions from a renal transplant patient and a lymphoma patient (37). All three patients had similar clinical presentations caused by fastidious mycobacteria with comparable microbiological properties.

Although the first recognized infections caused by *M. haemophilum* were in immunocompromised patients, in 1981 Dawson et al. described a case of submandibular lymphadenitis due to *M. haemophilum* in an otherwise apparently healthy child (16). This report documented that infection could occur in persons who were not overtly immunocompromised.

Five additional cases of *M. haemophilum* infection in patients who had undergone renal transplantation were documented in the 1980s (9, 15, 21, 38, 41). In 1983, a patient who had received a heterotopic heart transplant was described as presenting with chronic osteomyelitis, septic arthritis, and skin lesions (12). The first case of infection in an AIDS patient was reported in 1987 with the recovery of the organism from the synovial fluid of a 32-year-old man who presented with skin nodules and septic arthritis (33). Two additional cases of infection in AIDS patients were reported in the 1980s (40). *M. haemophilum* infection in bone marrow transplant recipients was first reported in 1993 (27). Two cases were described: one patient presented with pneumonitis, and the second presented with subcutaneous nodules and bacteremia.

In the last few years, 38 additional cases of *M. haemophilum* infection have been reported: 24 patients with AIDS (6, 18, 22, 24, 27, 29, 31, 36, 44–46, 48, 56); 6 children with lymphadenitis (4, 48); 3 bone marrow transplant recipients (53); 2 cardiac transplant recipients (12, 30); and 1 patient each who had coronary artery bypass surgery (35), non-Hodgkin's lymphoma (5), rheumatoid arthritis (14), or marrow hypoplasia (3).

As the growth requirements of *M. haemophilum* and its pathogenic potential in susceptible persons are better recognized, the number of reported cases continues to increase. The organism is now known to cause cutaneous and subcutaneous infection, septic arthritis, osteomyelitis, and pneumonitis in immunocompromised patients and lymphadenitis in apparently immunocompetent children. Reports have originated from Australia, Canada, France, Israel, the United States, and the United Kingdom. However, although our understanding of infections caused by *M. haemophilum* in humans has increased considerably, the natural habitat and means of acquisition of the organism remain unknown.

HUMAN INFECTIONS

M. haemophilum infects a wide variety of patients and can present with an array of symptoms ranging from focal involvement to widespread disease. Details of 54 cases in the peer-reviewed literature are listed in Table 2, and information about 10 cases from Arizona reported for the first time in this review is noted in Table 3. For the purposes of this review, illustrative case presentations from Tables 2 and 3 are used to introduce the clinical and histopathologic characteristics of the various patient groups, as well as the therapeutic approaches and outcome.

Lymphoma

The original report of documented *M. haemophilum* (42) infection describes a 51-year-old woman receiving treatment for Hodgkin's disease, who presented with generalized subcutaneous abscesses (Table 2). She also complained of painful swelling over her left elbow and knee. A biopsy of the skin nodules showed minute necrotic foci in the lower dermis, surrounded by areas of infiltration with granulocytes, lymphocytes, monocytes, fusiform cells, and giant cells. Acid-fast bacteria were seen on Ziehl-Neelsen stains.

She was released from the hospital without being treated but presented 2 months later with enlarging skin nodules. Pus from the nodules yielded acid-fast bacilli which grew in culture at 30 but not 37°C and required hemoglobin or hemin for growth. Biochemical and nucleic acid studies confirmed that the organism was a new species, subsequently named *M. haemophilum*. She received streptomycin, ethambutol, isoniazid, and topical *p*-aminosalicylic acid, and after 6 months she was entirely free of skin lesions.

Renal Transplant Recipients

A 48-year-old man received a renal transplant in April 1981, but it was rejected (21) (Table 2). He received a second transplant in August 1983, and this was followed by administration of anti-lymphocyte serum, azathioprine, and prednisolone. In April 1984, he complained of pain in the left knee, and *M. haemophilum* was isolated from a purulent synovial aspirate. Initial treatment with isoniazid and ethambutol was unsuccessful and was followed by rifampin plus minocycline. A second aspirate in May 1984 again contained AFB. Treatment with minocycline alone was continued until October 1984, when the knee had healed.

AIDS

A 37-year-old man with AIDS and three prior episodes of *Pneumocystis carinii* pneumonia presented with a 3-week history of diffuse, tender, pruritic, nodular skin lesions in September 1990 (27) (Table 2). Biopsy specimens of the arm and thigh lesions contained numerous AFB. He was initially treated with

TABLE 2. Information in the peer-reviewed literature on 54 patients with *M. haemophilum* infection

Patient no. and group	Reference(s)	Age (yr)/sex[a]	Country	Clinical presentation	Culture source	Treatment[b]	Outcome
Lymphoma							
1	42	51/F	Israel	Skin lesions	Skin	EMB, INH, SM	Responded
2	17, 37	58/M	Australia	Skin lesions, septic arthritis	Skin, synovial fluid	EMB, INH, RIF	Resolved
3	5	74/M	United States	Soft tissue abscess	Abscess	CIP, CLR, EMB, INH, RIF	Resolved
Renal transplant							
4	17, 50	46/F	Australia	Skin lesions	Skin	NA[c]	NA
5	17, 37	55/F	Australia	Skin lesions, septic arthritis	Skin, synovial fluid	EMB, INH, RIF	Persisted
6	15	32/F	United States	Skin lesions	Skin	INH, RIF	Resolved
7	41	47/F	Australia	Toe and ankle wounds	Wound drainage	EMB, INH, RIF	Resolved
8	38	38/F	Australia	Skin lesions	Skin	DOX, SMX, SXT	Responded
9	9	48/M	France	Skin lesions	Skin	EMB, INH, MIN, RIF	Resolved
10	21	48/M	France	Septic arthritis	Synovial fluid	EMB, INH, MIN, RIF	Resolved
Healthy children							
11	16	1/M	Australia	Submandibular lymphadenitis	Lymph node	Excision	Resolved
12	48	3/F	Canada	Cervical lymphadenitis	Lymph node	Excision	Resolved
13	4	1/M	Australia	Perihilar lymphadenitis	Lymph node	ERY, INH, PZA, RIF, SXT	Resolved
14	4	1/NA	Australia	Perihilar lymphadenitis	Lymph node	Excision	Resolved
15	4	16 mo./NA	Australia	Cervical lymphadenitis	Lymph node	Excision	Resolved
16	4	19 mo./NA	Australia	Submandibular lymphadenitis	Lymph node	Excision	Resolved
17	4	17 mo./NA	Australia	Cervical lymphadenitis	Lymph node	Excision	Resolved
AIDS							
18	33	32/M	United States	Subcutaneous nodules, septic arthritis	Synovial fluid	EMB, ETA, INH, PZA, RIF	Persisted
19	40	34/M	United States	Subcutaneous nodules	Skin, blood	EMB, INH, RIF	Persisted
20	40	38/M	United States	Skin lesions	Skin	EMB, INH, RIF	Persisted
21	48	55/M	Canada	Soft tissue swelling	Skin	NA	Persisted
22	29	36/M	United States	Skin lesions, septic arthritis	Synovial fluid, blood	PAS, RIF	Responded
23	24	37/M	United Kingdom	Skin lesions	Skin	ERY, MIN, RIF	Resolved
24	18	44/M	United States	Skin lesions	Bone marrow, skin, sputum	AMK, CIP, CLO, EMB, INH, RIF	Responded, then relapsed
25	18	28/M	United States	Abdominal pain	Periaortic node	CIP, CLO, EMB, INH, RIF	Responded, then relapsed
26	18	34/M	United States	Thigh plaques	Skin	CIP, INH, PZA, RIF	Responded, then relapsed
27	6	35/M	United States	Subcutaneous nodules	Skin	CIP, CLO, CYC, EMB, INH, RIF	Responded
28	56	31/F	United States	Skin papules, septic arthritis	Skin, synovial fluid	AMK, CIP, CLO, EMB, INH, PZA, RIF	Responded
29	27, 46, 56	37/M	United States	Skin lesions, septic arthritis	Skin, blood	AMK, CIP, CLO, DOX, EMB, INH RIF	Responded
30	27, 46	37/M	United States	Skin lesions, pneumonia	Skin, sputum	AMK, CIP, DOX, EMB, INH, RIF	Responded, then relapsed
31	46	32/F	United States	Skin lesions	Skin, bone	AMK, CIP, CLR, EMB, INH, MIN, RIF	Responded, then relapsed
32	46	38/M	United States	Skin lesions	Skin, bone	AMK, CIP, CLO, EMB, INH, RIF	Resolved
33	46	30/M	United States	Skin lesions	Skin, bone	AMK, CLR, CLO, DIC, EMB, INH, RIF	Responded, then relapsed
34	46	51/M	United States	Bronchitis	Sputum, bone	CIP, DOX, EMB, INH, PZA, RIF	Resolved
35	46	29/M	United States	Arthralgia	Synovial fluid	AMK, CIP, CLO, DOX, RIF	Improved
36	46	38/M	United States	Skin lesions	Skin	CIP, EMB, INH, RIF, SM	Persisted
37	46	31/M	United States	Sinusitis, skin lesions	Skin	AMK, CIP, DOX, EMB, MIN, RIF	Responded
38	46	31/F	United States	Skin lesions	Skin	CIP, CLO, EMB, RIF	Resolved

Continued on following page

TABLE 2—*Continued*

Patient no. and group	Reference(s)	Age (yr)/sex[a]	Country	Clinical presentation	Culture source	Treatment[b]	Outcome
39	44	41/M	United States	Skin lesions, arthritis	Elbow, sinus tract	INH, PZA, RIF	Resolved
40	36	40/M	United States	Skin lesions	Skin	INH, PZA, RIF, excision	Resolved
41	22	31/F	United States	Osteomyelitis, subcutaneous nodules	Skin	MIN, RIF	Responded
42	45	43/M	Australia	Osteomyelitis	Tissue	AMK, CIP, DOX, RIF	Responded
43	31	32/F	United States	Skin lesion	Skin	AMK, CIP, CLR, CLO, EMB, RIF	Responded
44	31	41/M	United States	Skin lesion	Skin	CIP, CLR, CLO, EMB, INH, RIF	Responded
Bone marrow transplant recipients							
45	27, 46	27/M	United States	Pneumonitis	Bronchial wash, lung, sputum	EMB, INH, PZA, RIF, SM	Died without response
46	27, 46	30/F	United States	Subcutaneous nodules	Skin, blood	CIP, DOX, EMB, INH, RIF	Responded
47	53	29/F	United States	Subcutaneous cyst	Skin	CIP, CLR, RIB, RIF	Responded
48	53	28/F	United States	Subcutaneous nodules	Skin	CIP, CLR, RIF	Responded
49	53	42/M	United States	Pneumonitis	Lung	AMK, CIP, CLR, EMB, INH, PZA	Died
Rheumatoid arthritis							
50	14	65/M	United States	Skin lesions	Skin	CIP, CLR, ERY, RIF	Responded
Coronary artery bypass surgery							
51	35	65/F	United States	Subcutaneous nodules	Skin	CIP, DOX, MIN	Resolved
Cardiac transplant recipients							
52	12	NA	Southern Africa	Osteomyelitis	NA	NA	Died
53	30	62/M	United States	Skin lesions	Skin	CLR, EMB, INH, OFL	Resolved
Marrow hypoplasia							
54	3	85/F	Australia	Skin lesions	Skin	INH, PZA, RIF	Responded

[a] F, female; M, male.
[b] AMK, amikacin; CIP, ciprofloxacin; CLO, clofazamine; CLR, clarithromycin; CYC, cycloserine; DIC, dicloxacillin; DOX, doxycycline; ERY, erythromycin; EMB, ethambutol; ETA, ethionamide; INH, isoniazid; MIN, minocycline; OFL, ofloxacin; PAS, *p*-aminosalicylic acid; PZA, pyrazinamide; RIB, rifabutin; RIF, rifampin; SM, streptomycin; SMX, sulfamethoxazole; SXT, sulfamethoxazole-trimethoprim.
[c] NA, not available.

isoniazid, ethambutol, and rifampin, but when *M. haemophilum* was recovered, the therapy was modified to doxycycline, rifampin, and amikacin. The patient showed marked regression of the cutaneous lesions for 10 months.

In June 1991, new lesions developed when antibiotics were withheld for idiopathic hepatitis. The lesions responded to reinstitution of rifampin and amikacin treatment with the addition of ciprofloxacin. However, in August 1991, he developed bilateral pneumonia, and *M. haemophilum* was isolated repeatedly from his sputum. With aggressive intravenous multidrug therapy, his pulmonary infiltrates improved but did not disappear; his sputum became negative for *M. haemophilum.*

He experienced progressive respiratory failure and died. Autopsy revealed pulmonary and esophagael lymphoma. *M. haemophilum* was recovered from a lymph node, the adrenal gland, and the spleen. The isolate now demonstrated resistance to the rifamycins (7).

Children

A 2-year-old girl presented with a 6-week history of unilateral swelling adjacent to the parotid gland (patient 4, Table 3). Initial evaluation included a positive tuberculin skin test but a negative chest roentgenogram. A physical examination revealed several fluctuant masses between 2 and 5 cm in size located between the mandible and the parotid gland (Fig. 1). The skin over one of the masses was erythematous and atrophic. The patient underwent excision, drainage, and curettage. Microscopy showed extensive necrosis, granulation tissue, areas of increased vascularity, and chronic inflammation, but no identifiable granulomas. Fluorochrome stains were positive for small numbers of AFB, and *M. haemophilum* was recovered on culture.

She did well until 3 weeks after the operation, when lesions reappeared. They were again debrided and excised. Histologic examination of the purulent debris and node showed necrotizing granulomatous inflammation. The granulomas were poorly formed but contained multinucleated giant cells (Fig. 2). Although the material was microscopically devoid of organisms, it yielded *M. haemophilum* on culture. The patient did well without specific antimycobacterial therapy, with no recurrence 5 years later.

Bone Marrow Transplant Recipients

A 28-year-old woman with acute promyelocytic leukemia in remission received a T-cell-depleted allogeneic bone marrow transplant from her HLA-matched sister (53) (Table 2). She

TABLE 3. Information about 10 cases of *M. haemophilum* infection from Arizona, previously unreported

Patient no.	Yr	Age (yr)/sex	Underlying condition(s)[a]	Clinical presentation and/or source of isolates[a]	Therapy and outcome[a]
1	1984	30/female	Initial: kidney transplant Subseq: AIDS	Initial: skin lesions, septic arthritis, osteomyelitis (hand) Subseq: pulmonary (sputum)	Initial: isoniazid, rifampin, ethambutol (resolution of lesions) Subseq: minocycline Died
2	1987	66/female	Rheumatoid arthritis (corticosteroid maintenance)	Initial: synovial fluid (hip) Subseq: recurrence as blind synovial pouch in gluteus (6 yr later)	Initial: doxycycline, rifampin (disappearance of symptoms); Subseq: excision; doxycycline, rifampin No recurrence
3	1989	8/female	None noted	Cervical lymphadenopathy	Excision only; no recurrence 6 yr later
4	1990	2/female	None noted	Initial: submandibular lymphadenopathy Subseq: recurrence after 6 wk	Initial: excision (no medication) Subseq: repeat excision only No recurrence 6 yr later
5	1990	77/male	T-cell lymphoma	Initial: synovial involvement with osteomyelitis (hand) Subseq: relapse 1 yr later	Initial: curettage only Subseq: NA Died of complications of lymphoma 2 months after second presentation
6	1992	56/male	AIDS	Synovial involvement (ankle)	Therapy: NA Died 2 months after initial presentation
7	1992	45/female	Renal transplant (corticosteroid maintenance)	Skin lesions (hand), synovial involvement (finger)	Ciprofloxacin, rifabutin Recovered (no recurrence 2.5 yr later)
8	1992	49/female	Rheumatoid arthritis (corticosteroid maintenance)	Cervical lymphadenopathy (bilateral)	Excision of one lesion; clarithromycin, trimethoprim-sulfamethoxazole Recovered to date
9	1993	33/male	AIDS, $CD4^+$ counts 27–36	Initial: synovial involvement (knee), blood X2 Subseq: relapse in knee, osteomyelitis, multiple skin lesions	Initial: ethambutol, clarithromycin, ciprofloxacin Subseq: same plus amikacin Died of AIDS complications
10	1994	39/female	Crohn's disease (corticosteroid maintenance)	Skin lesions (lower leg)	Amikacin for 1 week; minocycline No recurrence to date

[a] NA, not available; Subseq, subsequent.

received graft rejection prophylaxis with antithymocyte globulin and methylprednisolone for 2 weeks followed by a rapid taper. Her course was complicated by presumed grade I graft-versus-host skin disease, which was treated with topical corticosteroids. Five months after transplantation, she presented with a 1-month history of an erythematous nodule on her left upper thigh. She also had several palpable subcutaneous right upper thigh nodules on physical examination. A biopsy specimen of the initial nodule contained AFB as shown by direct staining, and *M. haemophilum* grew in culture.

The patient was given rifampin, ciprofloxacin, and clarithromycin. When in vitro susceptibility studies (7) showed a 10-fold-lower MIC of rifabutin than of other agents, rifabutin replaced rifampin in the therapeutic regimen. After 11 months of therapy, the patient presented with acute anterior uveitis attributed to rifabutin. All antimycobacterial therapy was discontinued, and her ocular complaints resolved completely. She remains free of disease more than 2 years after cessation of therapy.

Rheumatoid Arthritis

A 49-year-old woman, receiving chronic methotrexate therapy for rheumatoid arthritis, presented with an enlarging lymph node in the posterior cervical region (patient 8, Table 3). The area was slightly tender to palpation. Creamy yellow pus was drained from the fluctuant mass, and routine bacterial cultures were negative. She was given a 5-day course of azithromycin. After drainage, the mass persisted as a small nodule.

The patient presented a year later with an enlarging mass in a submandibular lymph node. At surgical excision, the node was noted to be fibrotic and markedly adherent to deep muscle and tissue and to have a suppurated central cavity containing purulent material. Culture of the excised node yielded *M. haemophilum.*

The patient received clarithromycin and trimethoprim-sulfamethoxazole. Her lymphadenitis subsided over a 2-month period, and she recovered within 4 months, at which time her antimicrobial therapy was stopped. She has had no recurrences 5 months after cessation of therapy, although she continues to take methotrexate.

Coronary Artery Bypass Surgery

A 65-year-old woman developed scaly erythematous papular eruptions on her right forearm several months after coronary artery bypass surgery (35) (Table 2). She had not received immunosuppressive therapy, but her human immunodeficiency

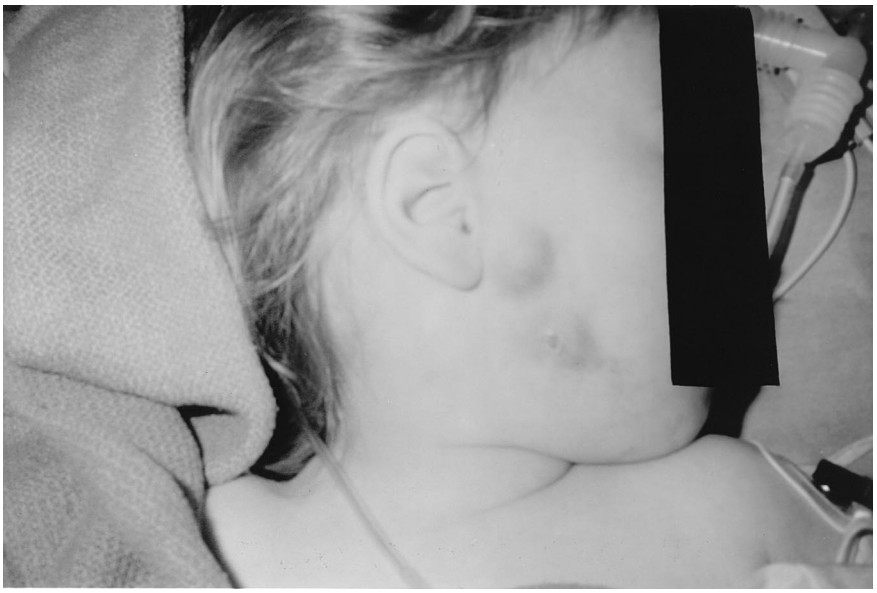

FIG. 1. Suppurating cutaneous lesions after a relapse of lymphadenitis in an otherwise seemingly normal child (patient 4, Table 3).

virus status was unknown. Biopsy specimens of the lesions contained epithelioid granulomas with multinucleated giant cells and a few AFB that were identified as *M. haemophilum*. She received ciprofloxacin, doxycycline, and minocycline; this led to complete resolution of the lesions.

Cardiac Transplant Recipient

A 62-year-old man underwent heart transplantation in May 1990, after which he received cyclosporine, prednisone, and azathioprine (30) (Table 2). He remained healthy until Feb-

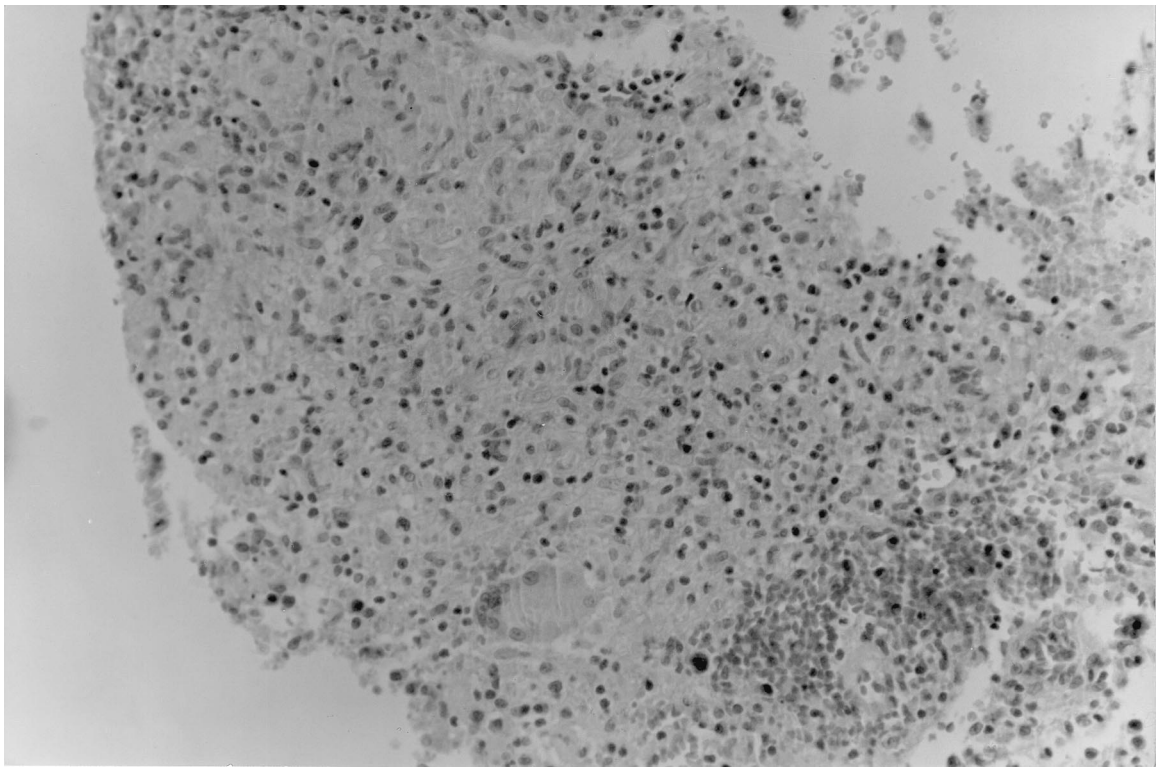

FIG. 2. Granulomatous histopathologic findings in the excised material from the patient in Fig. 1 (patient 4, Table 3). Granulomas are poorly formed but contain multinucleated giant cells.

ruary 1992, when he developed fever, dyspnea, and a dry cough. Bronchoscopic specimens were positive for cytomegalovirus and *P. carinii*. One month later, he presented with a 3-month history of a plaque on his left arm. A second plaque developed on his forehead, and biopsy specimens of both lesions were AFB smear positive and culture positive for *M. haemophilum*. He was given clarithromycin, isoniazid, ofloxacin, and ethambutol, and the lesions resolved within 10 weeks.

Marrow Hypoplasia

An 85-year-old woman with marrow hypoplasia was admitted with a 3-week history of diffuse joint pains and a painful skin eruption over her lower limbs (3) (Table 2). Examination revealed acute polyarthropathy and skin lesions consistent with erythema nodosum. Bilateral carpal tunnel syndrome was also noted. Histopathologic examination of a left-knee aspirate revealed lymphocyte predominance and no organisms. She was readmitted 3 weeks later following several falls. A right fourth nerve palsy and nystagmus were present. Multiple tender violaceous nodules were noted over her legs and upper arms. Biopsy specimens of a skin nodule contained AFB, later identified as *M. haemophilum*. Her skin lesions regressed after treatment with rifampin, isoniazid, and pyrazinamide for 10 weeks.

Crohn's Disease

A 39-year-old woman receiving corticosteroids for Crohn's disease developed skin lesions on her lower leg (patient 10, Table 3). *M. haemophilum* was cultured from a skin biopsy specimen, and the patient was treated successfully with amikacin and minocycline.

Both Renal Transplant and AIDS

A 30-year-old woman who had received a renal transplant 2 years earlier was admitted for evaluation of multiple, erythematous, tender, subcutaneous nodules of 6 weeks duration on her extremities (patient 1, Table 3). She had first noticed swelling and erythema of the left fourth finger (Fig. 3) and later noticed approximately 10 cutaneous lesions scattered over the lower and upper extremities (Fig. 4), including a lesion involving the right knee. A roentgenogram of the involved finger suggested osteomyelitis. On presentation, her medications included prednisone and azathioprine. Serosanguinous aspirates from the knee lesion revealed numerous AFB, and mycobacterial cultures were positive for *M. haemophilum*. The patient was given isoniazid, rifampin, and ethambutol until the skin lesions began to resolve, at which time she received minocycline alone. No alterations were made in her immunosuppressive therapy.

Her condition remained stable for 10 months, until she presented with progressively worsening fever, shortness of breath, a nonproductive cough, and right-sided pleuritic chest pain. A chest roentgenogram revealed bilateral infiltrates in a diffuse reticular nodular pattern. Skin lesions had not reappeared. Methenamine silver and auramine stains of bronchoscopically collected pulmonary secretions revealed *P. carinii* and AFB, respectively, and the culture was positive for *M. haemophilum*. She received trimethoprim-sulfamethoxazole and minocycline. Infection with HIV was now diagnosed. The patient did well for several months, but her condition later deteriorated and she died of other complications of AIDS.

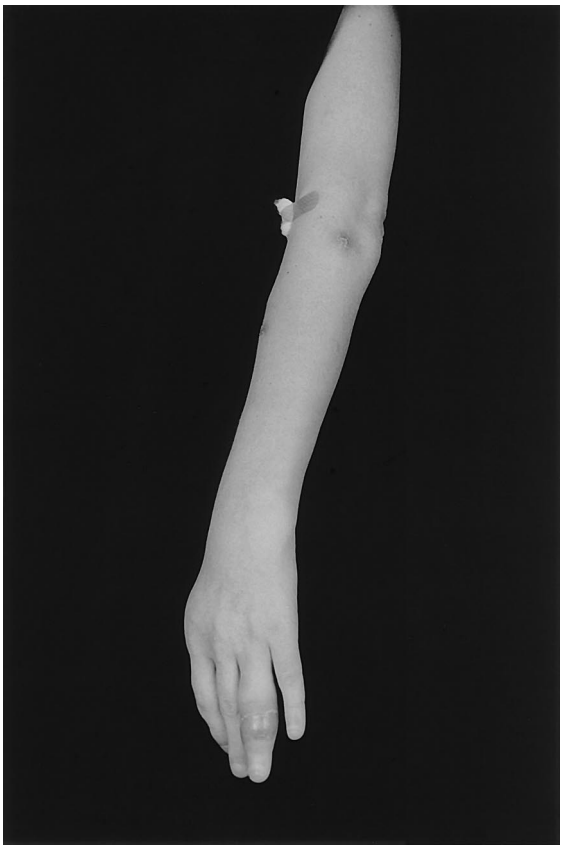

FIG. 3. Cutaneous lesions near the elbow and over the joint of the fourth digit of a renal transplant recipient. The synovial tissue and underlying bone were involved (patient 1, Table 3).

CLINICAL AND HISTOPATHOLOGIC CHARACTERISTICS

Of the 64 cases of *M. haemophilum* infection, 53 have been reported in immunocompromised adults (ages 27 to 85 years), 9 have been reported in children (ages 1 to 8 years), and 2 have been reported in adults whose risk factors were not known.

Immunocompromised Adults

The most common initial clinical presentation in adults is cutaneous lesions including nodules, cysts, and papules. The cutaneous lesions may vary in appearance. They may resemble scaly plaques or become erythematous, nodular, or ulcerated. Cellulitis and erythema have also been reported (29, 38, 40). The typical evolution of skin lesions involves progression from papules to pustules, commonly painless at first but culminating in deep ulcers that are potentially very painful. Although lesions may remain localized, they are usually diffuse and are most often found on the extremities, especially over joints. The distribution of lesions does not tend to follow the lymphatics, and the lesions are not sporotrichoid in appearance (46). Somewhat less common is septic arthritis with or without osteomyelitis. Pneumonitis is rare and may appear as the initial sign of infection (patients 45 and 49, Table 2) or may occur after diagnosis of cutaneous disease (patient 30, Table 2; patient 1, Table 3). Some infections are associated with bacteremia (patients 19, 22, 29, and 46, Table 2; patient 9, Table 3).

Microscopic examination of biopsy specimens and aspirates of *M. haemophilum* lesions reveals granulomatous panniculitis

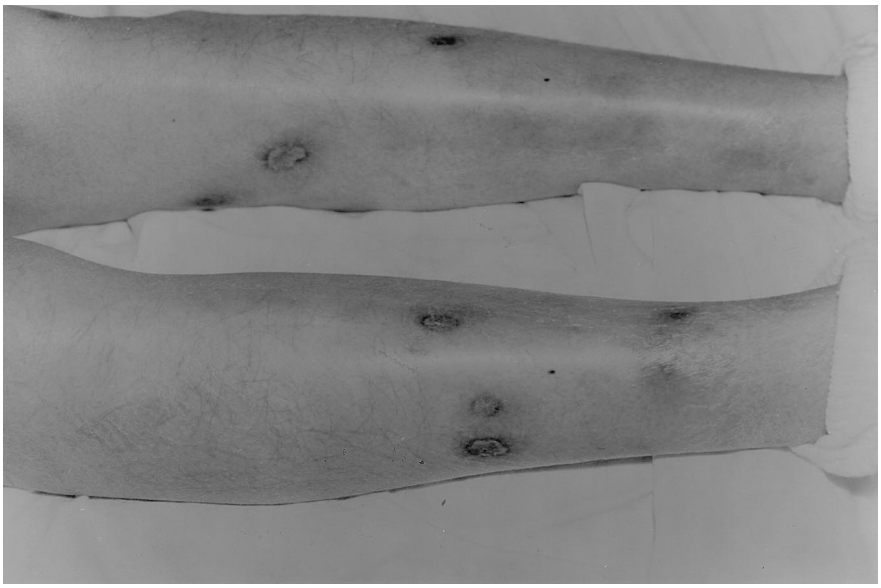

FIG. 4. Ulcerative lesions on the legs of the renal transplant patient in Fig. 3 (patient 1, Table 3).

(cutaneous disease) and granulomas with or without caseation (15, 29). Poorly formed granulomas are commonly seen in patients with AIDS (29, 40). Lesions may also reveal neutrophilic or mixed cellular infiltrates, including granulocytes, lymphocytes, monocytes, few multinucleated giant cells or Langhans' giant cells with or without foci of necrosis, and, less commonly, foamy macrophages (18, 32, 40, 42). The majority of lesions reveal few to numerous AFB, which are usually large and may be pleomorphic or curved. The organisms may occur singly or in clusters of cord-like formation.

Children

Lymph node involvement in children is primarily in the submandibular and cervical region and less commonly in the perihilar region (4, 16, 48, 54). Nodes vary in size, shape, firmness, and fluctuance and are usually unilateral. They may enlarge over several weeks to months before medical attention is sought. Children seldom complain of other symptoms, although slight fever may be present. The leukocyte count is within normal limits or slightly elevated (4, 16). Children with lymphadenitis caused by *M. haemophilum* may show a positive reaction with the purified protein derivative of *M. tuberculosis*, which may result in diagnostic confusion (16, 48).

THERAPY AND OUTCOME

Immunocompromised Adults

The outcome of treatment in adults appears to be greatly influenced by the ability to reverse underlying immunosuppression. Since this is frequently not clinically feasible, antimicrobial therapy is required to resolve the infection. Antimicrobial combinations reported to contribute to clinical response include rifampin with either isoniazid alone (15) or with ethambutol (41); or rifampin in combination with minocycline (9, 21), *p*-aminosalicylic acid (29), or erythromycin (24). Recently, three bone marrow transplant patients with cutaneous disease were successfully treated with antimicrobial regimens that included ciprofloxacin, clarithromycin, and rifampin (53). The drugs were chosen on the basis of results of a microdilution susceptibility test (34). Single agents such as trimethoprim-sulfamethoxazole, minocycline, erythromycin, and ciprofloxacin have been reported to have some efficacy (4, 21, 35, 38). Other agents used alone or in various combinations include pyrazinamide, streptomycin, ethionamide, cycloserine, amikacin, doxycycline, clarithromycin, clofazimine, clotrimoxazole, pyridoxine, dapsone, and rifabutin.

It is also unclear if single or combination therapy is required. Recent data (7) suggest that resistance can develop while the patient is undergoing therapy; therefore, at least two effective agents should be used. Patients with irreversible immunosuppression will probably require maintenance therapy for months to years, if not for the rest of their lives. For example, infection in patients with AIDS may persist or relapse even when the patients are taking agents shown to be effective in vitro (33, 40). Other patient populations have had relatively indolent clinical courses associated with complete resolution or nonprogression with or without specific antimicrobial therapy for *M. haemophilum.*

Children

Children with localized lymphadenitis do well with excision of the infected tissue alone. Total excision of affected lymph nodes is superior to incision and drainage, since the latter is associated with increased recurrence, chronic drainage, and possible scarring (16, 54). The role of antimicrobial therapy alone is uncertain, although no response was noted in two patients who were initially treated with erythromycin (4).

EPIDEMIOLOGY

We presently do not know the natural habitat of *M. haemophilum* or how the organism is spread to humans. The sporadic nature of the disease and the distinctive growth requirements of *M. haemophilum* have contributed to the lack of recognition of this bacterium as a cause of human morbidity, making the actual incidence of infection unknown.

The geographic distribution of reported *M. haemophilum* cases suggests that this bacterium is ubiquitous. The ability of the organism to grow over a wide range of pHs (5.4 to 7.4)

supports its potential for existence in diverse environments (38). An earlier observation that infections seemed clustered in cities near large bodies of water such as oceans or large lakes (6) is not corroborated by the new cases from Arizona presented in this review. Also, there are no reports of recovery of *M. haemophilum* from water or aquatic sources, although early culture methods may not have been adequate for recovery of this organism.

Opportunities to identify a source of infection arose when two cases occurred in the same hospital in France (21) and a cluster of cases occurred in New York City (27). In the first instance, two patients who had occupied neighboring beds and shared a bathroom in a renal dialysis unit developed *M. haemophilum* infection. One patient had just received a renal graft, and the other patient was receiving hemodialysis. The isolates were not compared by molecular fingerprinting to prove them identical; therefore, nosocomial person-to-person transmission could not be documented. Furthermore, *M. haemophilum* was not detected in water samples from the nephrology and hemodialysis units.

M. haemophilum has emerged as a significant pathogen in the New York City metropolitan area. Twenty-two cases from New York City have now been described in the literature (11, 27, 46, 53, 56) and at least 30 additional cases are known (25). Nosocomial transmission was suspected when two patients with AIDS and two bone marrow transplant recipients presented with *M. haemophilum* infection at Memorial Sloan-Kettering Cancer Center between August 1990 and March 1991 (27). The patients had several opportunities for contact either as inpatients or when visiting the outpatient clinics. These four cases and nine other cases from New York City hospitals were included in a case-control study conducted by the Centers for Disease Control and Prevention and personnel from the hospitals (46). No common epidemiologic elements between the cases was found.

Another evaluation considered the role of aerosolized pentamidine (53). There have now been six cases of *M. haemophilum* infection in bone marrow transplant patients at Memorial Sloan-Kettering Cancer Center (25, 27, 53), with two of the patients presenting with *M. haemophilum* pneumonia. Aerosolized pentamidine was a potential source of the organism, since all six patients had received prophylaxis with the aqueous solution used to prevent *P. carinii* pneumonia and this therapy was used in patients with AIDS at the same center. Culture for *M. haemophilum* in reconstituted pentamidine and water samples before and after respiratory nebulization failed to yield the organism. In addition, numerous other patients receiving aerosolized pentamidine have not contracted *M. haemophilum* infection.

A further attempt was made to elucidate the epidemiology of *M. haemophilum* infection by using restriction fragment length polymorphism analysis of 43 clinical isolates from 28 patients, most of whom were from New York City (28). Six distinct patterns were observed. Two patterns, types 1 and 2, accounted for 91% of the infections in patients from the New York City area. Of note, the isolates from Memorial Sloan-Kettering Cancer Center identified from two AIDS patients were type 1 and the five isolates tested from bone marrow transplant recipients were type 2, suggesting an epidemiologic association. Two isolates from Arizona had identical patterns and were distinct from the New York isolates, while the type strain from Israel had yet another pattern.

The epidemiologic diversity of *M. haemophilum* was also demonstrated by pulsed-field gel electrophoresis (55). Six patterns were observed. Of 16 isolates collected in the New York City metropolitan area, 12 had the same pattern, including all 6 isolates submitted from one hospital. Two different patterns were seen among the other four isolates, while individual isolates from upstate New York, Florida, and Texas had unique patterns.

As noted above, the epidemiology of *M. haemophilum* remains poorly defined. The 10 new cases from Arizona, presented in this review, appear sporadic and without any discernible common risk factors for acquisition of the organism. That the organism should be present in the environment, either animate or inanimate, seems likely. However, attempts at its recovery through environmental sampling have been unsuccessful. As diagnostic methods for *M. haemophilum* improve, the epidemiology, ecology, and mode of transmission of the organism should be elucidated.

MICROBIOLOGY

M. haemophilum (ATCC 29548), or the "blood-loving" mycobacterium, is an aerobic, slowly growing AFB (42). It has characteristics similar to other pathogenic mycobacteria. The optimal temperature for in vitro growth is 30 to 32°C, which is similar to that for *Mycobacterium marinum* and *Mycobacterium ulcerans*, other cutaneous pathogens. Colonies of *M. haemophilum* often show cord formation similar to that seen with *Mycobacterium tuberculosis*. The organism is biochemically inert, similar to the *Mycobacterium avium* complex. It has a specific phenolic glycolipid antigen and fatty acid pattern similar to that of *Mycobacterium leprae*. *M. haemophilum* also has its own distinct features including a unique requirement for supplementation of growth media with ferric iron-containing compounds such as ferric ammonium citrate or hemin.

Antigenic and Genetic Analysis

M. haemophilum DNA has relatively low G+C content, resembling the G+C contents of *M. marinum* and *M. ulcerans* (42, 43).

Species-specific antisera have been prepared against *M. haemophilum* and show little if any cross-reaction with other mycobacterial species (16, 17, 43, 51). Seroagglutination studies with antisera against *M. haemophilum* isolates recovered in Australia and Israel suggest a single serotype for the species (52). Antibody-coated protein A agglutination studies suggest that *M. haemophilum* shares some surface antigens with *M. marinum* and *M. ulcerans*. However, in conventional agglutination studies, anti-*M. haemophilum* antisera seemed to contain strong specific antibody (high homologous titers) and much weaker antibody to other mycobacterial species (low heterologous titers [43]).

Chromatographic Analysis

Chromatographic studies have shown that different isolates of *M. haemophilum* have common properties and that the organism is a distinct species. In two studies, all strains of *M. haemophilum* gave the same mycolic acid patterns when analyzed by thin-layer chromatography (13, 39). In one study, gas chromatography was used to show that a unique fatty acid profile supported the validity of classifying *M. haemophilum* as a distinct species (39). The fatty acid docosanoic acid is found in abundant quantities in *M. haemophilum* and *M. leprae*. This similarity between the two species is intriguing because *M. haemophilum* has also been shown to possess a specific phenolic glycolipid antigen that closely resembles the corresponding lipid of *M. leprae* (8).

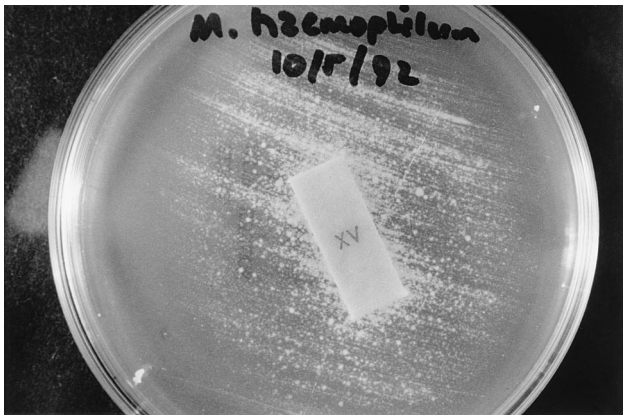

FIG. 5. Satellite growth of *M. haemophilum* around a paper strip containing hemin (X factor) placed on the surface of a Middlebrook 7H10 agar plate.

Culture Requirements

M. haemophilum is slowly growing and iron dependent and has an optimal growth temperature between 30 and 32°C. Growth at 37°C is nonexistent or poor, although an increased partial pressure of CO_2 may permit scanty growth. The original reports on *M. haemophilum* speculated that the temperature restriction is not caused by a temperature-sensitive enzyme, since temperature-resistant mutants have not been identified and enrichment of culture media does not allow growth at higher temperatures (42, 43). The optimal growth temperature of 32°C falls within the normal temperature of human skin and may explain the preference of *M. haemophilum* for cooler sites of the body.

As mentioned above, *M. haemophilum* is distinct from other mycobacteria in its requirement for ferric ions in the form of hemin, hemoglobin, or ferric ammonium citrate. Unlike more fastidious mycobacteria, the organism does not seem dependent on supplementation of media with mycobactin. It is unable to grow or grows poorly on routine media such as Lowenstein-Jensen, Middlebrook 7H9 and 7H10, and other media without adequate lysed erythrocytes. Several media have been used to recover the organism on primary isolation from specimens. Solid media include Lowenstein-Jensen medium with ferric ammonium citrate (17), Middlebrook 7H10 or 7H11 agar with hemin (29, 33, 38), Mueller-Hinton medium with Fildes supplement (9), chocolate agar (41), lysed horse blood agar (41), Columbia colistin-nalidixic acid agar with 5% sheep erythrocytes (21), and Centers for Disease Control and Prevention anaerobic blood agar (29). Broth media include BACTEC 7H12 broth supplemented with either 60 μM hemin, 1.5% ferric ammonium citrate, or 5% (vol/vol) hemolyzed fresh human blood (13). Not all media have equal capability for stimulating the growth of *M. haemophilum* (34), and success or speed to recovery may, to some degree, also depend on other growth conditions, including the CO_2 concentration.

A practical method for culture is to inoculate Middlebrook 7H10 (or similar medium) agar plates with the clinical specimen and to place a hemin-containing paper strip (X factor) on the agar surface (Fig. 5) (49). *M. haemophilum* will form satellite colonies near the paper strip to provide, in conjunction with an acid-fast stain, a presumptive identification of the organism. A potential problem with this method is the need for an adequate concentration of organisms in the primary inoculum; specimens with small numbers of organisms may not be successfully cultured.

Blood from three of four patients with *M. haemophilum* bacteremia (27, 29, 40) was collected into the Isolator lysis-centrifugation system (Wampole Laboratories, Cranbury, N.J.). In the laboratory, the Isolator concentrate was inoculated either onto Middlebrook 7H10 or 7H11 agar or into BACTEC 12B broth. Blood from a venipuncture of the fourth patient was inoculated directly into a BACTEC 13A blood culture bottle (56).

Identification

Microscopy. *M. haemophilum* does not stain by the Gram procedure but stains strongly acid fast by the Ziehl-Neelsen, Kinyoun, and fluorochrome acid-fast staining methods (42, 51). In culture, the organisms characteristically grow as short rods either singly or in clumps and occasionally curve. They range from 0.3 to 0.5 μm wide and from 1.2 to 2.2 μm long. Longer, thread-like bacilli may be seen among a population of predominantly short rods (42).

Culture. With appropriately supplemented primary culture media incubated at 30 to 32°C in 5 to 10% CO_2, colonies of *M. haemophilum* become visible within 2 to 3 weeks. Growth may occur several weeks later, however, especially if strong decontamination procedures are used when the specimen is processed (16, 29). Growth from subcultures is usually visible after incubation of the medium for 7 to 10 days, but it may be recognized in as little as 2 to 5 days (29, 38, 41, 42). Colonies are usually buff colored, rough, and nonpigmented, although both rough and smooth variants may appear together. Occasionally, colonies are eugonic, easily removed from the agar surface, and readily emulsified; they may also resemble colonies of *Mycobacterium fortuitum* more than colonies of *M. tuberculosis* (37).

Presumptive identification. *M. haemophilum* can be presumptively identified by its requirement for iron supplementation and by its comparatively good growth at 30 to 32°C and poor growth at 37°C. These characteristics are easily discernible in primary cultures or upon subculture. Rapid documentation may be made with BACTEC 7H12 radiometric broth (13) or by implementation of the simple commercial hemin disk or strip (49), as mentioned above.

Biochemical studies. Similar to *M. avium* and *M. intracellulare*, *M. haemophilum* is inert in most standard biochemical tests used to identify mycobacteria (Table 4). It is also, however, unable to produce either catalase or tellurite reductase. Although it resembles *M. marinum* in its ability to hydrolyze pyrazinamide and nicotinamide and *M. ulcerans* in its positive neutral red test (42), the species differ significantly in other biochemical activities, as well as with respect to the photochromogenicity of *M. marinum.*

HPLC. Analysis of mycolic acids of the mycobacterial cell wall has become a practical and rapid method of identifying mycobacteria isolated in the clinical laboratory (10, 47). Figure 6 depicts a typical high-pressure liquid chromatography (HPLC) chromatogram of a strain of *M. haemophilum* isolated from one of the patients in Arizona (10). In a recent study that included a variety of mycobacterial species, the use of HPLC allowed early identification of 16 strains of *M. haemophilum* by their characteristic chromatograms (47).

Pathogenicity

M. haemophilum is apparently of low virulence, since healthy mice and guinea pigs survived after intramuscular, intravenous, and subcutaneous inoculation with large numbers of bacilli (42). However, mice given prednisone and intravenous injections of the organisms developed ear lesions, containing AFB,

TABLE 4. Characteristics of *M. haemophilum*[a]

Characteristic	Reaction or activity[b]	
Growth temp (°C)		
20–25	±	
30	+	
32	+	
35–37	∓	
42	−	
Pigment	−	
Neutral red test	+	
Niacin accumulates	−	
5% NaCl tolerance	−	
Hemin, FAC, or other iron containing supplements required	+	
Resistance to isonicotinic acid hydrazide	+	
Enzymes		
Nicotinamidase	+	
Pyrazinamidase	+	
Acid phosphatase	−	(+)[c]
Nitrate reduction	−	(+)[d]
Urease (3 days)	−	
β-Galactosidase	−	
Catalase (45-mm foam and resists 68°C)	−	
Tween hydrolysis (10 days)	−	
Tellurite reduction (3 days)	−	
Arylsulfatase (3 and 14 days)	−	

[a] Compiled from references 9, 17, 21, 29, 33, 37–39, 41–43, and 51.
[b] +, positive; ±, considered positive but less than optimal growth; ∓, usually negative but positive at times under special circumstances; −, negative.
[c] Inconsistent reports: Males et al. (33) reported a positive reaction.
[d] Inconsistent reports: Dawson and Jennis (17), Mezo et al. (37), and Ryan and Dwyer (41) reported equivocal or weakly positive reactions.

that were grossly similar to the cutaneous lesions seen in human infections (1, 2).

In vitro, *M. haemophilum* seems to have a preference for growth in cultured human epithelial cells (20). A recently developed cell culture model suggests a greater intracellular replication at 33 than at 37°C and shows that the microorganisms are associated with cytotoxicity at the lower temperature (20).

Antimicrobial Susceptibility Tests

There is no standardized procedure for determining the in vitro antimicrobial susceptibility patterns of *M. haemophilum*. The nature of *M. haemophilum* requires the use of supplemented media for such studies, yet it has been recognized that the addition of iron-containing compounds (e.g., ferric ammonium citrate) to the test medium may interfere with the activity of antimycobacterial agents (1, 2, 29, 37). Previously described methods of testing the organism include (i) a proportional method involving Middlebrook 7H10 agar supplemented with hemin (46); (ii) a disk elution method with round-well cell culture plates, Middlebrook 7H10 broth, commercial antimicrobial disks, and commercial hemin disks (35); (iii) a microdilution method involving various concentrations of antimicrobics in Middlebrook 7H9 broth with and without hemin (48); and (iv) agar or broth dilution methods involving Middlebrook 7H11 agar or 7H12 broth with added hemin (23). Standard concentrations of the organism are added to the test systems, and results are usually available after incubation at 30 to 32°C for 1 to 3 weeks.

Although test methods have varied, there is some consensus about the susceptibility of *M. haemophilum* to certain antimicrobial agents (Table 5). The organism is most susceptible to ciprofloxacin, clarithromycin, rifabutin, and rifampin and is usually resistant to ethambutol, isoniazid, and pyrazinamide (26). However, resistance to the rifamycins developed in two patients who received therapy with several antimycobacterial agents, including the rifamycins (7).

SUMMARY AND RECOMMENDATIONS

M. haemophilum, an organism of low virulence, is emerging as a pathogen in patients who are severely immunocompro-

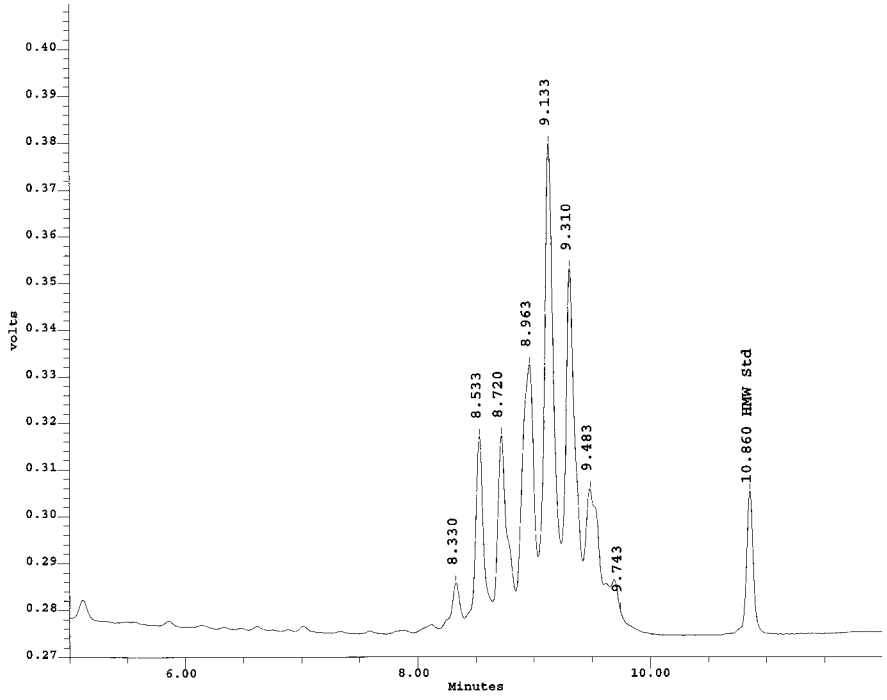

FIG. 6. Characteristic mycolic acid chromatogram of *M. haemophilum* obtained by HPLC as described in reference 10. HMW Std: standard.

TABLE 5. Consensus of in vitro antimicrobial susceptibility profiles of *M. haemophilum*[a]

Usually susceptible	Variable susceptibility	Usually resistant
Ciprofloxacin	Amikacin	Ethambutol
Clarithromycin	Cefoxitin	Isoniazid
Rifabutin	Doxycycline/minocycline	Pyrazinamide
Rifampin	Erythromycin	
	Ethionamide	
	Kanamycin	
	p-Aminosalicylic acid	
	Streptomycin	
	Trimethoprim-sulfamethoxazole	

[a] Compiled from references 23, 26, 30, 33–35, 38, 40, 42, and 48.

mised. Its restricted temperature requirements might explain the frequency of lesions on the superficial areas of the body, although deep-tissue and bone infections and pneumonia do occur.

The clustering of infections in certain patient groups and in certain parts of the world may only partly be explained by the heightened awareness of local clinicians and microbiologists or by the possibility of many cases not being reported. Since the source and means of acquisition of the organism are unknown, many questions about the epidemiology of the disease remain unanswered. For example, what is the reason for the comparatively large number of cases from Australia and Arizona, mostly in women who have received renal transplants or in apparently healthy children? The number of cases in patients with AIDS in the United States is not particularly surprising, but why have most cases been documented in New York City? Why, to date, have all of the cases in bone marrow transplant recipients come from one institution in New York City?

The timely diagnosis of *M. haemophilum* requires communication between clinicians and personnel in the microbiology laboratory. Since media must be supplemented with an iron source and incubated at 30 to 32°C, the laboratory must be informed when *M. haemophilum* infection is suspected. The urgency for making a diagnosis is illustrated by the two fatal cases in bone marrow transplant recipients in whom respiratory complaints and radiographic findings preceded the diagnosis. The infection progressed relentlessly despite empirical antibiotics for other processes. A delay in both the diagnosis and the institution of appropriate antibiotics probably contributed to the poor outcome in these patients.

What specimens should the laboratory routinely culture for the organism? Although situations may differ in different institutions or geographic areas, the use of conditions optimal for growth of *M. haemophilum* might be considered for (i) cutaneous ulcerations or septic arthritis in immunocompromised patients, (ii) undiagnosed pulmonary lesions in bone marrow transplant recipients, (iii) adenitis in children, and (iv) situations when acid-fast stains from immunocompromised patients are positive, particularly if stains from previous specimens were acid-fast positive and the culture was negative.

Treatment of *M. haemophilum* infection is best guided by the age and underlying disease of the patient. Children with adenitis respond well to excision of affected lymph nodes only. The outcome of treatment in adults is most influenced by the ability to enhance immune system function and the use of an antimicrobial regimen that includes some combination of ciprofloxacin, clarithromycin, and one of the rifamycins. Duration of therapy should also be guided by the patient's underlying condition and clinical response.

Standardization of in vitro methods for evaluation of the susceptibility of *M. haemophilum* to antimicrobial agents is needed, as is correlation of these results to clinical outcomes in patients. Until such studies are conducted, it is prudent to use in vitro susceptibility data cautiously in choosing an antimicrobial regimen when clinical circumstances warrant therapy.

It would be beneficial to study prospective new cases of *M. haemophilum* infections. Because of the sporadic nature of the disease and the small number of new cases recognized annually, it might be helpful to establish a registry of such cases that would accumulate data for appropriate evaluations of the disease and its epidemiology.

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